MECHANISMS UNDERPINNING THE LINK BETWEEN EMOTION, PHYSICAL HEALTH AND LONGEVITY

EDITED BY: Andrew H. Kemp
PUBLISHED IN: Frontiers in Psychology and Frontiers in Public Health

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-274-3
DOI 10.3389/978-2-88945-274-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

MECHANISMS UNDERPINNING THE LINK BETWEEN EMOTION, PHYSICAL HEALTH AND LONGEVITY

Topic Editor:
Andrew H. Kemp, Swansea University, United Kingdom; University of São Paulo, Brazil and University of Sydney, Australia

The 1990's was designated as 'the decade of the brain' and now, common mental disorders are described as 'brain disorders'. Yet intense research interest on the brain has largely side-lined the body as a passive observer, disconnecting mental from physical health and contributing to further societal stigma on the nature of psychiatric illness and mental distress. The biopsychosocial pathway to premature mortality or longevity is a complex one, involving a host of closely intertwined mechanisms and moderating factors, some of which are investigated in this special issue. All the articles published here provide new insights into the pathways linking emotion, physical health and longevity, highlighting the tight linkage between mind, brain and body.

Citation: Kemp, A. H., ed. (2017). Mechanisms Underpinning the Link between Emotion, Physical Health and Longevity. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-274-3

Table of Contents

Section 5: Interoception

Section 6: Positive health behaviours

Section 7: Social ties

frontiers in Psychology

EDITORIAL
published: 02 August 2017
doi: 10.3389/fpsyg.2017.01338

Editorial: Mechanisms Underpinning the Link between Emotion, Physical Health, and Longevity

Andrew H. Kemp [1, 2]*

[1] Department of Psychology and the Health and Wellbeing Academy, College of Human and Health Sciences, Swansea University, Swansea, United Kingdom, [2] School of Psychology and Discipline of Psychiatry, University of Sydney, Sydney, NSW, Australia

Keywords: emotion, health, wellbeing, morbidity and mortality, longevity, stress, vagal function, GENIAL model

Editorial on the Research Topic

Mechanisms Underpinning the Link between Emotion, Physical Health, and Longevity

The study of emotion has been likened to the 100 Years War between England and France (Lindquist et al., 2013), a conflict that may relate, in part, to intense research interest on the brain, largely side-lining the body as a passive observer. However, research on the link between the brain and body may help to develop a more informed basis on which to understand our emotions. Such research also has important implications for premature morbidity and mortality. Each article published in this special issue and research topic is now briefly described, focusing on the main findings and relevance for better understanding the links between emotion, physical health, and longevity.

In the first article of this issue, Kemp et al. report on associations between common mental disorders and coronary heart disease (CHD), conditions that impose considerable burden on society. In their large epidemiological cohort study on the Brazilian population, participants with psychiatric comorbidity display a threefold increase in CHD, while a 1.5- to 2-fold increase was observed for non-comorbid conditions. Also in the issue, the same authors demonstrate that tricyclic medications—a class of antidepressants freely dispensed in Brazilian public health pharmacies—are associated with a twofold increase in CHD, relative to non-use. These findings highlight an urgent need for better understanding how extreme negative emotions—and their treatments—might impact on physical health over the longer term, just as other studies discussed below point to the need for greater insight into how positive health behaviors more commonly associated with physical wellbeing (e.g., exercise, diet) can improve emotional and mental health.

The biopsychosocial pathway to premature morbidity and mortality is a complex one, involving a host of closely intertwined mechanisms (Kemp and Quintana, 2013; Kemp et al., 2016b, in press), some of which are investigated in contributions to this special issue. One of these mechanisms includes ongoing unconscious emotion processing, which may contribute to adverse health outcomes. In this regard, a study in the issue by van der Ploeg et al. examines the validity of the Implicit Positive and Negative Affect Test (IPANAT; Quirin et al., 2009; Brosschot et al., 2014) and investigates whether changes in unconscious emotions (as measured by the IPANAT) relate to changes in a variety of physiological parameters. The IPANAT requires participants to rate six artificial words (vikes, tunba, ronpe, belni, sukov, safme) on six emotional adjectives (happy, cheerful, energetic, helpless, tense, inhibited) using a six-point Likert scale. Higher implicit negative affect was associated with higher systolic blood pressure, lower heart rate variability (HRV) and total peripheral resistance, and slower recovery of diastolic blood pressure. Also in the issue by the same research group, (Mossink et al.) subjective stress, worry and affect were measured over a 24-h period in addition to cortisol levels in saliva.

OPEN ACCESS

Edited and reviewed by:
Marina A. Pavlova,
University of Tübingen, Germany

***Correspondence:**
Andrew H. Kemp
a.h.kemp@swansea.ac.uk

Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *21 June 2017*
Accepted: *20 July 2017*
Published: *02 August 2017*

Citation:
Kemp AH (2017) Editorial: Mechanisms Underpinning the Link between Emotion, Physical Health, and Longevity. Front. Psychol. 8:1338. doi: 10.3389/fpsyg.2017.01338

Implicit negativity bias was associated with increased cortisol levels, and higher levels of implicit sadness were associated with a stronger cortisol rise the next day. Research has seldom explored the physiological impacts of unconscious stress and further research in this area is needed.

A commonly measured variable in studies on stress is HRV. Unfortunately, researchers typically consider HRV as an epiphenomenon of the stress response (e.g., Friedman and Kern, 2014), rather than—as we would contend—a marker of vagal function responsible for a host of psychological and physiological processes that influence social ties and subsequent health and wellbeing (Kemp et al., in press). Also in the issue, a study by Porges et al. reports that individuals with lower vagal function respond with greater increases in salivary testosterone in response to observed violence. This finding was interpreted in line with a hierarchical model of autonomic activity (polyvagal theory; Porges, 2011) such that those with lower vagal function may be more vulnerable to environmental threat, increasing the potential for interpersonal conflict. The authors suggest that individuals with lower vagal function may have difficulties with emotional regulation, a suggestion that is supported by another article in the issue by Williams et al.

Reduced vagal function is also commonly reported in patients with a variety of psychiatric disorders, especially major depression (Kemp et al., 2010; Brunoni et al., 2013) and generalized anxiety disorder (Chalmers et al., 2014; Kemp et al., 2014). However, contradictory findings have also been reported, motivating another study in the issue by Kemp et al. Patients with melancholic depression were found to display increased heart rate and lower resting-state HRV relative to non-depressed controls. One explanation for the contradictory findings in the literature is that past studies have focused on major depressive disorder, rather than more homogeneous subtypes of depression such as melancholia. Critically, over the longer term these cardiac alterations may contribute to increased risk for premature morbidity and mortality. This possibility is supported by a recent meta-analysis on 21,988 participants without known cardiovascular disease (Hillebrand et al., 2013), reporting that vagal impairment predicts adverse cardiovascular events up to 15 years later.

Together, these findings have important implications for future research in this area, although it is also worth reminding newcomers about some of the methodological challenges, a motivation for including the paper by Quintana and Heathers in the issue.

Another area of research that has attracted considerable attention is the extent to which people are able to perceive and regulate visceral information from the body, a skill known as interoceptive ability. Interoceptive signals are communicated by the vagus nerve and a dedicated lamina-1 spinothalamocortical pathway to interoceptive centers in the brain (Craig, 2002, 2014; Critchley et al., 2004; Kemp et al., 2015). Atypical interoceptive sensitivity has been associated with psychopathology in adolescence and decreased socio-emotional competence in adulthood (Murphy et al., 2017). Another article in the issue by Jones et al. examined neural substrates for the volitional regulation of heart rate. Participants were instructed to either increase or decrease the level of an on-screen thermometer reflecting heart rate by either volitionally increasing or decreasing heart rate, respectively. Results demonstrate that heart rate during the arousal condition (76 bpm) significantly differed from the relaxation condition (72 bpm) indicating that instructions to participants had their desired effect. Decreases in heart rate were associated with activation of ventrolateral prefrontal and parietal cortices, regions known to be involved in cognitive reappraisal, and selective attention, respectively. Interestingly, participants could increase heart rate without any corresponding change in respiration, which may in part explain the relatively small observed changes in heart rate. Heart rate change also negatively correlated with anxiety scores. While no participant scored above the clinical threshold for anxiety, participants with more anxiety symptoms were less able to regulate their heart rate. Heart rate biofeedback training with a focus on prolonging the out-breath may help to diminish anxiety symptoms (Wells et al., 2012) and have a place in the management of anxiety disorders.

The study by Mallorquí-Bagué et al. in the issue examines the link between anxiety, affective reactivity and interoception in otherwise healthy people with joint hypermobility, an inherited condition associated with the expression of a common variation in the connective tissue protein, collagen. Hypermobility was assessed by asking participants whether they could touch the floor with their hands without bending their knees. Interestingly, joint hypermobility is associated with increased risk of anxiety and related disorders (Sanches et al., 2012). Interoception was found to mediate a positive association between joint hypermobility and state anxiety such that hypermobility is associated with heightened interoceptive sensitivity, which then contributes to state anxiety. Also reported was greater activation in the hypermobile group relative to controls during processing of sad vs. neutral images within a discrete set of brain regions associated with emotional processing. These findings highlight the dependence of emotional state on bodily context and further our understanding of how vulnerability to anxiety disorder might arise.

In another article of this issue, the study by Couto et al. examined two rare patients with respective damage of focal lesions to the insular cortex and to its subcortical tracts. Internally generated interoceptive streams were assessed through a heartbeat detection task, while externally triggered streams were examined via taste, smell, and pain recognition tasks. The patient with a lesion to the insula cortex displayed impaired internal signal processing while the patient with the subcortical lesion exhibited external perception deficits. The authors argue that the subcortical lesion may disrupt integrative contextual processing—spared in the patient with focal insula lesion—via a fronto-insulo-temporal network including the insula cortex as a critical hub. Importantly, this distinctive pattern was replicated when comparing patients' performance to that of subjects with lesions in other regions. This helps to confirm that the observed pattern of deficits relates to specific lesions rather than to nonspecific brain damage. These results point to a stratification of multimodal bodily signals that subsequently contribute to emotional awareness.

Considering evidence that antidepressant medications are not as safe as they were once believed (Licht et al., 2010; Kemp et al., 2014, 2016a), it is likely that positive health behaviors will play an increasingly important role in the management of mental health conditions. In their opinion article, Dash et al. make a strong argument in the issue for greater recognition that diet is a risk factor for common mental disorders and for public health strategies focused on dietary improvement. They argue that the physical health of patients should be given equal priority as a treatment target when considering patient mental health, and highlight the importance of a diet characterized by fruits, vegetables, whole grains, nuts, seeds, and fish while limiting intake of processed foods (Opie et al., 2015; see also: Jacka, 2017). Also in the issue, the study by Grung et al. focuses on the beneficial physiological effects of a fish diet on psychiatric inpatients including improved HRV, which are indices of emotional regulation as well as physical health. Two other papers in the issue focus on the effects of physical activity in bipolar disorder (Thomson et al.) and traumatic brain injury (TBI; Rzezak et al.). Also in the issue, the study by Garland et al. demonstrates that mindfulness may help to maintain trait positive affect and momentary positive cognitions in an upward spiral. Together, these articles highlight the utility of non-pharmacological options for improving health and wellbeing and provide some guidance on underlying mechanisms. Further research is needed to better inform and underpin the development of non-pharmacological interventions for mental-health conditions.

In the final study of the issue, IJzerman et al. suggest how relationship therapy might be modernized, a proposal that builds on Social Thermoregulation Theory (IJzerman et al., 2015). According to this theory, humans may adapt social behaviors and cognitions to temperature changes. For example, the authors describe how emotions like anxiety and sadness are associated with lower peripheral temperature, and that people respond to others' sadness with an increase in temperature. The authors explain that thermoregulation is crucial for survival and that individual differences in the need for thermoregulation may even predict attachment styles, health, and wellbeing. While the research in this area is still in its early stages, the links to social ties and health outcomes is intriguing, and is supported by an increasing body of evidence that was recently captured by the GENIAL model (Genomics—Environment—vagus Nerve—social Interaction—Allostatic regulation—Longevity; Kemp et al., in press).

In conclusion, all papers included in the special issue provide new insights into the pathways linking emotion, physical health, and longevity. Future research in this area would benefit from moving beyond the disciplinary dilemma, initiating multi-disciplinary exchange and facilitating new lines of interdisciplinary enquiry to better understand the complex pathways from emotion to longevity. The GENIAL model (Kemp et al., in press) is a first attempt to do exactly this and provides a foundation on which future research could be based.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

REFERENCES

Brosschot, J. F., Geurts, S. A. E., Kruizinga, I., Radstaak, M., Verkuil, B., Quirin, M., et al. (2014). Does unconscious stress play a role in prolonged cardiovascular stress recovery? *Stress Health* 30, 179–187. doi: 10.1002/smi.2590

Brunoni, A. R., Kemp, A. H., Dantas, E. M., Goulart, A. C., Nunes, M. A., Boggio, P. S., et al. (2013). Heart rate variability is a trait marker of major depressive disorder: evidence from the sertraline vs. electric current therapy to treat depression clinical study. *Int. J. Neuropsychopharm.* 16, 1937–1949. doi: 10.1017/S1461145713000497

Chalmers, J. A., Quintana, D. S., Abbott, M. J.-A., and Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. *Front. Psychiatry* 5:80. doi: 10.3389/fpsyt.2014.00080

Craig, A. D. (2002). How do you feel? *I*nteroception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655–666. doi: 10.1038/nrn894

Craig, A. D. B. (2014). *How Do You Feel?* Princeton, NJ: Princeton University Press.

Critchley, H. D., Wiens, S., Rotshtein, P., Öhman, A., and Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nat Neurosci.* 7, 189–195. doi: 10.1038/nn1176

Friedman, H. S., and Kern, M. L. (2014). Personality, well-being, and health. *Annu. Rev. Psychol.* 65, 719–742. doi: 10.1146/annurev-psych-010213-115123

Hillebrand, S., Gast, K. B., de Mutsert, R., Swenne, C. A., Jukema, J. W., Middeldorp, S., et al. (2013). Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace* 15, 742–749. doi: 10.1093/europace/eus341

IJzerman, H., Coan, J. A., Wagemans, F. M. A., Missler, M. A., van Beest, I., Lindenberg, S., et al. (2015). A theory of social thermoregulation in human primates. *Front. Psychol.* 6:464. doi: 10.3389/fpsyg.2015.00464

Jacka, F. N. (2017). Nutritional psychiatry: where to next? *EBioMedicine* 17, 24–29. doi: 10.1016/j.ebiom.2017.02.020

Kemp, A. H., and Quintana, D. S. (2013). The relationship between mental and physical health: insights from the study of heart rate variability. *Int. J. Psychophysiol.* 89, 288–296. doi: 10.1016/j.ijpsycho.2013.06.018

Kemp, A. H., Arias, J. A., and Fisher, Z. (in press). "Social ties, health and wellbeing: a literature review and model," in *Neuroscience and Social Science: The Missing Link,* eds A. Ibanez, L. Sedeno, and A. M. García (Springer International).

Kemp, A. H., Brunoni, A. R., Santos, I. S., Nunes, M. A., Dantas, E. M., Carvalho de Figueiredo, R., et al. (2014). Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil cohort baseline study. *Am. J. Psychiatry* 171, 1328–1334. doi: 10.1176/appi.ajp.2014.13121605

Kemp, A. H., Fráguas, R., Brunoni, A. R., Bittencourt, M. S., Nunes, M. A., Dantas, E. M., et al. (2016a). Differential associations of specific selective serotonin reuptake inhibitors with resting-state heart rate and heart rate variability. *Psychosom. Med.* 78, 810–818. doi: 10.1097/PSY.0000000000000336

Kemp, A. H., Krygier, J., and Harmon-Jones, E. (2015). "Neuroscientific perspectives of emotion," in *Handbook of Affective Computing,* eds R. A. Calvo, S. D'Mello, J. Gratch, and A. Kappas (New York, NY: Oxford University Press), 38–53.

Kemp, A. H., López, S. R., Passos, V. M. A., Bittencourt, M. S., Dantas, E. M., Mill, J. G., et al. (2016b). Insulin resistance and carotid intima-media thickness mediate the association between resting-state heart rate variability and executive function: a path modelling study. *Biol Psychol.* 117, 216–224. doi: 10.1016/j.biopsycho.2016.04.006

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., and Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart

rate variability: a review and meta-analysis. *Biol. Psychiatry* 67, 1067–1074. doi: 10.1016/j.biopsych.2009.12.012

Licht, C. M. M., de Geus, E. J. C., van Dyck, R., and Penninx, B. W. J. H. (2010). Longitudinal evidence for unfavorable effects of antidepressants on heart rate variability. *Biol. Psychiatry* 68, 861–868. doi: 10.1016/j.biopsych.2010.06.032

Lindquist, K., Siegel, E. H., Quigley, K. S., and Barrett, L. F. (2013). The hundred-year emotion war: are emotions natural kinds or psychological constructions? Comment on Lench, Flores, and Bench (2011). *Psychol Bull.* 139, 255–263. doi: 10.1037/a0029038

Murphy, J., Brewer, R., Catmur, C., and Bird, G. (2017). Interoception and psychopathology: a developmental neuroscience perspective. *Dev. Cogn. Neurosci.* 23, 45–56. doi: 10.1016/j.dcn.2016.12.006

Opie, R. S., O'Neil, A., Itsiopoulos, C., and Jacka, F. N. (2015). The impact of whole-of-diet interventions on depression and anxiety: a systematic review of randomised controlled trials. *Public Health Nutr.* 18, 2074–2093. doi: 10.1017/S1368980014002614

Porges, S. W. (2011). *The Polyvagal Theory: Neurophysiological Foundations of Emotions, Attachment, Communication, and Self-Regulation.* New York, NY: W. W. Norton & Company.

Quirin, M., Kazén, M., and Kuhl, J. (2009). When nonsense sounds happy or helpless: the Implicit Positive and Negative Affect Test (IPANAT). *J. Pers. Soc. Psychol.* 97, 500–516. doi: 10.1037/a0016063

Sanches, S. H. B., Osório Fde, L., Udina, M., Martín-Santos, R., and Crippa, J. A. S. (2012). Anxiety and joint hypermobility association: a systematic review. *Rev. Bras. Psiquiatr.* 34, 53–60. doi: 10.1016/S1516-4446(12)70054-5

Wells, R., Outhred, T., Heathers, J. A. J., Quintana, D. S., and Kemp, A. H. (2012). Matter over mind: a randomised-controlled trial of single-session biofeedback training on performance anxiety and heart rate variability in musicians. *PLoS ONE* 7:e46597. doi: 10.1371/journal.pone.0046597

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
PSYCHOLOGY

ORIGINAL RESEARCH ARTICLE
published: 25 February 2015
doi: 10.3389/fpsyg.2015.00187

The association between mood and anxiety disorders, and coronary heart disease in Brazil: a cross-sectional analysis on the Brazilian longitudinal study of adult health (ELSA-Brasil)

Andrew H. Kemp[1,2]*, Andre R. Brunoni[1], Maria A. Nunes[3], Itamar S. Santos[1], Alessandra C. Goulart[1], Antonio L. Ribeiro[4], Isabela M. Benseñor[1] and Paulo A. Lotufo[1]

[1] *Center for Clinical and Epidemiologic Research, University Hospital and Faculty of Medicine, University of São Paulo, São Paulo, Brazil*
[2] *School of Psychology and Discipline of Psychiatry, University of Sydney, Sydney, NSW, Australia*
[3] *Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*
[4] *Hospital das Clínicas and Faculty of Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Edited by:
Luiz Pessoa, University of Maryland, USA

Reviewed by:
Mario F. Juruena, University of Sao Paulo, Brazil
Linda Booij, Queen's University, Canada

****Correspondence:***
Andrew H. Kemp, Center for Clinical and Epidemiologic Research, Hospital Universitario at University of São Paulo, Av. Lineu Prestes 2565, 05508-000 São Paulo, Brazil
e-mail: andrew.kemp@hu.usp.br; andrew.kemp@sydney.edu.au; andrewhaddonkemp@gmail.com

Background: Associations between major depressive disorder (MDD) and coronary heart disease (CHD) have been established, and these associations increase risk of future morbidity and mortality. Prior research has been carried out in high-income countries. Here we examine associations between the mood and anxiety disorders, and CHD in a large cohort at baseline from Brazil, a country facing a variety of challenges that may affect these associations.

Methods: Participants included 15,105 civil servants aged 35 to 74 at baseline (2008–2010) from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). CHD ($N = 721$) included self-reported angina pectoris ($n = 305$), myocardial infarction ($n = 259$) and coronary revascularization ($n = 239$). Hierarchical logistic regression analyses were conducted to estimate odds ratios and confidence intervals.

Results: Major findings indicate that comorbid MDD and anxiety disorders ($n = 434$) are associated with a threefold increase in CHD, MDD alone ($n = 170$) with a twofold increase in CHD, while generalized anxiety disorder alone ($n = 1,394$) and mixed anxiety and depression disorder ($n = 1,844$) – symptoms present, but diagnostic threshold not reached – are associated with a 1.5-fold increase in CHD, after full adjustment for covariates.

Conclusion: The association with CHD is greatest in those with psychiatric comorbidity, while associations were also observed in MDD and generalized anxiety disorder without comorbidity. While findings are limited by the cross-sectional design of the study, given the known risks associated with comorbidity of the mood and anxiety disorders with CHD, findings reinforce the importance of comprehensive health assessment in Brazil.

Keywords: major depressive disorder, anxiety disorders, disorder comorbidity, coronary heart disease

INTRODUCTION

Major depressive disorder (MDD) and coronary heart disease (CHD) are leading burdens of disease and the relationship between these disorders is bidirectional (Nemeroff and Goldschmidt-Clermont, 2012; Ramasubbu et al., 2012a; Stapelberg et al., 2012; Lichtman et al., 2014): many patients experience depression decades before CHD is manifest, while MDD in patients with CHD is associated with increased morbidity and mortality. However, it remains unclear whether MDD, anxiety disorder or their comorbidity are most associated with CHD. Research has begun to highlight associations between the anxiety disorders and CHD (Tully and Cosh, 2013; Tully et al., 2013). A study on participants recruited for the Netherlands Study of Depression and Anxiety ($N = 2807$) reported that persons with current anxiety disorders with or without depression have a threefold increased prevalence of CHD, while no associations were observed for those with depressive disorders alone, or for depressive and anxiety disorders in remission (Vogelzangs et al., 2010). Research has been restricted to high-income countries including Australia, Canada, Denmark, Finland, Netherlands, UK, and USA (Vogelzangs et al., 2010; Tully and Cosh, 2013), highlighting a need for research in low to middle-income countries. Given the lack of data on the associations between common mental disorders (CMD), specific mood and anxiety disorders, and CHD in Brazil, an upper-middle-income country, the present study sought to address this need.

It is unclear whether the associations between the mood and anxiety disorders, and CHD parallel those or differ from the findings reported in developed countries such as the Netherlands (e.g., Vogelzangs et al., 2010). Research has demonstrated that Brazil is characterized by higher rates of mental disorders

(Andrade et al., 2012), while rates of antidepressant use (Brunoni et al., 2013) are lower than high-income countries. Brazil faces significant social challenges that may contribute to psychological distress (de Jesus Mari, 2014), which may affect observed associations. For example, Brazil has one of the highest levels of income inequality in the world and recent data from this country (Filho et al., 2013) indicate that this inequality is associated with mental disorders, especially depression. The authors argued that inequality is associated with adverse social comparisons, leading to psychological distress, and increases in disease and mortality, consistent with the 'relative income hypothesis' (Wilkinson, 2002). Income inequality is likely one of many psychosocial stressors that may affect the associations between the mood and anxiety disorders and CHD in Brazil. We hypothesized a relationship between CMD and CHD, and predicted that the prevalence of CHD would be most robust in those with comorbid MDD and anxiety disorders after controlling for relevant confounding factors including antidepressant medications, socio-demographic issues, physical inactivity, obesity and smoking, as well as risk factors including dyslipidemia, hypertension, and diabetes. Here we defined CHD as participants reporting a medical history of stable angina pectoris, myocardial infarction (MI) or coronary revascularisation.

MATERIALS AND METHODS

PARTICIPANTS

ELSA-Brasil is a cohort of 15,105 civil servants aged 35 to 74 enrolled between August, 2008 and December, 2010 at six cities (Belo Horizonte, Porto Alegre, Rio de Janeiro, Salvador, Sao Paulo, and Vitoria) designed to investigate the relationship between cardiovascular diseases and diabetes, their social determinants and risk factors. The study design and sampling procedures of ELSA-Brasil have been reported previously (Aquino et al., 2012; Schmidt et al., 2014). Exclusion criteria comprised current or recent pregnancy (within 4 months of first interview), intention to quit working at the institution in the near future, severe cognitive or communication impairment, and if retired, residence outside of a study centre's metropolitan area.

ETHICS STATEMENT

The ethics committees of the participating universities approved the research protocol. All participants provided written informed consent after a complete description of the study. The study design and sampling procedures of ELSA-Brasil have been reported previously (Aquino et al., 2012; Schmidt et al., 2014).

PSYCHIATRIC EVALUATION

Mental disorders were determined by trained interviewers using the Portuguese version (Nunes et al., 2012) of the Clinical Interview Schedule-Revised (CIS-R; Lewis et al., 1992). This is a structured interview used for diagnosis of current, common, non-psychotic psychiatric conditions in the community. The complete CIS-R version was applied; CMDs were defined as participants with CIS-R scores $\geq$12. The following ICD-10 categories were also determined: MDD, agoraphobia, social phobia, specific phobia, generalized anxiety disorder (GAD), panic disorder, social anxiety disorder, and mixed anxiety and depressive disorder (MADD). The ICD-10 refers to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems, a medical classification list by the World Health Organization. These diagnostic groupings were then reduced to six categories: MDD, GAD, all phobias, panic disorder, a comorbid group comprised of participants with MDD and any anxiety disorder, and MADD. The latter two categories are distinct; the former comprises participants that meet criteria for both MDD and any anxiety disorder, while the latter comprises participants who display symptoms of both anxiety and depression, but do not meet the criteria of either diagnosis separately.

CORONARY HEART DISEASE ASSESSMENT

Coronary heart disease included participants who reported a medical diagnosis of stable angina pectoris, MI or coronary revascularization determined through questionnaire- and intensive interview-based assessment focusing on medical history.

COVARIATES

Sociodemographic information included age, sex, years of education (less than high-school, high-school, university) and race (white, mixed, black, Asiatic, indigenous). Lifestyle and behavioral characteristics included smoking status (never, past/current), physical activity (measured with the International Physical Activity Questionnaire (Craig et al., 2003) and categorized according to low, moderate, or high activity based on scoring guidelines: http://www.ipaq.ki.se/scoring.pdf) and body mass index (BMI; weight in kilograms divided by height in meters squared). Established risk factors for CHD were also considered in analysis; these included hypertension, diabetes mellitus (DM), and dyslipidemia. Hypertension was defined as systolic blood pressure $\geq$140 mm Hg, or diastolic blood pressure $\geq$90 mm Hg, or use of antihypertensive medications. Resting blood pressure was measured three times in the seated position after 5 min rest, and the average of the second and third measurements were used in analyses. Diabetes was defined as self-reported or fasting blood glucose $\geq$126 mg/dL or 2-h oral glucose tolerance test $\geq$200 mg/dL or glycated hemoglobin $\geq$6.5%. Dyslipidemia was defined as LDL-cholesterol $\geq$130 mg/dL or use of lipid reductor. We identified antihypertensive treatments, medications for diabetes, and antidepressants on the basis of prescription and over-the-counter drugs in the past 2 weeks determined through pill bottle review. Antidepressant use was added as a covariate considering that antidepressant medications may also contribute to morbidity and mortality (Smoller et al., 2009; Whang et al., 2009; Hamer et al., 2011).

STATISTICAL ANALYSIS

Statistical analysis was conducted using IBM SPSS Statistics Version 21. Participant characteristics were examined using independent samples *t*-tests and one-way analyses of variance (ANOVA) for contrasts involving continuous dependent measures, and χ^2 statistics for categorical variables. Tukey's HSD was used to correct for multiple comparisons and aid interpretation of ANOVA's, while standardized residuals (*z*-scores) were used to help interpret chi-square tests on larger contingency tables (as per Field, 2013). A series of logistic regression analyses were then used to estimate the odds ratios and 95% confidence intervals for the association

between CMD and CHD, before and after adjustment for covariates. These initial analyses were followed up by sensitivity analysis on the specific mental disorder groupings. Unadjusted analyses (Model 1) were conducted on CMD, as well as specific mental disorder groupings, with no other predictors. Adjusted multivariate analyses (Model 2) involved hierarchical (blockwise) logistic regression analysis on *a priori* selected covariates (as described in previous section). Each covariate grouping (i.e., sociodemographic information, lifestyle/behavioral characteristics, risk factors, and medications) was entered into a successive block; mental disorders were entered into the final block. Variables in each block were entered simultaneously using the forced entry method to assess the independent contribution of each predictor over and above all other predictors entered in the same block. Finally, additional sensitivity analyses were conducted for subtypes of CHD including stable angina pectoris, MI, and coronary revascularisation to determine the consistency of associations across these distinct categories of CHD. Again, these analyses involved hierarchical (blockwise) logistic regression analysis adjusting for covariate groupings (as per Model 2).

RESULTS

PARTICIPANT CHARACTERISTICS

Table 1 summarizes participant characteristics by CHD status. Compared to participants without CHD ($n = 14{,}366$), those with CHD ($n = 721$) were older, black, less educated, and male. They engaged in less physical activity, were heavier, more likely to have smoked (or to be a smoker), have DM, hypertension, dyslipidemia, or a CMD and to be medicated with antidepressants. When groupings of mental disorders were examined, comorbid MDD and anxiety was significantly associated with CHD. **Table 2** presents participant characteristics according to whether or not participants were identified as having a CMD. Compared to those without CMD ($n = 11{,}058$), participants with CMD ($n = 4{,}036$) were more likely to be younger, less educated, and female. They were less likely to be white, engaged in less physical activity, and were heavier. They were more likely to have smoked (or to be a smoker), to have DM or CHD, and to be medicated with antidepressants. **Table 3** provides finer grained detail on participant characteristics by specific mental disorder groupings including MDD ($n = 179$), GAD ($n = 1{,}458$), phobias ($n = 156$), PD ($n = 74$), MADD ($n = 1914$), comorbid MDD, and anxiety disorders ($n = 451$) as well as controls.

ASSOCIATION BETWEEN MENTAL DISORDERS AND CHD

Table 4 describes the results of unadjusted and fully adjusted logistic regression analyses assessing the association of CMD as well as mental disorder groupings relative to those without mental disorders. Model 1 provides the results of unadjusted analyses conducted for CMD [model $\chi^2(1) = 26.53$, $p = 0.001$] and specific mental disorder groupings [model $\chi^2(6) = 32.16$, $p < 0.001$]. Model 2 refers to adjusted analyses for CMD [model $\chi^2(17) = 708.25$, $p < 0.001$; block $\chi^2(1) = 43.76$, $p < 0.001$] and mental disorder groupings [model $\chi^2(22) = 686.87$, $p < 0.001$; block $\chi^2(6) = 43.06$, $p < 0.001$]. In the fully adjusted model (model 2), the odds of CHD were most robust for those with comorbid MDD and anxiety disorders (OR: 2.99, 95% CI = 2.10–4.28), followed by those participants with MDD (OR: 2.14, 95% CI = 1.17–3.95), participants with GAD (OR: 1.41, 95% CI = 1.07–1.85), and participants that displayed symptoms of both anxiety and depression, but did not meet the criteria of either diagnosis separately (MADD; OR: 1.53, 95% CI = 1.20–1.94). Participants with comorbid MDD and anxiety disorders display a greater association with CHD, than GAD and MADD (i.e., confidence intervals do not overlap), but not MDD.

ASSOCIATION BETWEEN MENTAL DISORDERS AND CHD SUBTYPES

Table 5 reports results for additional sensitivity analyses on stable angina pectoris, MI, and coronary revascularization.

For angina, there was a significant association with CMD [model $\chi^2(17) = 199.51$, $p < 0.001$; block $\chi^2(1) = 35.49$, $p < 0.001$], and mental disorder groupings [model $\chi^2(22) = 189.43$, $p < 0.001$; block $\chi^2(6) = 27.57$, $p < 0.001$] after full adjustment. Results indicate that MDD, GAD, MADD, and comorbid MDD and anxiety disorders are associated with angina; odds were most robust for the comorbid group (OR: 2.98, 95% CI = 1.83–4.83), followed by MDD (OR: 2.87, 95% CI = 1.36–6.04), GAD (OR: 1.58, 95% CI = 1.09–2.31), and MADD (OR: 1.64, 95% CI = 1.18–2.29). It should be noted that confidence intervals are overlapping for each of these groups, so we cannot say with certainty that one group displays a 'greater' association with CHD over another.

For MI, there was a significant association with CMD [model $\chi^2(17) = 447.07$, $p < 0.001$; block $\chi^2(1) = 10.32$, $p < 0.001$), and mental disorder groupings [model $\chi^2(22) = 442.72$, $p < 0.001$; block $\chi^2(6) = 18.41$, $p = 0.005$] after full adjustment. Results indicate that MADD and Comorbid groupings are associated with MI; odds were most robust for the Comorbid group (OR: 3.051, 95% CI = 1.74–5.34) followed by the MADD group (OR: 1.54, 95% CI = 1.05–2.26). Again, confidence intervals are overlapping for Comorbid and MADD groupings, thus we cannot say that the Comorbid group displays a 'greater' association with MI, than MADD. For coronary revascularisation, there was a significant association with CMD [model $\chi^2(17) = 483.15$, $p < 0.001$; block $\chi^2(1) = 4.88$, $p = 0.027$], and mental disorder groupings [model $\chi^2(22) = 479.79$, $p < 0.001$; block $\chi^2(6) = 15.01$, $p = 0.02$] after full adjustment. Results indicate that the Comorbid group only is associated with coronary revascularisation (OR: 3.087, 95% CI = 1.67–5.71)

DISCUSSION

The goal of this study was to determine the associations between CMD, specific mood and anxiety disorder groupings, and CHD in a large cohort of participants recruited for the ELSA-Brasil project. Major findings highlight a significant association between CMD and CHD (OR: 1.83, 95% CI = 1.53–2.18), even after full adjustment for sociodemographic variables, lifestyle and behavioral characteristics, and additional risk factors including dyslipidemia, hypertension, diabetes, and medication use. Further analysis revealed that this association is most apparent in MDD, GAD, MADD and comorbid MDD, and anxiety. No significant associations were observed for phobias or PD. The association was most robust for those with comorbid MDD and anxiety (OR: 2.99, 95%

Table 1 | Participant characteristics by coronary heart disease (CHD) status[1].

Characteristics	No CHD ($n = 14366$, 93%)	CHD ($n = 721$, 7%)	Statistic
Age, mean (SD)	51.75 (8.97)	58.71 (8.75)	$t(15085) = 20.34$, $p < 0.001$
Women (%)	54.9	45.5	$\chi^2(1) = 24.28$, $p < 0.001$
Education (%)			$\chi^2(2) = 85.03$, $p < 0.001$
Less than high-school	12.1	23.6	
High-school	34.7	33.6	
College	53.2	42.9	
Race (%)			$\chi^2(4) = 7.76$, $p = 0.101$
White	52.4	50.1	
Mixed	28.2	27.1	
Black	15.9	19.2	
Asiatic	2.5	2.1	
Indigenous	1.0	1.6	
Smoker (past or current) (%)	42.5	55.9	$\chi^2(1) = 50.39$, $p < 0.001$
Physical activity			$\chi^2(2) = 8.59$, $p = 0.014$
Low	76.9	77.7	
Moderate	13.9	16.0	
High	9.2	6.3	
Body mass index (kg/m^2), mean (SD)	26.95 (4.74)	28.40 (4.82)	$t(15079) = 7.99$, $p < 0.001$
Antidepressant status (yes) (%)	6.0	8.7	$\chi^2(1) = 8.39$, $p = 0.004$
Diabetes mellitus (DM; yes) (%)	18.8	37.7	$\chi^2(1) = 156.38$, $p < 0.001$
Hypertension (yes) (%)	34.0	70.0	$\chi^2(1) = 384.40$, $p < 0.001$
Dyslipidemia (yes) (%)	56.9	75.9	$\chi^2(1) = 101.50$, $p < 0.001$
Mental disorder status (%)			$\chi^2(1) = 28.04$, $p < 0.001$
No mental disorder	73.7	64.8	
Common mental disorder (CMD)	26.3	35.2	
Mental disorder groupings[2] (%)			$\chi^2(1) = 20.05$, $p = 0.003$
MDD	1.2	2.0	
GAD	9.9	10.2	
Phobias	1.0	1.5	
PD	0.5	0.7	
MADD	13.0	14.6	
Comorbid	2.9	6.6	

[1] *Some proportions might not add up to 100% due to rounding, and small numbers of participants with missing data.* [2] *Participants categorized into major depressive disorder (MDD), generalized anxiety disorder (GAD), and panic disorder (PD) did not have additional comorbidity. The Phobias category includes Agoraphobia with or without panic disorder, Social Phobias and Specific Phobias. mixed anxiety and depressive disorder (MADD) includes participants who display symptoms of both anxiety and depression, but do not meet the criteria of either diagnosis separately, while the Comorbid group (comorbid anxiety and depression) includes participants that meet criteria for MDD and any anxiety disorder.*

CI = 2.10–4.28), followed by MDD (OR: 2.14, 95% CI = 1.17–3.95), MADD (OR: 1.53, 95% CI = 1.1.20–1.94), and GAD (OR: 1.41, 95% CI = 1.07–1.85). Notably, participants with comorbid MDD and anxiety disorders display a greater association with CHD, than GAD and MADD, but not MDD, a specific finding that we discuss further below. Examination of CHD subtypes also revealed important information; while MDD, GAD, MADD and comorbid MDD, and anxiety were associated with a significant increase in odds for angina, only those with comorbid MDD and anxiety disorders and MADD displayed an increase in odds for MI and coronary revascularisation. In summary, findings highlight (1) strong associations between psychiatric comorbidity and CHD in Brazil, (2) associations for MDD and GAD, in addition to MADD and the comorbid group, with angina (without a history of MI or revascularisation), emphasizing the need for close monitoring of these individuals to avoid further adverse events, and (3) that psychiatric comorbidity is associated with MI and coronary revascularization.

Table 2 | Participant characteristics by CMD status[1].

Characteristics	No CMD ($n = 11058$, 73%)	CMD ($n = 4036$, 27%)	p-value
Age, mean (SD)	52.60 (9.22)	50.70 (8.53)	$t(7703.15) = 11.85$, $p < 0.001$
Women (%)	49.2	68.7	$\chi^2(1) = 451.06$, $p < 0.001$
Education (%)			$\chi^2(2) = 160.27$, $p < 0.001$
Less than high-school	12.3	14.0	
High-school	32.0	41.7	
College	55.7	44.3	
Race (%)			$\chi^2(4) = 133.98$, $p < 0.001$
White	54.7	45.3	
Mixed	26.6	32.4	
Black	15.0	19.1	
Asiatic	2.8	1.8	
Indigenous	0.9	1.4	
Smoker (past or current) (%)	42.3	45.2	$\chi^2(1) = 10.26$, $p < 0.001$
Physical activity			$\chi^2(2) = 164.39$, $p < 0.001$
Low	74.4	84.1	
Moderate	15.3	10.3	
High	10.4	5.5	
Body mass index (kg/m^2), mean (SD)	26.84 (4.61)	27.58 (5.08)	$t(6602.94) = 8.31$, $p < 0.001$
Antidepressant status (yes) (%)	4.6	10.5	$\chi^2(1) = 180.53$, $p < 0.001$
DM (yes) (%)	19.1	21.2	$\chi^2(1) = 8.078$, $p = 0.004$
Hypertension (yes) (%)	35.7	36.0	$\chi^2(1) = 0.082$, p = 0.775
Dyslipidemia (yes) (%)	58.3	56.4	$\chi^2(1) = 4.64$, $p = 0.031$
CHD status (%)			$\chi^2(1) = 28.04$, $p < 0.001$
No	95.8	93.7	
Yes	4.2	6.3	

[1] *Some proportions might not add up to 100% due to rounding, and small numbers of participants with missing data.*

The most robust associations with CHD were observed in those individuals with psychiatric comorbidity. It is worth noting here that the combined presence of anxious and depressive symptoms is associated with more than a twofold increased risk of mortality in patients with ischemic heart disease (Doering et al., 2010), relative to those who were free from psychiatric symptoms. We have previously shown that reduced heart rate variability – a predictor of future mortality – is most reduced in MDD patients with GAD (Kemp et al., 2012), demonstrating the adverse effects of distinctive clinical features including a depressive episode, persistent worry, and hypervigilance on cardiac function (see also Kemp et al., 2014). In the present study, we also observed that only disorders with concurrent anxiety and depression (comorbid MDD and anxiety, and MADD) also displayed associations with 'hard' CHD endpoints including MI and coronary revascularisation. While our findings are consistent with studies from high-income countries (e.g., Vogelzangs et al., 2010), highlighting greater associations with comorbid anxiety and depression, our study also highlights associations with CHD in participants without psychiatric comorbidity. In particular, we observed associations between MDD and GAD, and CHD, further highlighting the importance of non-comorbid, chronic depression, and generalized anxiety, rather than disorders involving heightened phasic anxiety (the phobias and panic disorder). These findings further suggest that characteristic features of depression (anhedonia), generalized anxiety (anxious apprehension), and psychiatric comorbidity (trait negative affect) may all contribute to the associations we observed here in Brazil.

Prior research has indicated that Brazil is a country where depression (and presumably anxiety) is most often somaticized (Simon et al., 1999; see also Mari et al., 2013). While this suggests that the associations we observed here may in part, reflect an overlap of somatic symptoms observed in mental illness and physical disease, the importance of this finding should not be minimized. Prognosis for patients with angina is not benign; in fact, it is no better than those with MI and/or revascularization (e.g., Buckley and Murphy, 2009). Some limitations of this study are worth noting here. These include the cross-sectional design of the study, generalizability of the sample beyond civil servants in Brazil, and the possibility of undetected or undiagnosed CHD

Table 3 | Participant characteristics by mental disorder groupings[1,2].

Characteristics	CTL ($n = 10412$)	MDD ($n = 179$)	Phobias ($n = 156$)	PD ($n = 74$)	GAD ($n = 1458$)	MADD ($n = 1914$)	Comorbid ($n = 451$)	*p*-value
Age, mean (SD)	52.66 (9.23)	50.87 (8.99)	50.15* (8.40)	52.69 (8.48)	50.92* (8.50)	50.72* (8.71)	51.25* (8.44)	$F(6,14650) = 20.58$, $p < 0.001$
Women (%)	48.4*	74.9*	53.5	66.2	69.6*	66.4*	75.1*	$\chi^2(6) = 508.12$, $p < 0.001$
Education (%)								$\chi^2(12) = 165.07$, $p < 0.001$
Less than high-school	12.4	14.0	17.8	24.3*	12.3	12.7	16.3*	
High-school	31.8*	37.4	45.9*	44.6	39.4*	38.8*	48.5*	
College	55.8*	48.6	36.3*	31.1*	48.3*	48.6*	35.2*	
Race (%)								$\chi^2(24) = 160.13$, $p < 0.001$
White	54.8*	55.1	43.5	25.7*	49.9	44.5*	41.6*	
Mixed	26.6*	27.0	33.1	36.5	29.5	32.9*	35.4*	
Black	14.9*	14.6	22.7*	33.8*	16.7	19.6*	19.2	
Asiatic	2.7	2.8	0*	2.7	2.6	1.9	1.5	
Indigenous	0.9	0.6	0.6	1.4	1.3	1.2	2.2*	
Smoker (past or current), %	42.2	50.8	45.2	52.7	44.4	42.9	49.6*	$\chi^2(12) = 19.35$, $p = 0.004$
Physical activity								$\chi^2(12) = 149.18$, $p < 0.001$
Low	74.3*	85.1	85.3	78.1	80.1	83.8*	88.6*	
Moderate	15.3*	9.1	9.6	15.1	12.6	10.3*	7.6*	
High	10.4*	5.7	5.1	6.8	7.3*	6.0*	3.8*	
BMI (kg/m^2), mean (SD)	26.81 (4.58)	27.90* (5.11)	27.71 (5.34)	27.01 (4.71)	27.36* (5.03)	27.48* (5.03)	27.93* (5.32)	$F(6,14644) = 12.17$, $p < 0.001$
Hypertension (yes) (%)	35.7	34.6	38.9	47.3	36.6	35.0	36.6	$\chi^2(6) = 6.07$, $p = 0.42$
Diabetes (yes) (%)	19.0	22.9	24.2	29.7*	18.8	21.3	22.9	$\chi 2(6) = 17.57$, $p = 0.007$
Dyslipidemia (yes) (%)	58.5	53.7	53.8	60.8	57.2	56.4	55.8	$\chi^2(6) = 6.89$, $p = 0.331$
Antidepressant Use (yes) (%)	4.4*	13.6*	8.9	9.5	10.9*	7.3*	15.8*	$\chi^2(6) = 217.86$, $p < 0.001$
CHD (yes) (%)	4.3*	7.8	6.4	6.8	4.8	5.2	10.0*	$\chi^2(6) = 39.47$, $p < 0.001$

[1] *Some proportions might not add up to 100% due to rounding, and small numbers of participants with missing data. *Refers to one-way ANOVA in which disorder is compared to controls (Tukey's HSD, $p < 0.05$) or standardized residuals (z-scores) from χ^2 statistics lying outside ± 1.96 reflecting a significance of value of $p < 0.05$.*

Table 4 | Unadjusted (model 1) and adjusted (model 2) associations of mental disorders with CHD.

	CHD: model 1[a] ($n = 721$)				CHD: model 2[b] ($n = 721$)			
Predictor	***N***	**OR**	**95% CI**	***p*-value**	***N***	**OR**	**95% CI**	***p*-value**
Common mental disorder (CMD)								
No CMD	11050		REF		10631		REF	
CMD	4026	1.526	1.304–1.787	<0.001	3869	1.829	1.535–2.179	<0.001
Mental disorder groupings								
No mental disorder	10405		REF		10012		REF	
MDD	179	1.908	1.097–3.320	0.022	170	2.144	1.165–3.945	0.014
Phobias	156	1.540	0.806–2.944	0.191	151	1.555	0.763–3.169	0.224
PD	74	1.630	0.654–4.059	0.845	73	1.433	0.553–3.708	0.459
GAD	1457	1.135	0.877–1.469	0.337	1394	1.405	1.068–1.849	0.015
MADD	1914	1.240	0.992–1.549	0.059	1844	1.528	1.203–1.941	0.001
Comorbid	451	2.492	1.806–3.440	<0.001	434	2.994	2.096–4.275	<0.001

[a] *Model 1 relates to separate unadjusted analyses for CMD and specific mental disorder groupings including MDD, Phobias, panic disorder (PD), generalized anxiety disorder (GAD), mixed anxiety and depressive disorder (MADD), and Comorbid MDD and any anxiety disorder.* [b] *Model 2 relates to analyses adjusted for sociodemographic variables (age, sex, years of education, ethnicity), lifestyle and behavioral characteristics (smoking status, physical activity, body mass index), risk factors (hypertension, diabetes, dyslipidemia), and medications for lipid reduction, hypertension, diabetes, and mental disorders (antidepressants).*

Table 5 | Association between mental disorders and CHD subtypes fully adjusted for sociodemographic variables, lifestyle and behavioral characteristics, risk factors, and medications.

	Angina ($n = 305$)				Myocardial infarction (MI) ($n = 259$)				Coronary revascularisation ($n = 239$)			
Predictor	***N***	**OR**	**95% CI**	***p*-value**	***N***	**OR**	**95% CI**	***p*-value**	***N***	**OR**	**95% CI**	***p*-value**
Common mental disorder (CMD)												
No CMD	10358		REF		10634			REF	10635			REF
CMD	3755	2.134	1.671–2.724	<0.001	3877	1.615	1.213–2.151	0.001	3878	1.431	1.048–1.954	0.024
Mental disorder groupings												
No mental disorder	9754		REF		10014			REF	10015		REF	
MDD	165	2.866	1.359–6.043	0.006	170	2.142	0.818–5.611	0.121	170	0.427	0.057–3.215	0.408
Phobias	146	1.616	0.585–4.469	0.355	152	2.191	0.843–5.696	0.107	152	0.470	0.063–3.490	0.461
PD	71	2.117	0.648–6.914	0.214	73	0.615	0.081–4.653	0.637	73	1.691	0.381–7.503	0.489
GAD	1362	1.588	1.092–2.310	0.015	1395	1.219	0.759–1.957	0.413	1395	1.330	0.818–2.162	0.250
MADD	1795	1.639	1.175–2.286	0.004	1847	1.536	1.045–2.255	0.029	1847	1.447	0.951–2.201	0.084
Comorbid	412	2.976	1.831–4.834	<0.001	436	3.051	1.743–5.342	>0.001	436	3.087	1.668–5.713	<0.001

considering that classification of CHD was based on self-report. The cross-sectional design of the study makes it impossible to determine whether the mood and anxiety disorders preceded CHD or vice versa. That said, the literature suggests that the relationship between MDD (and anxiety) and CHD is bidirectional and reciprocal (Nemeroff and Goldschmidt-Clermont, 2012; Ramasubbu et al., 2012a; Stapelberg et al., 2012). We also note that the ELSA-Brasil cohort captures the racial, social, and regional diversity in the Brazilian population (see Schmidt et al., 2014), highlighting the relevance of the present study's findings for developing countries. It is possible, however, that non-diagnosed CHD is *lower in our sample*, than for the general Brazilian population, as the civil servants who participated in our study have more access to health services. It is possible therefore that the associations we observe here are lower than those that might be observed in the general Brazilian population. Indeed, it has been noted previously that there is a low identification rate for depression and other CMDs in Brazil (Mari et al., 2013; see also Mari et al., 1987; Busnello et al., 1999).

There are a number of potential explanations for the associations we observed between the CMD (and specific diagnostic groupings) and CHD including psychosocial and biological factors. Brazil currently faces major social challenges, which may contribute to chronic stress and increased morbidity (de Jesus Mari, 2014), strengthening the associations between mental disorders and CHD we observed here, in comparison to high-income countries. In addition to income inequality (Filho et al., 2013), personal safety remains an issue of great concern. For example,

findings from a representative sample of 15 to 75-year old residents of São Paulo and Rio de Janeiro in Brazil indicated that psychiatric disorders and traumatic events, especially violence, are extremely common (Ribeiro et al., 2013). Nearly 90% of participants surveyed in that study faced a lifetime exposure to actual or threatened death, and these traumatic events correlated with all psychiatric diagnoses. These results are particularly relevant in light of other research (Russ et al., 2012) highlighting a dose-response association between psychological distress and increased risk of mortality from a variety of causes including CVD, cancer and external causes over 8 years. Biological factors may also underpin associations between the mood and anxiety disorders and CHD including autonomic dysfunction, a hyperactive hypothalamic-pituitary-adrenal axis, increased activity in the sympatho-adreno-medullary activity, increased inflammation, and platelet activation and aggregation (Nemeroff and Goldschmidt-Clermont, 2012; Ramasubbu et al., 2012a; Stapelberg et al., 2012; Lichtman et al., 2014). Decreases in heart rate variability indicative of autonomic dysfunction may provide a structural link between emotional disturbance and failure to appropriately regulate inflammatory processes leading to increased morbidity and mortality from a host of disorders including CVD's (Kemp and Quintana, 2013). Disturbances in the immune system may also lead to depression (Dantzer et al., 2008), highlighting the bidirectional relationships between the mental disorders, and physical disease.

Our study is characterized by a number of strengths including a focus on a relatively large and well-characterized sample of the Brazilian population, consideration of a variety of specific mood and anxiety disorder groupings, application of a structured clinical interview – rather than self-report – to determine psychiatric diagnoses, and adjustment for a host of covariates known to contribute to metabolic and cardiovascular risk. Recently, we reported that that use of tricyclic antidepressants is associated with a twofold increase in prevalent CHD, above and beyond severity of mood and anxiety symptoms (Kemp et al., 2015). Importantly, we demonstrate here that the association between mood and anxiety disorders, and CHD is above and beyond use of antidepressant medications including tricyclic antidepressants. A recent statement by the Canadian Network for Mood and Anxiety Treatments (CANMAT) task force (Ramasubbu et al., 2012b) highlights the bidirectional relationship between the mood and anxiety disorders, and a variety of medical conditions including cardiovascular disease, cerebrovascular disease, cancer, human immuno-deficiency virus, hepatitis C virus, migraine, multiple sclerosis, epilepsy, and osteoporosis. Given the increased risks for morbidity and mortality associated with comorbid psychiatric and medical conditions, future studies are needed to better understand the pathways underpinning their comorbidity, with a goal toward developing more effective preventive strategies and treatments. Interested readers are referred to recent guidelines for pharmacological and psychosocial management in low and middle-income countries (Mari et al., 2013). In conclusion, the present study provides important new information on the association between the mental disorders and CHD in Brazil. Findings indicate that the most robust association with CHD relates to symptomatic and diagnostic, psychiatric comorbidity, although an association with MDD and GAD was also observed. Both MDD and GAD were found to be associated with an increase in the odds for CHD overall as well as angina pectoris, which may have adverse implications for the future health of ELSA-Brasil participants. Considering that comorbid psychiatric illness and CHD is associated with increased morbidity and mortality (Lichtman et al., 2014), findings reinforce a need for, and the importance of, comprehensive health assessment in Brazil, a country that faces major social challenges and inequities.

AUTHOR CONTRIBUTIONS

AK conducted the literature search, identified the research questions, and clarified the hypotheses. He analyzed the data, interpreted the results and wrote the paper. MN adapted the Clinical Interview Schedule for use in our project and was responsible for the psychiatric evaluations of participants recruited in ELSA-Brasil. She also helped to refine the research questions and finalize the article for publication. AB assisted AK with reviewing the literature, and clarifying research questions and hypotheses. He played a key role in interpreting the results and writing the paper. IS and AG were involved in data collection, data analysis, interpretation of results, and writing the manuscript. AR was also involved in data collection and helped to critically revise the manuscript for publication. IB and PL have been involved in the ELSA-Brasil project since its inception, and secured the funding to initiate and conduct the project. They were involved in all aspects of the present study including research design, data collection, analysis, interpretation, and writing the manuscript.

ACKNOWLEDGMENTS

AK is supported by an International Research Professorship from the Universidade de São Paulo, Brazil. AR, IB, and PL are recipients of an award for established researchers from CNPq. AR is also a supported by a research grant (Pesquisador Mineiro) from FAPEMIG, the research agency of the State of Minas Gerais, Brazil. AB is a recipient of a young investigator award from FAPESP, São Paulo, Brazil. The ELSA-Brasil baseline study was supported by the Brazilian Ministry of Health (Science and Technology Department) and the Brazilian Ministry of Science and Technology (Financiadora de Estudos e Projetos and CNPq National Research Council; grants 01 06 0010.00 RS, 01 06 0212.00 BA, 01 06 0300.00 ES, 01 06 0278.00 MG, 01 06 0115.00 SP, 01 06 0071.00 RJ). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors would also like to acknowledge the participation of the 15,105 individuals recruited for this study without which this study and those based on the ELSA-Brasil cohort would not have been possible.

REFERENCES

Andrade, L. H., Wang, Y.-P., Andreoni, S., Silveira, C. M., Alexandrino-Silva, C., Siu, E. R., et al. (2012). Mental disorders in megacities: findings from the São Paulo megacity mental health survey, Brazil. *PLoS ONE* 7:e31879. doi: 10.1371/journal.pone.0031879

Aquino, E. M. L., Barreto, S. M., Benseñor, I. M., Carvalho, M. S., Chor, D., Duncan, B. B., et al. (2012). Brazilian longitudinal study of adult health (ELSA-Brasil): objectives and design. *Am. J. Epidemiol.* 175, 315–324. doi: 10.1093/aje/kwr294

Brunoni, A. R., Nunes, M. A., Figueiredo, R., Barreto, S. M., da Fonseca Mde, J., Lotufo, P. A., et al. (2013). Patterns of benzodiazepine and antidepressant

use among middle-aged adults. The Brazilian longitudinal study of adult health (ELSA-Brasil). *J. Affect. Disord.* 151, 71–77. doi: 10.1016/j.jad.2013.05.054

Buckley, B., and Murphy, A. (2009). Do patients with angina alone have a more benign prognosis than patients with a history of acute myocardial infarction, revascularisation or both? Findings from a community cohort study. *Heart* 95, 461–467. doi: 10.1136/hrt.2008.146944

Busnello, E. D., Tannous, L., Gigante, L., Ballester, D., Hidalgo, M. P., Silva, V., et al. (1999). Diagnostic reliability in mental disorders of the International Classification of Diseases in primary care. *Rev. Saude Publica* 33, 487–494. doi: 10.1590/S0034-89101999000500008

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., et al. (2003). International physical activity questionnaire: 12-country reliability and validity. *Med. Sci. Sports Exerc.* 35, 1381–1395. doi: 10.1249/01.MSS.0000078924.61453.FB

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

de Jesus Mari, J. (2014). Mental healthcare in Brazil: modest advances and major challenges. *Adv. Psychiatr. Treat.* 20, 113–115. doi: 10.1192/apt.bp.113.011593

Doering, L. V., Moser, D. K., Riegel, B., McKinley, S., Davidson, P., Baker, H., et al. (2010). Persistent comorbid symptoms of depression and anxiety predict mortality in heart disease. *Int. J. Cardiol.* 145, 188–192. doi: 10.1016/j.ijcard.2009.05.025

Field, A. (2013). *Discovering Statistics using IBM SPSS Statistics*, 4 Edn, London: SAGE, 1.

Filho, A. D. P. C., Kawachi, I., Wang, Y. P., Viana, M. C., and Andrade, L. H. S. G. (2013). Does income inequality get under the skin? a multilevel analysis of depression, anxiety and mental disorders in São Paulo, Brazil. *J. Epidemiol. Community Health* 67, 966–972. doi: 10.1136/jech-2013-202626

Hamer, M., David Batty, G., Seldenrijk, A., and Kivimaki, M. (2011). Antidepressant medication use and future risk of cardiovascular disease: the scottish health survey. *Eur. Heart. J.* 32, 437–442. doi: 10.1093/eurheartj/ehq438

Kemp, A. H., Brunoni, A. R., Bittencourt, M. S., Nunes, M. A., Benseñor, I. M., and Lotufo, P. A. (2015). The Association between antidepressant medications and coronary heart disease in Brazil: a cross-sectional analysis on the Brazilian Longitudinal Study of adult health (ELSA-Brazil). *Front. Public Health* 3:9. doi: 10.3389/fpubh.2015.00009

Kemp, A. H., Brunoni, A. R., Santos, I. S., Nunes, M. A., Dantas, E. M., Carvalho de Flqueiredo, R., et al. (2014). Effects of depression, anxiety, comorbidity and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil cohort baseline study. *Am. J. Psychiatry* doi: 10.1176/appi.ajp.2014.13121605 [Epub ahead of print].

Kemp, A. H., and Quintana, D. (2013). The relationship between mental and physical health: insights from the study of heart rate variability. *Int. J. Psychophysiol.* 89, 288–296. doi: 10.1016/j.ijpsycho.2013.06.018

Kemp, A. H., Quintana, D. S., Felmingham, K. L., Matthews, S., and Jelinek, H. (2012). Depression, comorbid anxiety disorders, and heart rate variability in physically healthy, unmedicated patients: implications for cardiovascular risk. *PLoS ONE* 7:e30777. doi: 10.1371/journal.pone.0030777

Lewis, G., Pelosi, A. J., Araya, R., and Dunn, G. (1992). Measuring psychiatric disorder in the community: a standardized assessment for use by lay interviewers. *Psychol. Med.* 22, 465–486. doi: 10.1017/S0033291700030415

Lichtman, J. H., Froelicher, E. S., Blumenthal, J. A., Carney, R. M., Doering, L. V., Frasure-Smith, N., et al. (2014). Depression as a risk factor for poor prognosis among patients with acute coronary syndrome: systematic review and recommendations: a scientific statement from the american heart association. *Circulation* 129, 1350–1369. doi: 10.1161/CIR.0000000000000019

Mari, J., Iacoponi, E., Williams, P., Simões, O., and Silva, J. B. (1987). Detection of psychiatric morbidity in the primary medical care setting in Brazil. *Rev. Saude. Publica* 21, 501–507. doi: 10.1590/S0034-89101987000600006

Mari, J., Tófoli, L. F., Noto, C., Li, L. M., Diehl, A., Claudino, A. M., et al. (2013). Pharmacological and psychosocial management of mental, neurological and substance use disorders in low- and middle-income countries: issues and current strategies. *Drugs 73*, 1549–1568. doi: 10.1007/s40265-013-0113-4

Nemeroff, C., and Goldschmidt-Clermont, P. (2012). Heartache and heartbreak—the link between depression and cardiovascular disease. *Nat. Rev. Cardiol.* 9, 526–539. doi: 10.1038/nrcardio.2012.91

Nunes, M. A., de Mello Alves, M. G., Chor, D., Schmidt, M. I., and Duncan, B. (2012). Adaptação transcultural do CIS-R (clinical interview schedule- revised version) para o português no estudo longitudinal De Saúde Do Adulto. (ELSA). *Revista HCPA* 31, 487–490.

Ramasubbu, R., Beaulieu, S., Taylor, V. H., Schaffer, A., and McIntyre, R. S. (2012a). The CANMAT task force recommendations for the management of patients with mood disorders and comorbid medical conditions: diagnostic, assessment, and treatment principles. *Ann. Clin. Psychiatry* 24, 82–90.

Ramasubbu, R., Taylor, V. H., Samaan, Z., Sockalingham, S., and Li, M. (2012b). The canadian network for mood and anxiety treatments (CANMAT). task force recommendations for the management of patients with mood disorders and select comorbid medical conditions. *Ann. Clin. Psychiatry* 24, 91–109.

Ribeiro, W. S., de Jesus Mari, J., Quintana, M. I., Dewey, M. E., Evans-Lacko, S., Vilete, L. M. P., et al. (2013). The impact of epidemic violence on the prevalence of psychiatric disorders in sao paulo and rio de Janeiro, Brazil. *PLoS ONE* 8:e63545. doi: 10.1371/journal.pone.0063545

Russ, T. C., Stamatakis, E., Hamer, M., Starr, J. M., Kivimaki, M., Batty, G. D., et al. (2012). Association between psychological distress and mortality: individual participant pooled analysis of 10 prospective cohort studies. *BMJ* 345, e4933. doi: 10.1136/bmj.e4933

Schmidt, M. I., Duncan, B. B., Mill, J. G., Lotufo, P. A., Chor, D., and Barreto, S. M., (2014). Cohort profile: longitudinal study of adult health (ELSA-Brasil). *Int. J. Epidemiol.* 44, 1–8. doi: 10.1093/ije/dyu027

Simon, G. E., VonKorff, M., Piccinelli, M., Fullerton, C., and Ormel, J. (1999). An international study of the relation between somatic symptoms and depression. *N. Engl. J. Med.* 341, 1329–1335. doi: 10.1056/NEJM199910283411801

Smoller, J. W., Allison, M., Cochrane, B. B., Curb, J. D., Perlis, R. H., Robinson, J. G., et al. (2009). Antidepressant use and risk of incident cardiovascular morbidity and mortality among postmenopausal women in the women's health initiative study. *Arch. Intern. Med.* 169, 2128–2139. doi: 10.1001/archinternmed.2009.436

Stapelberg, N. J., Hamilton-Craig, I., Neumann, D. L., Shum, D. H. K., and McConnell, H. (2012). Mind and heart: heart rate variability in major depressive disorder and coronary heart disease – a review and recommendations. *Aust. N. Z. J. Psychiatry* 46, 946–957. doi: 10.1177/0004867412444624

Tully, P. J., Cosh, S. M. (2013). Generalized anxiety disorder prevalence and comorbidity with depression in coronary heart disease: a meta analysis. *J. Health Psychol.* 18, 1601–1616. doi: 10.1177/1359105312467390

Tully, P. J., Cosh, S. M., and Baune, B. T. (2013). A review of the affects of worry and generalized anxiety disorder upon cardiovascular health and coronary heart disease. *Psychol. Health Med.* 18, 627–644. doi: 10.1080/13548506.2012.749355

Vogelzangs, N., Seldenrijk, A., Beekman, A. T. F., van Hout, H. P. J., de Jonge, P., and Penninx, B. W. (2010). Cardiovascular disease in persons with depressive and anxiety disorders. *J. Affect. Disord.* 125, 241–248. doi: 10.1016/j.jad.2010.02.112

Whang, W., Kubzansky, L. D., Kawachi, I., Rexrode, K. M., Kroenke, C. H., Glynn, R. J., et al. (2009). Depression and risk of sudden cardiac death and coronary heart disease in women. *J. Am. Coll. Cardiol.* 53, 950–958. doi: 10.1016/j.jacc.2008.10.060

Wilkinson, R. (2002). *Unhealthy Societies.* London: Routledge, 1.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The Review Editor Mario F. Juruena declares that, despite being affiliated to the same institution as the author Andrew Kemp, the review process was handled objectively.

Received: 15 September 2014; accepted: 05 February 2015; published online: 25 February 2015.

Citation: Kemp AH, Brunoni AR, Nunes MA, Santos IS, Goulart AC, Ribeiro AL, Benseñor IM and Lotufo PA (2015) The association between mood and anxiety disorders, and coronary heart disease in Brazil: a cross-sectional analysis on the Brazilian longitudinal study of adult health (ELSA-Brasil). Front. Psychol. ***6****:187. doi: 10.3389/fpsyg.2015.00187*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in
PUBLIC HEALTH

ORIGINAL RESEARCH ARTICLE
published: 22 January 2015
doi: 10.3389/fpubh.2015.00009

The association between antidepressant medications and coronary heart disease in Brazil: a cross-sectional analysis on the Brazilian longitudinal study of adult health (ELSA-Brazil)

Andrew H. Kemp[1,2]*, Andre R. Brunoni[1], Marcio S. Bittencourt[1], Maria A. Nunes[3], Isabela M. Benseñor[1] and Paulo A. Lotufo[1]

[1] *Faculty of Medicine, University Hospital, University of São Paulo, São Paulo, Brazil*
[2] *School of Psychology and Discipline of Psychiatry, University of Sydney, Sydney, NSW, Australia*
[3] *Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil*

Edited by:
Yuanzhang Li, Walter Reed Army Institute of Research, USA

Reviewed by:
Arthur Eumann Mesas, Universidade Estadual de Londrina, Brazil
Wenlong Gao, Lanzhou University, China

***Correspondence:**
Andrew H. Kemp, Center for Clinical and Epidemiologic Research, University Hospital, University of São Paulo, Av. Prof. Lineu Prestes 2565, São Paulo CEP 05508-000, Brazil
e-mail: andrew.kemp@hu.usp.br; andrew.kemp@sydney.edu.au

Background: Recent studies have highlighted associations between use of antidepressant medications and coronary heart disease (CHD). Tricyclic antidepressants (TCA) are not recommended in patients with CHD as they may increase morbidity and mortality. However, this class of antidepressants is freely prescribed in public health pharmacies, while access to other classes of antidepressants is restricted in Brazil. Here, we examine the associations between antidepressant use and prevalent CHD in a large cohort from Brazil.

Methods: Participants included 14,994 civil servants aged 35–74 years from the baseline assessment of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). CHD ($n = 710$) included stable angina, myocardial infarction, and coronary revascularization. Univariate (unadjusted) and multivariate (adjusted) logistic regression analyses were conducted to estimate odds ratios and confidence intervals.

Results: After full adjustment for covariates, TCA use ($n = 156$) was associated with a twofold increase in prevalent CHD, relative to non-use ($n = 14{,}076$). Additional sensitivity analysis revealed a threefold association for myocardial infarction (OR: 2.96, 95% CI: 1.41–6.21) and coronary revascularization (OR: 2.92, 95% CI: 1.28–6.66). There were no significant associations between antidepressant use and stable angina pectoris.

Conclusion: Findings highlight a strong association between TCA use and prevalent CHD. While the cross-sectional design is an important limitation of the present study, findings have important implications for the treatment of cardiac patients in Brazil.

Keywords: tricyclic antidepressants, coronary heart disease, Brazil, cross-sectional design, clinical epidemiology, TCA, CHD

INTRODUCTION

Coronary heart disease (CHD) and major depressive disorder (MDD) are leading burdens of disease (1) and the relationship between these disorders is bidirectional: patients with CHD have more MDD than the general population, while those with MDD are more likely to develop CHD (2, 3). Critically, comorbidity between CHD and MDD increases risk of further morbidity and mortality (4). Other studies have highlighted the association between CHD and the anxiety disorders (5, 6). Antidepressant use in patients with CHD is controversial. While use of tricyclic antidepressants (TCA) is not recommended (7), research indicates that all classes of antidepressant medications may have adverse cardiac effects (8, 9) [but see Ref. (10, 11) in regards to the selective serotonin reuptake inhibitor, or SSRI, antidepressant class]. The selective serotonin reuptake inhibitors are generally considered to be the safest class of antidepressants for use in cardiac patients (12) when needed. Here, we examine the association between antidepressant use and prevalent CHD in a large epidemiological cohort from Brazil.

The cardiovascular risks of TCA are well known (13). Although initially it was believed that TCAs could suppress arrhythmias in depressed patients with pre-existing arrhythmias, this belief was revised more than 20 years ago (13). The TCAs are also potent antagonists of muscarinic acetylcholine receptors (14) at the sinoatrial node of the heart leading to a decrease in parasympathetic activity, disinhibition of sympathetic nervous system activity, and tachycardia (15), which may lead to adverse cardiovascular events. While an earlier case–control study (16) reported that TCAs only increased the risk of myocardial infarction within the initial 28 days of antidepressant use, more recent research (17) demonstrated that use of TCAs are associated with a 35% increased risk of cardiovascular death over an 8-year follow-up period in initially healthy individuals. Consistent with this body of literature, a recent consensus statement from the National Heart Foundation

of Australia (7), a high-income country, recommends that TCA be avoided in patients with CHD and depression.

While recommendations are helpful, they are difficult to apply in less-developed countries. Brazil is an upper-middle-income country facing major social challenges that may impact on the associations between CHD and antidepressant use. We have reported previously (18) that only 14 and 16.5% of patients in Brazil with generalized anxiety and MDD, respectively, take antidepressant medication. We also observed that while SSRIs were prescribed twice more frequently than tricyclic medications, antidepressant use was related to having private health insurance. TCA are freely dispensed in public health pharmacies in Brazil, while most of the SSRI medications are not, with the exception of fluoxetine and, in some regions of Brazil including São Paulo, sertraline (18, 19). (The list of medicines supplied by the Brazilian Unified Health System, or SUS, in São Paulo is available here: http://www2.hu.usp.br/confira-lista-de-medicamentos-do-sus/.) TCA therefore play an important role in treating depression, as well as a variety of other conditions including neuropathic pain and fibromyalgia (20) in Brazil. This context provides an important background for the current study, which sought to determine the associations between use of antidepressant medications and prevalent CHD in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) (21, 22) cohort. We examine associations in the cohort at baseline to provide an important foundation for future prospective analyses on this cohort.

MATERIALS AND METHODS

PARTICIPANTS

ELSA-Brasil is a cohort of 15,105 civil servants aged 35–74 years enrolled between August 2008 and December 2010 at six cities (Belo Horizonte, Porto Alegre, Rio de Janeiro, Salvador, Sao Paulo, and Vitoria) designed to investigate the relationship between cardiovascular diseases and diabetes, their social determinants, and risk factors. The study design and sampling procedures of ELSA-Brasil have been reported previously (21, 22). A total of 14,994 participants are reported here after dropping a relatively small number of cases ($n = 111$) with missing data on variables that were included in analyses.

ETHICS STATEMENT

The ethics committees of the participating universities as well as the National Research Ethics Committee approved the research protocol. All participants provided written informed consent after a complete description of the study.

PSYCHIATRIC EVALUATION

Mental disorders were determined by trained interviewers using the Portuguese version (23) of the Clinical Interview Schedule-Revised (CIS-R) (24). This is a structured interview used for diagnosis of common, non-psychotic psychiatric conditions in the community. The complete CIS-R version was applied, severity scores were obtained, and common mental disorder status (CIS-R scores $\geq$12), determined. Antidepressant medications [Anatomical Therapeutic Chemical (ATC) Classification code: N06A] included the selective serotonin reuptake inhibitors (SSRI, ATC code: N06AB), the serotonin and noradrenaline reuptake inhibitors (SNRIs, ATC codes: N06AX16/N06AX23/N06AX21), the TCA (ATC code: N06AA), and other antidepressants (N06AX22, N06AX12, N06AA21, N06AX05, N06AX11). Individuals taking at least one antidepressant medicine continuously in the past 2 weeks were classified as users.

CORONARY HEART DISEASE ASSESSMENT

Coronary heart disease (CHD) included participants with stable angina pectoris, myocardial infarction, and coronary revascularization determined through questionnaire- and intensive interview-based assessment focusing on medical history. All prior CHD were self-referred by the patients during the structured interview. For the current analysis, prevalent CHD was defined as a prior history of a physician diagnosed myocardial infarction, a prior percutaneous coronary intervention including balloon angioplasty with or without stent placement, a prior surgical revascularization consisting of either arterial or venous grafts and the history of stable angina as defined by a physician taking care of the participant prior to the inclusion in the ELSA study. The outcome of coronary revascularization was defined as either a percutaneous coronary intervention or a surgical revascularization as previously described.

STATISTICAL ANALYSIS

Statistical analysis was conducted using IBM SPSS Statistics Version 21. Participant characteristics were examined using independent samples t-tests and one-way analyses of variance (ANOVA) for contrasts involving continuous dependent measures, and χ^2 statistics for categorical variables. Degrees of freedom were corrected when Levene's Test for Equality of Variances was violated. Tukey's HSD is reported to correct for multiple comparisons and aid interpretation of ANOVA's, while standardized residuals (z-scores) were used to help interpret chi-square tests on larger contingency tables [as per Ref. (25)].

A series of univariate and multivariate, binary, logistic regression analyses were then used to estimate the odds ratios and 95% confidence intervals for the association between antidepressant use as the independent variable (IV) and prevalent CHD (no, yes) as the dependent variable (DV), before and after adjustment for covariates. Unadjusted (univariate) and adjusted (multivariate) analyses were also conducted on specific classes of antidepressants (IV) including the SSRIs, the SNRIs, tricyclic medications and others, and prevalent CHD (no, yes) (DV). Unadjusted univariate, binary, logistic regression analyses (model 1) were conducted on antidepressant use (no versus yes), as well as classes of antidepressants, with no other predictors. Adjusted multivariate analyses (model 2) involved binary logistic regression analysis in which covariates were entered into the first block using the enter method, and antidepressant use was entered into the second (final) block, a technique known as sequential logistic regression analysis. Covariates included age, sex, education (less than high-school, high-school, university), smoking status (never, past/current), body mass index (BMI; weight in kilograms divided by height in meters squared), hypertension (systolic blood pressure $\geq$140 mm Hg, or diastolic blood pressure $\geq$90 mm Hg, or use of antihypertensive medications), diabetes mellitus (DM) (self-reported or fasting blood glucose $\geq$126 mg/dL, 2-h oral glucose tolerance

test ≥200 mg/dL, or glycated hemoglobin ≥6.5%), and severity of mood and anxiety disorders. Sequential, logistic regression analysis is a common approach that allows for the independent contribution of antidepressant use over and above covariates to be determined (26). Statistics from multivariate analyses are reported for the overall model and block (after adjusting for covariates). The block statistic indicates whether or not the IV of interest (antidepressant use/class) is significantly associated with the dependent measure, CHD. Sensitivity analyses were conducted for specific CHD events to determine the consistency of associations across distinct categories of CHD. Sensitivity analyses involved sequential, binary logistic regression analysis in which covariates were entered into block 1 using the enter method, followed by antidepressant use in block 2 (as per model 2).

RESULTS

PARTICIPANT CHARACTERISTICS

Table 1 summarizes participant characteristics by CHD status, while **Table 2** presents participant characteristics according to antidepressant use. Prevalent CHD was characterized by older age, more men, less education, more smokers, higher BMI, more individuals with DM, hypertension and common mental disorder, higher CIS-R score, and antidepressant use. Antidepressant use was characterized by older age, more women, more education, more smokers, more individuals with CHD and common mental disorder, and higher CIS-R score. **Table 3** provides a more detailed breakdown of participant characteristics by antidepressant class. Notably, TCA use is characterized by more women, fewer individuals with college-level education and more with CHD and common mental disorder, and a higher CIS-R score.

ASSOCIATION BETWEEN ANTIDEPRESSANT USE AND PREVALENT CHD

Table 4 describes results of analyses assessing the association of antidepressant medications with prevalent CHD. Model 1 relates to the results of unadjusted analyses for antidepressant use [model $\chi^2(1) = 7.12$, $p = 0.008$] and antidepressant class [model $\chi^2(4) = 14.03$, $p = 0.007$], while model 2 relates to results adjusted for covariates [antidepressant use,

Table 1 | Participant characteristics by CHD status (*N* = 14,994).

Characteristics	No CHD (*n* = 14,284, 95%)	CHD (*n* = 710, 5%)	Statistic
Age, mean (SD)	51.75 (8.97)	58.66 (8.74)	$t(14{,}992) = 20.07$, $p < 0.001$
Women (%)	54.8	45.5	$\chi^2(1) = 23.45$, $p < 0.001$
Education (%)			$\chi^2(2) = 85.16$, $p < 0.001$
Less than high-school	12.1	23.7*	
High-school	34.8	33.8	
College	53.1	42.5*	
Smoker (past or current) (%)	42.5	56.1	$\chi^2(1) = 50.77$, $p < 0.001$
Body mass index (kg/m^2), mean (SD)	26.95 (4.74)	28.37 (4.80)	$t(14{,}992) = 7.76$, $p < 0.001$
Diabetes mellitus (yes) (%)	18.7	37.9	$\chi^2(1) = 157.21$, $p < 0.001$
Hypertension (yes) (%)	34.0	70.0	$\chi^2(1) = 382.49$, $p < 0.001$
Common mental disorder (yes) (%)	26.2	35.5	$\chi^2(1) = 29.86$, $p < 0.001$
CIS-R Score, mean (SD)	8.08 (7.86)	10.34 (9.61)	$t(756.82) = 6.18$, $p < 0.001$
Antidepressant use (yes) (%)	6.0	8.6	$\chi^2(1) = 7.91$, $p = 0.005$

*CIS-R, Clinical Interview Schedule-Revised. *Refers to categories with standardized residuals (z-scores) lying outside ± 1.96 reflecting a significance value of p < 0.05.*

Table 2 | Participant characteristics by antidepressant use (*N* = 14,994).

Characteristics	No (*n* = 14,076, 94%)	Yes (*n* = 918, 6%)	*p*-Value
Age, mean (SD)	51.97 (9.07)	53.70 (9.14)	$t(14{,}992) = 5.59$, $p < 0.001$
Women (%)	52.9	76.4	$\chi^2(1) = 191.32$, $p < 0.001$
Education (%)			$\chi^2(2) = 34.90$, $p < 0.001$
Less than high-school	13.0	7.8*	
High-school	35.0	31.2	
College	52.0	61.0*	
Smoker (past or current) (%)	42.9	47.1	$\chi^2(1) = 6.15$, $p = 0.013$
Body mass index (kg/m^2), mean (SD)	27.01 (4.75)	27.09 (4.76)	$t(14{,}992) = 0.48$, $p = 0.632$
Diabetes mellitus (yes) (%)	19.8	17.8	$\chi^2(1) = 2.20$, $p = 0.138$
Hypertension (yes) (%)	35.6	36.5	$\chi^2(1) = 0.28$, $p = 0.596$
CHD status (yes) (%)	4.6	6.7	$\chi^2(1) = 8.39$, $p = 0.004$
Common mental disorder (yes) (%)	25.4	45.6	$\chi^2(1) = 180.59$, $p < 0.001$
CIS-R Score, mean (SD)	7.92 (7.75)	12.27 (9.90)	$t(991.68) = 13.06$, $p < 0.001$

*CIS-R, Clinical Interview Schedule-Revised. *Refers to categories with standardized residuals (z-scores) lying outside ± 1.96 reflecting a significance value of p < 0.05.*

Table 3 | Participant characteristics by antidepressant grouping ($N = 14,994$).

Characteristics	CTL ($n = 14,076$)	SSRI ($n = 567$)	SNRI ($n = 100$)	TCA ($n = 156$)	Other ($n = 95$)	*p*-Value
Age, mean (SD)	51.97 (9.07)	53.70* (9.36)	55.48* (8.60)	53.42 (8.45)	52.26 (9.33)	$F(4,14,993) = 9.41$, $p < 0.001$
Women,%	52.9*	77.8*	81.0*	75.0*	65.3	$\chi^2(4) = 197.48$, $p < 0.001$
Education (%)						$\chi^2(8) = 80.32$, $p < 0.001$
Less than high-school	13.0	6.7*	4.0*	15.4	6.3	
High-school	35.0	28.6*	23.0*	47.4*	28.4	
College	52.0	64.7*	73.0*	37.2*	65.3	
Smoker (past or current) (%)	42.9	47.1	40.0	47.4	53.7	$\chi^2(4) = 9.89$, $p = 0.042$
BMI (kg/m^2), mean (SD)	27.01 (4.75)	27.12 (4.89)	27.68 (4.62)	26.83 (4.06)	26.69 (5.21)	$F(4,14,993) = 0.731$, $p = 0.571$
Hypertension (yes)%	35.6	34.9	45.0	38.5	33.7	$\chi^2(4) = 4.64$, $p = 0.33$
Diabetes (yes)%	19.8	17.3	18.0	20.5	15.8	$\chi^2(4) = 3.27$, $p = 0.51$
CHD (yes) (%)	4.6	6.3	3.0	10.9*	5.3	$\chi^2(4) = 17.61$, $p = 0.001$
Common mental disorder (yes) (%)	25.4*	45.0*	43.0*	46.2*	51.6*	$\chi^2(4) = 182.81$, $p < 0.001$
CIS-R Score, mean (SD)	7.92 (7.75)	12.17 (9.70)*	12.19 (10.51)*	12.48 (10.24)*	12.55 (9.94)*	$F(4,14,993) = 65.42$, $p < 0.001$

**Refers to one-way ANOVA in which each group is compared to controls (Tukey's HSD, $p < 0.05$) or standardized residuals (z-scores) from χ^2 statistics lying outside ± 1.96 reflecting a significance value of $p < 0.05$.*

Table 4 | Unadjusted (model 1)[a] and adjusted (model 2)[b] associations between antidepressant use and CHD ($N = 14,994$).

Predictor	*N*	CHD: model 1[a] ($n = 710$)			*N*	CHD: model 2[b] ($n = 710$)		
		OR	95% CI	*p*-Value		OR	95% CI	*p*-Value
Any antidepressant use								
No	14,076		REF		14,076		REF	
Yes	918	1.47	1.12–1.93	0.005	918	1.28	0.96–1.71	0.093
Antidepressant groupings								
None	14,076		REF		14,076		REF	
SSRI	567	1.40	0.99–1.98	0.056	567	1.26	0.87–1.81	0.218
SNRI	100	0.64	0.20–2.02	0.447	100	0.47	0.15–1.52	0.209
TCA	156	2.53	1.52–4.21	<0.001	156	2.15	1.24–3.71	0.006
Other	95	1.15	0.47–2.84	0.763	95	1.03	0.40–2.65	0.949

[a]Model 1 relates to separate unadjusted analyses for any antidepressant use and specific classes of antidepressant including SSRI (selective serotonin reuptake inhibitors), SNRI (serotonin and noradrenaline reuptake inhibitors), TCA (tricyclic antidepressants), and other.

[b]Model 2 relates to analyses adjusted for covariates.

model $\chi^2(10) = 724.85$, $p < 0.001$; block $\chi^2(1) = 2.69$, $p = 0.101$; antidepressant class, model $\chi^2(10) = 732.04$, $p < 0.001$; block $\chi^2(4) = 9.88$, $p = 0.042$]. Antidepressant use was associated with a 1.5-fold increase in the odds of prevalent CHD (95% CI: 1.12–1.93) (model 1), although this was reduced to a 1.3-fold increase when adjusting for covariates (95% CI: 0.96–1.71) (model 2). Sensitivity analysis revealed that use of TCAs (OR = 2.53, 95% CI: 1.52–4.21, model 1; OR = 2.15, 95% CI = 1.24–3.71) in particular is significantly associated with prevalent CHD.

ASSOCIATION BETWEEN ANTIDEPRESSANT USE AND CHD SUBTYPES

Table 5 reports results for the additional specificity analyses on stable angina pectoris [antidepressant use: model $\chi^2(10) = 223.26$, $p < 0.001$; block $\chi^2(1) = 0.02$, $p = 0.891$; antidepressant class: model $\chi^2(13) = 223.26$, $p < 0.001$; block $\chi^2(4) = 3.02$, $p = 0.555$], myocardial infarction [antidepressant use: model $\chi^2(10) = 426.90$, $p < 0.001$; block $\chi^2(1) = 0.83$, $p = 0.361$; antidepressant class: model $\chi^2(13) = 437.14$, $p < 0.001$; block $\chi^2(4) = 11.07$, $p = 0.026$], and coronary revascularization [antidepressant use: model $\chi^2(10) = 440.68$, $p < 0.001$; block $\chi^2(1) = 6.02$, $p = 0.014$; antidepressant class: model $\chi^2(13) = 448.41$, $p < 0.001$; block $\chi^2(4) = 13.75$, $p = 0.008$]. While there were no significant associations observed for stable angina, use of tricyclic medications were associated with a threefold increase in odds for myocardial infarction as well as coronary revascularization. These additional findings indicate that the association between TCA use and prevalent CHD is specific to "hard" CHD events including myocardial infarction and coronary revascularization.

DISCUSSION

The goal of this study was to determine the associations between use of antidepressant medications and prevalent CHD in a cohort of civil servants from Brazil. This is an important goal because

Table 5 | Fully adjusted association between antidepressant use and CHD subtypes ($N = 14,994$).

Predictor	*N*	Stable angina ($n = 312$)[a]			*N*	Myocardial infarction ($n = 267$)			*N*	Coronary revascularization ($n = 255$)		
		OR	95% CI	*p*-Value		OR	95% CI	*p*-Value		OR	95% CI	*p*-Value
Any antidepressant use												
No	13,715			REF	14,076			REF	14,076			REF
Yes	881	0.85	0.54–1.36	0.501	918	1.25	0.78–2.01	0.349	918	1.79	1.15–2.77	0.009
Antidepressant groupings												
None	13,715				14,076			REF	14,076		REF	
SSRI	546	1.00	0.58–1.71	0.989	567	1.12	0.59–2.10	0.735	567	1.96	1.16–3.34	0.013
SNRI	98	0.30	0.04–2.16	0.229	100	n/a	n/a	0.996	100	1.15	0.27–4.85	0.845
TCA	143	1.01	0.37–2.79	0.986	156	2.96	1.41–6.21	0.004	156	2.92	1.28–6.66	0.011
Other	94	1.65	0.59–4.64	0.340	95	0.54	0.07–4.03	0.547	95	n/a	n/a	0.996

[a]*This model excludes 398 participants with myocardial infarction or coronary revascularization.*

Brazil currently faces many socioeconomic inequities, which may impact on antidepressant usage. Major findings indicate that use of TCA is associated with: (1) a twofold increase in the odds for prevalent CHD and (2), a threefold increase in the odds for myocardial infarction and coronary revascularization, after adjustment for covariates. The associated 95% confidence intervals for TCA use – all of which excluded the null value of 1 – provide sets of likely values for the odds ratio on which for a repeated study would most likely fall (on average, a five-in-six chance) (27). Values close to the sample estimates, however, are ~7 times more likely to reflect the true population estimate (μ), than values near the limits of the interval (27). These considerations and the size of the effects obtained, enhance our confidence in the reported findings reported here.

While our study highlights a strong relationship between TCA use and "hard" CHD events, it is important to acknowledge that some participants in our study may have been using low-dose TCAs to treat conditions other than mental disorders, such as chronic/neuropathic pain and sleep issues. Importantly, research has demonstrated a dose-related increase in sudden cardiac death in current users of TCAs from 0.97 for doses lower than 100 mg (amitriptyline or its equivalent) to 2.53 for doses of 300 mg or more, highlighting that doses of <100 mg does not increase risk (at least for sudden cardiac death). However, current recommendations indicate that TCAs should be avoided completely in cardiac patients (7). In Brazil, access to TCAs is free (18, 19), while access to other classes of antidepressants is restricted suggesting socioeconomic reasons that may increase the association between TCA and prevalent CHD. It is notable that elderly Brazilian patients with psychiatric disorders are 5.3 times more likely to be using inappropriate medications (28). We suggest that these previous findings (28) may help to understand the findings that we report here, which may indicate problems associated with ongoing health care of cardiac patients in the Brazilian population.

It is important to acknowledge the cross-sectional design as an important limitation of the present study. This limitation precludes any conclusions over the causal relationship between TCA use and CHD. For instance, it is equally possible that TCA use preceded the development of CHD consistent with research that suggests TCAs lead to cardiovascular events [e.g., Ref. (17)] (i.e., a biological explanation) beyond that explained by psychiatric illness, or that TCAs were prescribed after CHD was diagnosed consistent with research that suggests patients may be inappropriately medicated in Brazil [e.g., Ref. (28)] (i.e., a sociodemographic explanation). However, regardless of the causal direction of the relationship between TCA use and CHD, our findings still have important implications for the treatment of cardiac patients in Brazil. It is notable here that research from the Netherlands (5), a high-income country, did not observe a significant association between use of TCAs and CHD. The authors noted that while the adverse cardiovascular effects of TCAs are well known, a null finding might reflect the contraindication of TCA use in heart patients. We suggest here that socioeconomic inequities in Brazil may over-ride recommendations to avoid these medications in cardiac patients as patients have easier and free access to this class of antidepressant medications through free public health care.

Longitudinal research (8) from the Netherlands Study of Depression and Anxiety ($N = 2,114$) indicates that all classes of antidepressants may have adverse effects on heart function, determined by reductions in heart rate variability, a psychophysiological predictor of future cardiovascular mortality [see in Ref. (29) for review]. Adverse effects were greatest for the TCAs, followed by the SNRIs, and then the SSRIs, relative to no antidepressant use (8) [see also in Ref. (30)]. This study also reported that these effects disappear when antidepressants are discontinued. However, the adverse effects reported for the SSRI class were small, which may, in part, explain the contradictory findings reported previously for the association between SSRIs and CHD (9, 17). SSRI antidepressants are generally considered to have a more favorable cardiovascular profile than the TCA (and the serotonin and noradrenaline reuptake inhibitors, or SNRIs). The SSRIs may exert cardiovascular benefits through direct action on the biological substrates of the stress response (31), including a blunting of blood pressure, myocardial responses, and cortisol reactivity under stress. The SSRIs also have antiplatelet properties, which will reduce the risk for thrombus formation (2, 3). While we did not observe a relationship between SSRIs and prevalent CHD, we did observe

a twofold increase association for SSRIs and coronary revascularization. A possible explanation for the null association between SSRIs and myocardial infarction is the restricted access to antidepressants from the SSRI class of antidepressants, resulting perhaps from the socioeconomic inequities of Brazilian mental health care (28, 32, 33) and high cost of these newer medications.

In conclusion, the present study provides important new information on the association of antidepressant use and prevalent CHD in Brazil. While a limitation of our study is its cross-sectional design, this limitation does not undermine the importance of our findings, as TCA use in patients with CHD increases risk of future morbidity and mortality. While it is possible that some of our participants were on low-dose TCAs for conditions other than depression and anxiety, recommendations from high-income countries suggest that these medications should be avoided in cardiac patients. Our study is characterized by a number of strengths including a focus on a relatively large and well-characterized sample of the Brazilian population, application of a structured clinical interview to determine psychiatric diagnosis and disorder severity, and adjustment for a host of covariates known to contribute to metabolic and cardiovascular risk. Our findings indicate a strong relationship between TCA use and prevalent CHD. We will further examine the impact of the different antidepressant classes in a longitudinal follow-up study of the ELSA-Brasil cohort once data collection is complete.

AUTHOR CONTRIBUTIONS

Andrew H. Kemp conducted the literature search, identified the research questions, and clarified the hypotheses. He analyzed the data, interpreted the results, and wrote the paper. Maria A. Nunes adapted the Clinical Interview Schedule for use in our project and was responsible for the psychiatric evaluations of participants recruited in ELSA-Brasil. Isabela M. Bensen or and Paulo A. Lotufo have been involved in the ELSA-Brasil project since its inception, and secured the funding to initiate and conduct the project. They were involved in all aspects of the present study including research design, data collection, analysis, and interpretation of results. All authors reviewed and approved the manuscript for publication.

ACKNOWLEDGMENTS

Andrew H. Kemp is supported by an International Research Professorship from the Universidade de São Paulo, Brazil. Isabela M. Bensen or and Paulo A. Lotufo are recipients of an award for established researchers from CNPq. Andre R. Brunoni is a recipient of a young investigator award from FAPESP, São Paulo, Brazil. The ELSA-Brasil baseline study was supported by the Brazilian Ministry of Health (Science and Technology Department) and the Brazilian Ministry of Science and Technology (Financiadora de Estudos e Projetos and CNPq National Research Council) (grants 01 06 0010.00 RS, 01 06 0212.00 BA, 01 06 0300.00 ES, 01 06 0278.00 MG, 01 06 0115.00 SP, 01 06 0071.00 RJ). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors would also like to acknowledge the participation of the 15,105 individuals recruited for this study without which this study and those based on the ELSA-Brasil cohort would not have been possible.

REFERENCES

1. Murray C, Vos T, Lozano R, Naghavi M, Flaxman AD. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* (2013) **380**:2197–223. doi:10.1016/S0140-6736(12)61689-4
2. Nemeroff C, Goldschmidt-Clermont PJ. Heartache and heartbreak – the link between depression and cardiovascular disease. *Nat Rev Cardiol* (2012) **9**(9):526–39. doi:10.1038/nrcardio.2012.91
3. Stapelberg NJ, Hamilton-Craig I, Neumann DL, Shum DHK, McConnell H. Mind and heart: heart rate variability in major depressive disorder and coronary heart disease – a review and recommendations. *Aust N Z J Psychiatry* (2012) **46**(10):946–57. doi:10.1177/0004867412444624
4. Lichtman JH, Froelicher ES, Blumenthal JA, Carney RM, Doering LV, Frasure-Smith N, et al. Depression as a risk factor for poor prognosis among patients with acute coronary syndrome: systematic review and recommendations: a scientific statement from the American Heart Association. *Circulation* (2014) **129**(12):1350–69. doi:10.1161/CIR.0000000000000019
5. Vogelzangs N, Seldenrijk A, Beekman ATF, van Hout HPJ, de Jonge P, Penninx BWJH. Cardiovascular disease in persons with depressive and anxiety disorders. *J Affect Disord* (2010) **125**(1–3):241–8. doi:10.1016/j.jad.2010.02.112
6. Tully PJ, Cosh SM. Generalized anxiety disorder prevalence and comorbidity with depression in coronary heart disease: a meta-analysis. *J Health Psychol* (2013) **18**:1601–16. doi:10.1177/1359105312467390
7. Colquhoun DM, Bunker SJ, Clarke DM, Glozier N, Hare DL, Hickie IB, et al. Screening, referral and treatment for depression in patients with coronary heart disease. *Med J Aust* (2013) **198**(9):483–4. doi:10.5694/mja13.10153
8. Licht CMM, de Geus EJC, van Dyck R, Penninx BWJH. Longitudinal evidence for unfavorable effects of antidepressants on heart rate variability. *Biol Psychiatry* (2010) **68**(9):861–8. doi:10.1016/j.biopsych.2010.06.032
9. Whang W, Kubzansky LD, Kawachi I, Rexrode KM, Kroenke CH, Glynn RJ, et al. Depression and risk of sudden cardiac death and coronary heart disease in women. *J Am Coll Cardiol* (2009) **53**(11):950–8. doi:10.1016/j.jacc.2008.10.060
10. Kemp AH, Quintana DS, Malhi GS. Effects of serotonin reuptake inhibitors on heart rate variability: methodological issues, medical comorbidity, and clinical relevance. *Biol Psychiatry* (2011) **69**(8):e25–6. doi:10.1016/j.biopsych.2010.10.035
11. Kemp AH. Depression, antidepressant treatment and the cardiovascular system. *Acta Neuropsychiatr* (2011) **23**(2):82–3. doi:10.1111/j.1601-5215.2011.00535.x
12. Pizzi C, Rutjes AWS, Costa GM, Fontana F, Mezzetti A, Manzoli L. Meta-analysis of selective serotonin reuptake inhibitors in patients with depression and coronary heart disease. *Am J Cardiol* (2011) **107**(7):972–9. doi:10.1016/j.amjcard.2010.11.017
13. Glassman AH. The safety of tricyclic antidepressants in cardiac patients risk-benefit reconsidered. *JAMA* (1993) **269**(20):2673–5. doi:10.1001/jama.269.20.2673
14. Richelson E. Synaptic effects of antidepressants. *J Clin Psychopharmacol* (1996) **16**(3):1S–7S. doi:10.1097/00004714-199606002-00002
15. Coupland N, Wilson S, Nutt D. Antidepressant drugs and the cardiovascular system: a comparison of tricylics and selective serotonin reuptake inhibitors and their relevance for the treatment of psychiatric patients with cardiovascular problems. *J Psychopharmacol* (1997) **11**(1):83–92. doi:10.1177/026988119701100118
16. Tata LJ, West J, Smith C, Farrington P, Card T, Smeeth L, et al. General population based study of the impact of tricyclic and selective serotonin reuptake inhibitor antidepressants on the risk of acute myocardial infarction. *Heart* (2005) **91**(4):465–71. doi:10.1136/hrt.2004.037457
17. Hamer M, David Batty G, Seldenrijk A, Kivimaki M. Antidepressant medication use and future risk of cardiovascular disease: the Scottish health survey. *Eur Heart J* (2011) **32**(4):437–42. doi:10.1093/eurheartj/ehq438
18. Brunoni AR, Nunes MA, Figueiredo R, Barreto SM, da Fonseca M, de JM, et al. Patterns of benzodiazepine and antidepressant use among middle-aged adults. the Brazilian longitudinal study of adult health (ELSA-Brasil). *J Affect Disord* (2013) **151**(1):71–7. doi:10.1016/j.jad.2013.05.054
19. Burr RL. Interpretation of normalized spectral heart rate variability indices in sleep research: a critical review. *Sleep* (2007) **30**(7):913–9.
20. Moore RA, Derry S, Aldington D, Cole P, Wiffen PJ. Amitriptyline for neuropathic pain and fibromyalgia in adults. *Cochrane Database Syst Rev* (2012) **12**:CD008242. doi:10.1002/14651858.CD008242.pub2

21. Aquino EML, Barreto SM, Benseñor IM, Carvalho MS, Chor D, Duncan BB, et al. Brazilian longitudinal study of adult health (ELSA-Brasil): objectives and design. *Am J Epidemiol* (2012) **175**(4):315–24. doi:10.1093/aje/kwr294
22. Schmidt MI, Duncan BB, Mill JG, Lotufo PA, Chor D, Barreto SM, et al. Cohort profile: longitudinal study of adult health (ELSA-Brasil). *Int J Epidemiol* (2014). doi:10.1093/ije/dyu027
23. Nunes MA, de Mello Alves MG, Chor D, Schmidt MI, Duncan BB. Adaptação transcultural do CIS-R (clinical interview schedule-revised version) para o português no Estudo Longitudinal De Saúde Do Adulto (ELSA). *Revista HCPA* (2012) **31**(4):487–90.
24. Lewis G, Pelosi AJ, Araya R, Dunn G. Measuring psychiatric disorder in the community: a standardized assessment for use by lay interviewers. *Psychol Med* (1992) **22**(2):465–86. doi:10.1017/S0033291700030415
25. Field A. *Discovering Statistics using IBM SPSS Statistics*. 4 ed. London: SAGE (2013). 1 p.
26. Tabachnick BG, Fidell LS. *Using Multivariate Statistics*. 6 ed. Boston: Pearson (2012).
27. Cumming G. The new statistics: why and how. *Psychol Sci* (2014) **25**(1):7–29. doi:10.1177/0956797613504966
28. de Lima TSJV, Garbin CAAS, Garbin ANJS, Sumida DRH, Saliba O. Potentially inappropriate medications used by the elderly: prevalence and risk factors in Brazilian care homes. *BMC Geriatr* (2013) **13**(1):52. doi:10.1186/1471-2318-13-52
29. Kemp AH, Quintana DS. The relationship between mental and physical health: insights from the study of heart rate variability. *Int J Psychophysiol* (2013) **89**(3):288–96. doi:10.1016/j.ijpsycho.2013.06.018
30. Kemp AH, Brunoni AR, Santos IS, Nunes MA, Dantas EM, Carvalho de Figueiredo R, et al. Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil cohort baseline study. *Am J Psychiatr* (2014) **171**:1328–34. doi:10.1176/appi.ajp.2014.13121605
31. Straneva-Meuse PA, Light KC, Allen MT, Golding M, Girdler SS. Bupropion and paroxetine differentially influence cardiovascular and neuroendocrine responses to stress in depressed patients. *J Affect Disord* (2004) **79**(1–3):51–61. doi:10.1016/S0165-0327(02)00352-X
32. Bertoldi AADM, Helfer AP, Camargo AL, Tavares NMUL, Kanavos P. Is the Brazilian pharmaceutical policy ensuring population access to essential medicines? *Global Health* (2012) **8**(1):1–1. doi:10.1186/1744-8603-8-6
33. Cruz L, Lima AFDS, Graeff-Martins A, Maia CRM, Ziegelmann P, Miguel S, et al. Review article mental health economics: insights from Brazil. *J Ment Health* (2013) **22**(2):111–21. doi:10.3109/09638237.2012.759193

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 October 2014; accepted: 07 January 2015; published online: 22 January 2015.

*Citation: Kemp AH, Brunoni AR, Bittencourt MS, Nunes MA, Benseñor IM and Lotufo PA (2015) The association between antidepressant medications and coronary heart disease in Brazil: a cross-sectional analysis on the Brazilian longitudinal study of adult health (ELSA-Brazil). Front. Public Health **3**:9. doi: 10.3389/fpubh.2015.00009 This article was submitted to Epidemiology, a section of the journal Frontiers in Public Health.*

frontiers in Psychology

ORIGINAL RESEARCH
published: 30 March 2016
doi: 10.3389/fpsyg.2016.00425

The Implicit Positive and Negative Affect Test: Validity and Relationship with Cardiovascular Stress-Responses

Melanie M. van der Ploeg[1]*, *Jos F. Brosschot*[1], *Julian F. Thayer*[2] and *Bart Verkuil*[3]

[1] Health, Medical and Neuropsychology Unit, Institute of Psychology, Leiden University, Leiden, Netherlands, [2] Department of Psychology, The Ohio State University, Columbus, OH, USA, [3] Clinical Psychology Unit, Institute of Psychology, Leiden University, Leiden, Netherlands

Self-report, i.e., explicit, measures of affect cannot fully explain the cardiovascular (CV) responses to stressors. Measuring affect beyond self-report, i.e., using implicit measures, could add to our understanding of stress-related CV activity. The Implicit Positive and Negative Affect Test (IPANAT) was administered in two studies to test its ecological validity and relation with CV responses and self-report measures of affect. In Study 1 students ($N = 34$) viewed four film clips inducing anger, happiness, fear, or no emotion, and completed the IPANAT and the Positive And Negative Affect Scale at baseline and after each clip. Implicit negative affect (INA) was higher and implicit positive affect (IPA) was lower after the anger inducing clip and vice versa after the happiness inducing clip. In Study 2 students performed a stressful math task with ($n = 14$) or without anger harassment ($n = 15$) and completed the IPANAT and a Visual Analog Scale as an explicit measure afterwards. Systolic (SBP), diastolic (DBP) blood pressure, heart rate (HR), heart rate variability (HRV), and total peripheral resistance (TPR) were recorded throughout. SBP and DBP were higher and TPR was lower in the harassment condition during the task with a prolonged effect on SBP and DBP during recovery. As expected, explicit negative affect (ENA) was higher and explicit positive affect (EPA) lower after harassment, but ENA and EPA were not related to CV activity. Although neither INA nor IPA differed between the tasks, during both tasks higher INA was related to higher SBP, lower HRV and lower TPR and to slower recovery of DBP after both tasks. Low IPA was related to slower recovery of SBP and DBP after the tasks. Implicit affect was not related to recovery of HR, HRV, and TPR. In conclusion, the IPANAT seems to respond to film clip-induced negative and positive affect and was related to CV activity during and after stressful tasks. These findings support the theory that implicitly measured affect can add to the explanation of prolonged stress-related CV responses that influence CV health.

Keywords: stress, prolonged cardiovascular activity, reactivity/recovery, harassment, unconscious stress, implicit affect, implicit measures, IPANAT

OPEN ACCESS

Edited by:
Andrew Kemp,
Swansea University, UK

Reviewed by:
Tim Outhred,
The University of Sydney, Australia
Belinda Jayne Liddell,
University of New South Wales, Australia

***Correspondence:**
Melanie M. van der Ploeg
m.m.van.der.ploeg@fsw.leidenuniv.nl

Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *18 August 2015*
Accepted: *10 March 2016*
Published: *30 March 2016*

Citation:
van der Ploeg MM, Brosschot JF, Thayer JF and Verkuil B (2016) The Implicit Positive and Negative Affect Test: Validity and Relationship with Cardiovascular Stress-Responses. Front. Psychol. 7:425. doi: 10.3389/fpsyg.2016.00425

INTRODUCTION

Psychosocial stressors such as marital stress and job stress are increasingly recognized as contributors to the development or progress of cardiovascular (CV) disease (see for example McEwen, 1998, 2003; Rozanski et al., 1999; Rosengren et al., 2004; Strike and Steptoe, 2004; Brotman et al., 2007; Chida and Hamer, 2008; Dimsdale, 2008; Lu et al., 2013). Still, studies have been inconclusive on the mechanisms underlying the relationship between psychosocial stress and CV diseases (Dimsdale, 2008; Brindle et al., 2014). This might be related to the inability of the used measurements of psychological stress to explain CV activity (Gerin et al., 2006; Key et al., 2008; Pieper et al., 2010). The current paper addresses this issue by validating a test that indirectly assesses affect and is expected to more closely relate to psychophysiological responses; the Implicit Positive and Negative Affect Task (IPANAT; Quirin et al., 2009a; Quirin and Lane, 2012).

The reactivity hypothesis of stress has been the main focus of the field and emphasizes the acute physiological responses during a stressor. However, accumulating literature suggests that prolonged stress responses and not, or to a lesser extent, the reactivity during stressors, determine the detrimental consequences for health. In other words, measuring the CV activity during stressors might not fully represent that part of the physiological stress response that explains the development of CV or other diseases. Slow recovery from stressors and anticipatory responses to them might be of equal or even greater importance (Haynes et al., 1991; Linden et al., 1997; Ursin and Eriksen, 2004; Pieper and Brosschot, 2005; Koolhaas et al., 2011; Panaite et al., 2015). Moreover, this prolonged activity leads to a pathological state that is often described as "allostatic load" (McEwen, 1998) and is the final biological pathway to organic disease. Earlier research focusing on reactivity to a stressor has overlooked these different forms of the maladaptive stress response, i.e., prolonged physiological activation. These forms of prolonged activation have been attributed to ongoing cognitive representation of the stressors, which is known as perseverative cognition. Perseverative cognition, often manifested as rumination or worry, has been associated with prolonged CV activity (Brosschot et al., 2006, 2007; Pieper et al., 2007; Juster et al., 2012; see for reviews Verkuil et al., 2010; Ottaviani et al., 2016).

The assessment of psychological stress to explain related CV responses is typically done through self-report methods such as keeping a worry and mood diary or completing questionnaires like work stress scales or trait questionnaires of worry, anxiety, or general negative affect (e.g., Gerin et al., 2006; Brosschot et al., 2007; Pieper et al., 2007, 2010; Key et al., 2008; Verkuil et al., 2012). However, several findings indicate that these measures do not fully explain the prolonged CV responses to stressors (Gerin et al., 2006; Key et al., 2008; Pieper et al., 2010). Brosschot et al. (2007) for example found that individuals that experienced stressors and worry during the day displayed increased cardiac activity during sleeping at night, when conscious worry and affect related cognitions are absent. Moreover, Pieper et al. (2010) demonstrated that cardiac effects of worry in real life continued after worry episodes ceased and were not due to negative affect or bio-behavioral variables such as movement or smoking. Additionally, Gerin et al. (2006; Key et al., 2008) found that slow blood pressure (BP) recovery after an experimental stressor was not due to explicit worrisome thoughts. These findings seem to indicate that part of the psychological stress response affects the CV system in a way that is not addressed by self-report measures. Brosschot and colleagues (Brosschot, 2010; Brosschot et al., 2010) have hypothesized that this part is explained by ongoing unconscious (or "implicit") stress-related cognition. This unconscious stress-related cognition would represent a general negative state that one is unable to express, but that does affect physical wellbeing. Concepts related to unconscious stress-related cognition have already been widely used within cognitive and social psychology, such as implicit affective attitudes, self-esteem, and emotion (see for example Kihlstrom, 1987; Fazio and Olson, 2003; Bargh and Morsella, 2008; Gyurak et al., 2011), and have been demonstrated to influence for example decision making processes (Dijksterhuis et al., 2006) and affective evaluation (Zajonc, 1980). Implicit stress-related cognition cannot be measured with self-report methods, because for these methods deliberate processing of the assessed construct is required (De Houwer et al., 2009).

Various instruments have been designed to measure affective processing at an implicit level, i.e., implicit measures, such as the affective Implicit Association Test (IAT; Egloff et al., 2002; Verkuil et al., 2014) and the Implicit Positive and Negative Affect Task (IPANAT; Quirin et al., 2009a). In the current study, we examined the IPANAT as an implicit measure of stress-related cognition operationalized as implicit affect (Quirin et al., 2009a). The IPANAT is suggested to operate as an implicit measure of affect through the process of affect misattribution (Zajonc, 1980; Forgas, 1995; Payne et al., 2005; Quirin and Bode, 2014). Similar to the original studies of Zajonc and colleagues in the IPANAT (1980) ambiguous stimuli are presented, namely a set of nonsense words, of which the affective value is rated on a six point scale for 12 emotional adjectives. The assumption is that the participants, again as in Zajonc's studies, respond in accordance with their current affective state, without being fully aware of the construct being measured (Quirin et al., 2009a). The implict negative affect scale (INA) of the IPANAT has been shown to predict cortisol responses to a speech stressor and increases in circadian cortisol concentrations (Quirin et al., 2009b). The latter was recently partly replicated by Mossink et al. (2015). In Brosschot et al. (2014, Study 2) INA, measured with the IPANAT, was related to slower recovery of BP after a math stressor with anger harassment, whereas explicit negative affect (ENA) showed no significant relationship. However, in that study no control group for extra negative affective changes due to harassment was used, which limits inferences on the application of the IPANAT as implicit measure of stress-related cognition. In the current study, the harassment manipulation was again tested and a control group with only a math task was added to the design to test whether it is the specific affective component of anger harassment that affects INA and IPA as measured with the IPANAT.

The present studies address two issues. First, the IPANAT's content validity has hitherto only been tested with simple

affective stimuli, namely pictorial emotional stimuli. Furthermore, although associations of the IPANAT with physiological measures have been found its relationship with explicit measures of affect are underappreciated (for a review see Quirin et al., 2009a; Quirin and Bode, 2014). For example Quirin et al. (2009b) found a relationship between the negative, but not the positive, subscales of implicit and explicit affect. However, this observational study measured changes in cortisol levels, but not in affect. Thus, the interpretation of both the relationship between implicit and explicit affect and the ability of the IPANAT to capture direct changes in affect due to stressful experiences cannot readily be applied to the current ideas about unconscious stress-related cognition. In the current two studies content validity was examined under more realistic conditions by providing negative and positive emotional film clips in one study, which are more ecologically valid than simple pictures and have been suggested to elicit prolonged affective responses compared with pictures (e.g., Gross and Levenson, 1995; Rottenberg et al., 2007; Schaefer et al., 2010), and by deploying a more naturalistic stressor, namely a math task with and without anger harassment in a second study. Moreover, in the first study we assessed the IPANAT's ability to detect changes in (implicit) affect and in the second study we relate the IPANAT subscales to physiological parameters to more specifically address the theory that changes in these parameters can be related to affect measured implicitly. We expected that the emotional film clips and especially anger harassment would evoke affect-congruent changes on the IPANAT subscales that are at least partly independent of explicit affect. Second, it addresses whether CV responses during a stressor and recovery from it, as a model of prolonged CV activation, are associated with implicit affect as measured with the IPANAT and whether this association is at least partly independent of that of explicit affect. More precisely, we expected that INA would be related to a higher reactivity to a stressor and slower recovery from it, and vice versa for implicit positive affect (IPA).

Furthermore, we expected stronger affective and CV effects for the math stressor with harassment. CV recovery is typically longer after emotional stressors than after physical or neutral stressors, while reactivity (i.e., responses during these stressors) is often equally high (e.g., Brosschot et al., 2014, Study 1; Linden et al., 1997). This difference in recovery is taken to be due to prolonged explicit stress-related cognition, or high ENA or low explicit positive affect (EPA), or both. Here, we hypothesized that it is also due to implicitly measured affect, that is high INA or low IPA, or both. Consequently, we expected that a more strongly negative emotional stressor (math with harassment) would lead to slower CV recovery and higher negative and lower positive affect, measured explicitly and implicitly, than a relatively more neutral stressor (math without harassment). We also expected that the slower CV recovery after harassment would be explained by the stronger affective responses, and that implicit affect explains CV recovery over and above explicit affect.

In sum, previous findings suggest that the IPANAT might be a suitable implicit measure of stress-related affective cognition, but its content validity and its ability to explain CV activity, expressed as reactivity and recovery to an emotional stressor, have not been thoroughly examined. In the present article two studies are reported that tested whether the IPANAT is able to detect changes in affective state induced by emotional film clips (Study 1) and whether it can explain CV responses to a stressor beyond explicit measures of affect (Study 2). In addition, it was tested whether the IPANAT scores were related to the general and differential CV responses to a stressor with and without anger harassment and to CV recovery after these stressors.

STUDY 1

Methods

Participants and Procedure

A total of 34 [64.7% female; mean age of 24.0 (SD = 8.51)] students of Leiden University with sufficient understanding of the Dutch language enrolled in the experiment for course credits or five euro. Participants provided informed consent and received the standard instructions for the questionnaires after which they were seated in front of a computer and were asked to put on a Sennheiser HD201 headphone. In random order, four film clips were shown that were previously validated to elicit anger, happiness, fear and a neutral state. The film clips were English versions identical to code 15 (1:17 min.), 24 (2:45 min.), 65 (3:57 min.) and 55 (0:40 min.), respectively, from the FilmStim database (Schaefer et al., 2010). The volume accompanying the film fragments was set at medium (45–55 dB). The IPANAT and Positive And Negative Affect Scale (PANAS; Watson et al., 1988) were administered at baseline and after each video clip (see **Figure 1**). In one case the PANAS was not completed after the anger film clip. The study was approved by the Independent Ethics Committee of the Institute of Psychology of Leiden University, under number 5148415681.

Implicit and Explicit Affect

A Dutch translation of The Implicit Positive and Negative Affect Test (IPANAT) as a measure of implicit affect was provided (Quirin et al., 2009a; Brosschot et al., 2014). Respondents rated six artificial words (vikes, tunba, ronpe, belni, sukov, safme) for emotional adjectives on a six-point Likert scale. In the version we used, the IPANAT for discrete emotions (Quirin and Bode, 2014), 12 emotional adjectives are used. The mean scores per adjective for all artificial words were computed and summarized in the mean scores of INA (sad, gloomy, unhappy, annoyed, irritated, angry, afraid, frightened, scared) and IPA (joyful, cheerful, happy). In this particular study the IPANAT was used as a repeated measure by providing the entire IPANAT at baseline and two nonsense words, randomly selected from the pool of six words, after each film clip. Repeated presentation of the same full test was likely to cause carryover and training effects or boredom, resulting in erroneous scoring. Filling out the full version IPANAT takes about 5 min and as a repeated measure about 2 min for each administration. In the current sample the IPANAT administered at baseline was found to be reliable with Cronbach's $\alpha = 0.75$ for INA and Cronbach's $\alpha = 0.89$ for IPA, which is comparable to the reliability found by Quirin et al. (2009a).

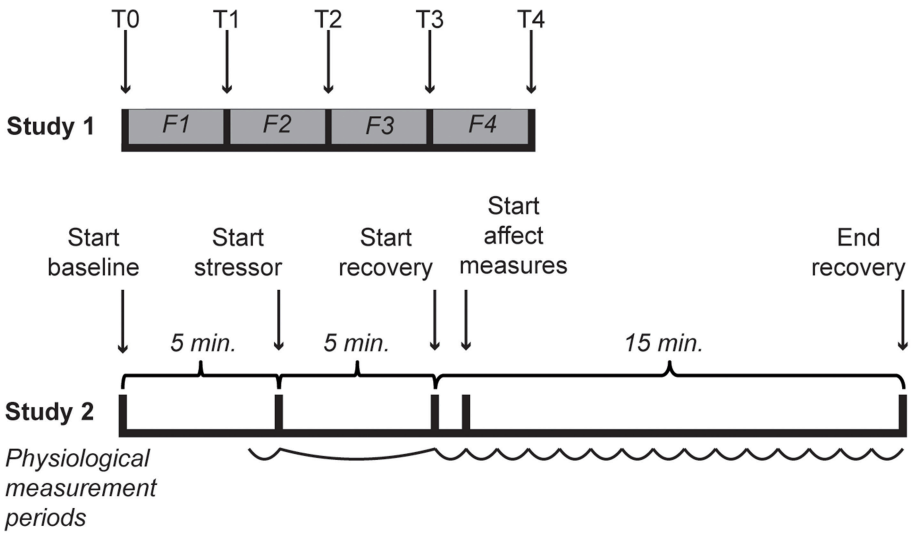


FIGURE 1 | Timelines of both studies. In Study 1 T0 represents the baselines measurement of affect, while T1–T4 represent the affect measures after each film clip (indicated with F1–F4). During Study 2 cardiovascular activity was measured throughout. For analyses the last minute of baseline, the 5-min stressor and 15 separate min of the recovery were used, as indicated with a curved line.

At all measurement points explicit affect was measured with the PANAS, which measures positive and negative affect on two 10 item scales with emotional adjectives (Watson et al., 1988). Participants indicate on a five-point scale the extent to which the items apply to their current affective state. The PANAS was found reliable in this sample with Cronbach's $\alpha = 0.90$ for ENA, Cronbach's $\alpha = 0.87$ for explicit positive affect (EPA), which is comparable with reliability found by Crawford and Henry (2004) in a large non-clinical sample. The implicit and explicit affective responses to video clips were compared with the affective responses at baseline.

Results

The demographical information of all participants is provided in **Table 1**. Mean affect scores are displayed in **Table 2**. In this within-subjects design, the effect of the film clips on affect was determined with four one-way repeated measures ANOVA's, one for each affect measure. There were significant differences between film clips on all affect measures [INA: Wilks' $\lambda = 0.51$, $F_{(4, 30)} = 7.32$, multivariate partial $\eta^2 = 0.49$; IPA: Wilks' $\lambda = 0.44$, $F_{(4, 30)} = 9.64$, multivariate partial $\eta^2 = 0.56$; ENA: Wilks' $\lambda = 0.28$, $F_{(4, 29)} = 18.6$, multivariate partial $\eta^2 = 0.72$; EPA: Wilks' $\lambda = 0.47$, $F_{(4, 29)} = 8.31$, multivariate partial $\eta^2 = 0.53$, all $p < 0.001$].

Subsequently, affect after each film clip was compared with baseline through planned comparisons, tested one sided since our hypotheses had a clear direction (e.g., Ludbrook, 2013). The results were corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10% (Simes, 1986; Benjamini and Hochberg, 1995; McDonald, 2014). Results, displayed in **Table 3**, indicated that compared with baseline ($M = 2.55$, $SD = 0.53$) INA scores were significantly higher after the anger inducing film clip ($M = 3.00$, $SD = 1.01$) and lower after the happiness inducing clip ($M = 2.14$, $SD = 0.77$), $t_{(33)} = 2.79$, $p = 0.009$, $d = 0.56$ and $t_{(33)} = -3.22$, $p = 0.003$, $d = 0.62$, respectively. INA was not significantly different after the fear inducing ($M = 2.79$, $SD = 0.80$) and neutral film ($M = 2.59$, $SD = 0.81$) clips compared with baseline, $t_{(33)} = 1.59$, $p = 0.122$, $d = 0.35$ and $t_{(33)} = 0.22$, $p = 0.830$, $d = 0.06$, respectively. Similarly, compared with baseline ($M = 3.20$, $SD = 0.88$), IPA was significantly lower after the anger inducing clip ($M = 2.51$, $SD = 1.20$), $t_{(33)} = -2.83$, $p = 0.008$, $d = 0.66$ and significantly lower after the fear inducing clip ($M = 2.67$, $SD =$

TABLE 1 | Baseline characteristics of the total sample ($N = 34$) of Study 1.

Measure	*M*	*SD*
DEMOGRAPHICS		
Age, years	24.0	8.51
Female sex[a]	23	(70)
BMI	21.5	4.73
In a relationship[a]	19	(56)
BIOBEHAVIORAL VARIABLES		
Smoke[a]	4	(12)
Smoked units today	0.08	0.28
Cafeine use[a]	29	(85)
Caffeine units today	0.45	1.03
Alcohol use[a]	12	(86)
Alcohol units last 24 h	0.39	1.77
Drug use[a]	4	(12)
Drugs today[a]	0	(0)
Current mental health complaints	2	(6)
Current psychological treatment	3	(9)

BMI, Body Mass Index.
[a] *Indicated with number of positive responses (percentage).*

TABLE 2 | Mean affect scores at baseline and after every film fragment in Study 1.

Phase	Implicit				Explicit			
	NA		PA		NA		PA	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Baseline	2.55	0.53	3.20	0.88	1.48	0.67	2.88	0.54
Anger	3.00	1.01	2.51	1.20	2.52[a]	0.90	2.38[a]	0.50
Happy	2.14	0.77	3.70	1.06	1.44	0.59	2.75	0.67
Fear	2.79	0.80	2.67	0.84	2.37	0.75	2.52	0.45
Neutral	2.59	0.81	2.95	1.06	1.45	0.54	2.30	0.61

N = 34. NA, Negative affect; PA, Positive affect.
[a] *N = 33.*

TABLE 3 | Planned comparisons between affect at baseline and after each film clip in Study 1.

Comparisons	*M diff*	*SE*	*t*	*d*
IMPLICIT NA				
Anger	0.453	0.16	2.79**	0.56
Happy	−0.407	0.13	−3.22**	0.62
Fear	0.245	0.15	1.59	0.35
Neutral	0.033	0.15	0.22	0.06
IMPLICIT PA				
Anger	−0.691	0.24	−2.83**	0.66
Happy	0.495	0.20	2.46*	0.51
Fear	−0.534	0.21	−2.60*	0.62
Neutral	−0.255	0.23	−1.12	0.26
EXPLICIT NA				
Anger[a]	1.027	0.17	5.90***	1.31
Happy	−0.032	0.11	−0.30	0.06
Fear	0.891	0.15	5.96***	1.25
Neutral	−0.029	0.10	−0.29	0.04
EXPLICIT PA				
Anger[a]	−0.521	0.11	−4.92***	0.96
Happy	−0.12	0.11	−1.19	0.21
Fear	−0.359	0.09	−4.00***	0.72
Neutral	−0.582	0.12	−4.87***	1.01

N = 34. d is calculated with original means and standard deviations. Tests were performed one sided and corrected for multiple comparisons using the Benjamini-Hochberg procedure (Simes, 1986; Benjamini and Hochberg, 1995) with the false discovery rate set at 10%. NA, Negative affect; PA, Positive affect.
[a] *N = 33.*
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.*

TABLE 4 | Pearsons product-moment correlations between changes in implicit and explicit affect in Study 1.

Affect	Fragment	*r*	
		ENA	EPA
INA	Anger	0.26	0.10
	Happy	0.01	−0.32+
	Fear	−0.07	0.11
	Neutral	−0.01	0.33+
IPA	Anger	−0.06	0.06
	Happy	−0.06	0.32+
	Fear	0.28	−0.21
	Neutral	0.10	−0.34+

N = 34. INA, Implicit negative affect; IPA, Implicit positive affect; ENA, Explicit negative affect, EPA, Explicit positive affect.
+*$p < 0.10$.*

0.84), $t_{(33)} = -2.60$, $p = 0.014$, $d = 0.62$. IPA was significantly higher after the happiness inducing film clip ($M = 3.70$, $SD = 1.06$), $t_{(33)} = 2.46$, $p = 0.019$, $d = 0.51$. IPA was not significantly changed after the neutral film clip [$M = 2.95$, $SD = 1.06$; $t_{(33)} = 1.12$, $p = 0.272$, $d = 0.26$].

ENA scores were, compared with baseline ($M = 1.48$, $SD = 0.67$), significantly higher after the anger inducing clip ($M = 2.52$, $SD = 0.90$) and the fear inducing clip ($M = 2.37$, $SD = 0.75$) with $t_{(32)} = 5.90$, $p < 0.001$, $d = 1.31$ and $t_{(33)} = 5.96$, $p < 0.001$, $d = 1.25$, respectively. ENA was not significantly changed after the happiness inducing ($M = 1.44$, $SD = 0.59$) and neutral film clips ($M = 1.45$, $SD = 0.54$), with $t_{(33)} = -0.30$, $p = 0.767$, $d = 0.06$ and $t_{(33)} = -0.29$, $p = 0.772$, $d = 0.04$, respectively. Finally, compared with baseline ($M = 2.88$, $SD = 0.54$), EPA was significantly lower after the anger inducing film clip ($M = 2.38$, $SD = 0.50$), the fear inducing film clip ($M = 2.52$, $SD = 0.45$) and the neutral film clip ($M = 2.30$, $SD = 0.61$) with $t_{(32)} = -4.92$, $p < 0.001$, $d = 0.96$, $t_{(33)} = -4.00$, $p < 0.001$, $d = 0.72$ and $t_{(33)} = -4.87$, $p < 0.001$, $d = 1.01$, respectively. EPA was not significantly changed after the happiness inducing film clip ($M = 1.75$, $SD = 0.67$), $t_{(33)} = -1.19$, $p = 0.241$, $d = 0.21$. Furthermore, there were no significant correlations between changes in implicit affect and explicit affect as displayed in **Table 4**.

Discussion

In this study we tested whether the IPANAT is able to detect changes in affective state. The film clips instigated affect-congruent changes on the IPANAT subscales that were unrelated to changes in self-reported affect. These results add to the evidence for the IPANAT's validity by using stimuli that are more "ecologically valid" than the pictures used in the original studies (Quirin et al., 2009a).

Notably, the fear inducing clip lowered IPA, but did not change INA, while the anger evoking clip did change both scales in the expected directions. The fear inducing clip might not have effectively evoked the targeted emotion, anxiety. Still, although not significantly, it did change INA in the expected direction, and yielded expected and significant explicit NA changes. Moreover, in the film clip pool (Schaefer et al., 2010) the same clip yielded a comparable mean ENA of 2.40. Together, this seems to indicate that the negative affect induced by the fear clip was not captured by the INA subscale of the IPANAT. Similarly, although explicit affect changed in an affect-congruent fashion, no changes in EPA were found after the happiness inducing clip. However, considering that EPA did not only decrease after the two negative clips, but also after the neutral film clip, the absence of an affect after the happiness inducing clip can be interpreted as an affect-congruent effect. An alternative explanation could be that the sample had a relatively high positive affect at baseline that did not change after the happiness inducing clip, as it was congruent with the dominant affective state, but did decrease to a relatively more neutral state after the neutral film. Furthermore, one could argue that the differences in length of the film clips elicited different intensities of the induced affect (Gross and Levenson, 1995). However, longer exposure time to a film clip did not increase the effect of the film clips, i.e., the fear inducing film clip was the longest but did not elicit the largest effect.

In sum, the results suggest that the IPANAT is able to measure changes in affect after emotion induction using films that are congruent with the valence of these stimuli. Moreover, it measures changes independently of explicit measures.

STUDY 2

Methods

Participants

Thirty three Dutch undergraduate students from Leiden University, The Netherlands were recruited and received eight Euro or course credits for participation. Participants were randomly assigned to the stressor with harassment and stressor without harassment conditions (see below). Two participants had current CV disease and/or psychological problems, in one case the experiment failed due to technical difficulties and one participant had consumed over 5 units of alcohol in the 24 h before the experiment. These cases were excluded from the analysis. The final sample with a mean age of 21.0 ($SD = 2.29$) consisted of 18 females (62.1%). The study was approved by the Independent Ethics Committee of the Institute of Psychology of Leiden University, under number 3145923676.

Implicit and Explicit Affect

The Dutch full version IPANAT was used in this study as a single measure 1 min after the termination of the stressor. The artificial word "safme" was omitted as subjects reported it was associated with "save me," and thus possibly not sufficiently ambiguous. Leaving out one of the words did not affect reliability; Cronbach's α was 0.93 for INA and 0.92 for IPA, which is in line with previous findings (Quirin et al., 2009a; Brosschot et al., 2014).

As an explicit measure of affect a Visual Analog Scale (VAS) was provided. Participants were asked to what extent they felt a certain emotion (e.g., "How annoyed are you at this moment?"), using the same emotional adjectives as in the IPANAT. At the bottom of the screen a horizontal line of 10 cm was shown, with "not at all" on the left and "very much" on the right on which the participants could indicate their affect, resulting in a score in the range of −100 to +100, with a higher rating indicating increased levels of the adjective. Scores were averaged into ENA and EPA in a similar fashion as the IPANAT. With respect to reliability Cronbach's α's were 0.90 and 0.96 for ENA and EPA, respectively.

Cardiovascular Activity

The physiological data were measured continuously throughout the experiment. Averages of each outcome measurement were calculated over the last minute of baseline, the 5-min stressor phase and separately for all 15 min of the recovery. Systolic BP (SBP) and diastolic BP (DBP) [in millimeters of mercury (mmHg)] were measured with the Portapres Model-2 (Finapres Medical Systems, Amsterdam, The Netherlands), a non-invasive method to measure BP by placing a finger cuff on the middle finger of the non-dominant hand. The electrocardiogram (ECG) was recorded with Kendall® 200 Covidien electrodes at a sample rate of 200 Hz with BIOPAC MP150, Biopac Systems, Goleta, CA, USA and visually inspected as well as corrected for movement artifacts with Acqknowledge 3.9.1.4. SBP, DBP and HR (in beats per minute, bpm) were extracted with a tailor made toolbox in Matlab R2012b. A low-pass filter (20 Hz, Blackman 40 coeffients) was applied to the BP signal. The ECG signal was up sampled to 1000 Hz and a comb filter (50 Hz, $Q = 5$) was applied. Root mean squared successive differences (RMSSD; ms) was derived from the ECG signal as a measure of HRV (Berntson et al., 2007; Nussinovitch et al., 2011; Smith et al., 2013; Munoz et al., 2015). Total peripheral resistance (TPR; in mmHg.min/L) was derived using an approximation of cardiac output (CO) by the formula $CO = (0.002*(SBP - DBP))*HR$ (Sun et al., 2005; Hill et al., 2011, 2012). From MAP and the approximated CO, using the formula $TPR = (MAP/CO)$, estimated TPR was then obtained (Sherwood et al., 1990). To avoid redundancy, only the outcome measure of interest, TPR, is reported.

Stress Induction

All participants were instructed to perform a mathematical task; calculating backwards from 9000 in steps of 17 out loud. Emotional stress was induced by an anger harassment procedure in the stressor with harassment condition only; participants received seven pre-recorded remarks in an angry tone at set times (0:30; 1:00; 1:30; 2:30; 2:40; 4:00; and 4:55) during the 5 min duration of the stressor phase. These harassing remarks, such as "You are counting too slow, try to speed up." and "Could you really try to focus now?", were similar to those used by Radstaak et al. (2011) and others (e.g., Glynn et al., 2002; Mauss et al., 2007). Participants in the stressor without harassment condition did not receive any harassing remarks, but all participants received the instruction to start at 0:00.

Procedure

The study was run by two experimenters, of which one monitored the physiological measurements and the other was in contact with the participant. The procedure was explained to the participants after which they signed an informed consent before starting with the experiment. Demographics and biobehavioral variables were obtained followed by placement of the finger cuff and electrodes. The tasks and tests were presented via computer (E-Prime 2.0.8.90). A 5 min baseline period started during which participants could read a magazine with neutral content and were asked to sit quietly (e.g., Gerin, 2011). This was followed by the stress induction as described above. The immediately ensuing recovery started with a minute during which participants did not perform any tasks and were instructed to remain seated for measurement purposes. This was considered to be different from baseline since cognitive representations of the stressor were assumed to be present. After the first minute of recovery the IPANAT started, followed by the VAS. When finished with the tasks within 15 min after the stressor, participants would wait until the 15 min had passed (See **Figure 1**). Finally, the finger cuff and electrodes were removed and participants were asked about their thoughts and experiences during and about the experiment before they were given a debriefing on the actual purpose of the study and constructs assessed with the IPANAT.

Statistical Analyses

To represent reactivity, but not recovery, change scores were calculated by subtracting baseline values from those during the stressors for all CV outcomes (Llabre et al., 1991) and effects of condition (i.e., stressor with and without harassment) were analyzed with one sided *t*-tests since our hypotheses had a specific direction (e.g., Ludbrook, 2013). Hierarchical multiple regression was used to assess the association between affect measures and physiological outcome variables, after controlling for condition. Recovery was analyzed with multilevel analyses for SBP and DBP (Lehman et al., 2015), as it has various advantages over repeated measures ANOVAs when analyzing effects of time, such as a better handling of missing data and including individual slopes into the model and thus is able to consider multiple levels in the data (e.g., Llabre et al., 2001). The mean of the CV measure during the stressors was included as covariate in the basic growth model. The model fit did not increase when adding both the baseline and task-related activity and by applying a random slope we already corrected for inter-individual variance unrelated to the stressor (Llabre et al., 2001; Singer and Willett, 2003; Lehman et al., 2015). Grand mean centering was applied to all predictors and covariates. For SBP and DBP separate models were built, but for all models Time was the level 1 variable, representing the measurements' course over 15 min (Model 1). Level 2 represented the person level, which included implicit (Model 2) or explicit affect (Model 3) or both (Model 4). The fit of the models was determined by significant changes in the Akaike information criterion (AIC) and Bayesian information criterion (BIC; Llabre et al., 2001). The data did not allow for multilevel analysis on HR, RMSSD, and TPR as visual inspection showed that recovery of these outcome measures occurred within 1 min after the stressor. Accordingly, for these outcome measures instead of multilevel analyses partial correlations were performed on the first minute of the recovery phase with the affect measures while correcting for CV activity during the stressors. All analyses were done with SPSS 21.0.

Results

The data were inspected for collection errors, missing values, outliers (>3 *SD*s from the mean) and violation of assumptions for all performed analyses. The distribution of RMSSD was skewed and a square root transformation was applied. One participant displayed a high SBP at rest (>175 mmHg) and throughout the experiment, which was considered extreme. To be conservative, these data points were not included in analyses. Furthermore, one participant provided too many identical responses, i.e., 1-1-1-1-1 on the IPANAT, and that data was excluded from the data set. As suggested by Quintana and Heathers (2014) differences between conditions regarding demographical and bio-behavioral variables were examined but none were observed, nor were there differences found between conditions in CV outcome measures as displayed in **Table 5**.

Explicit and Implicit Affect

To examine the effect of the stressor with and without harassment on affect independent samples *t*-tests were performed, one-sided (e.g., Ludbrook, 2013), and corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10% (Simes, 1986; Benjamini and Hochberg, 1995; McDonald, 2014). In response to the stressor higher levels of ENA were reported by participants after the stressor with harassment ($M = -46.2$, $SD = 37.6$) compared with the stressor without harassment [$M = -74.97$, $SD = 21.89$; $t_{(26)} = 2.47$, $p = 0.020$, 95% *CI* [4.83, 52.6], $d = 0.93$]. Furthermore, after the stressor with harassment lower EPA ($M = -7.60$, $SD = 43.2$) was reported compared with the stressor without harassment [$M = 31.9$, $SD = 36.3$; $t_{(27)} = -2.67$, $p = 0.013$, 95% *CI* [−69.9, −9.15], $d = 0.99$]. However, there was no condition effect on INA [with harassment: $M = 2.97$, $SD = 0.54$, without harassment: $M = 2.97$, $SD = 0.46$; $t_{(26)} = 0.030$, $p = 0.976$, 95% *CI* [−0.38, 0.39], $d = 0.01$], nor on IPA [with harassment: $M = 3.34$, $SD = 0.75$, without harassment: $M = 3.43$, $SD = 0.52$; $t_{(26)} = -0.37$, $p = 0.713$, 95% *CI* [−0.59, 0.41], $d = 0.14$]. In sum, there was no condition effect on implicit affect, but there was an expected condition effect on ENA.

As exploratory analyses the associations between the affect measures were examined. INA was not significantly related to IPA or EPA ($rs < -0.20$, $ps > 0.05$) ENA [$r_{(28)} = 0.16$, $p > 0.05$, IPA was not significantly related to ENA [$r_{(28)} = -0.20$, $p > 0.05$], and marginally significantly related to EPA [$r_{(28)} = 0.32$, $p = 0.09$]. ENA and EPA showed a strong inverse relationship [$r_{(28)} = -0.83$, $p < 0.001$].

Cardiovascular Reactivity

First, we examined whether there were statistically significant changes in CV activity from baseline during both tasks using paired *t*-tests, one-sided (e.g., Ludbrook, 2013), and corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10% (Simes, 1986; Benjamini

TABLE 5 | Baseline characteristics for the total sample of Study 2 by condition.

Measure	Harassment ($n = 14$)		No harassment ($n = 15$)		
	M	*SE*	*M*	*SE*	t/χ^2
DEMOGRAPHICS					
Age, years	20.6	0.69	21.3	0.52	−0.73
Female sex[a]	7	(50)	11	(73)	1.68
BMI	21.7	0.91	22.2	1.07	−0.30
In a relationship					
BIOBEHAVIORAL VARIABLES					
Smoke[a]	2	(14)	1	(6)	−0.45
Daily Smoking	0.93	0.73	0.60	0.60	0.35
Cafeine use[a]	11	(79)	9	(60)	−1.17
Daily caffeine intake[c]	1.50	0.49	0.90	0.26	1.09
Alcohol use[a]	12	(86)	13	(87)	−0.01
Weekly alcohol consumption	3.09	0.76	2.72	0.97	0.30
Drug use[a]	1	(7)	0	(0)	−1.11
Exercice[a]	11	(79)	13	(87)	−0.33
Weekly exercise (hours)	3.11	0.75	3.37	0.96	−0.21
Visits to GP (last 6 months)	0.79	0.21	1.00	0.45	−0.43
CARDIOVASCULAR MEASURES					
SBP[b]	129.2	3.23	124.5	3.55	0.97
DBP	68.3	2.02	68.5	1.95	−0.16
HR	72.2	2.01	79.4	3.27	−1.93$^+$
RMSSD[b]	6.14	0.41	5.78	0.35	0.66
TPR[b]	3.17	0.06	3.19	0.10	−0.16

A square root transformation was applied to RMSSD. There were no significant differences between the conditions. BMI, Body Mass Index; GP, General practitioner; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HR, Heart Rate; RMSSD, Root Mean Square of Successive Differences; TPR, Total Peripheral Resistance.

[a]Indicated with number of positive responses (percentage), Pearson χ^2 was used as test statistic.

[b]$N = 28$.

[c]Levene's Test indicated unequal variances, $df = 19.9$.

$^+p < 0.10$, tested two-sided.

and Hochberg, 1995; McDonald, 2014). Compared to baseline in both conditions there was an increase in SBP, DBP and HR and a decrease in TPR (see **Table 6**). No significant decrease was found for RMSSD. Second, we examined the effect of the stressor with and without harassment on the CV measures using independent samples *t*-tests, again one-sided (e.g., Ludbrook, 2013) and with the Benjamini-Hochberg correction. These tests indicated that the stressor with harassment elicited significantly higher SBP ($M = 23.3$, $SD = 9.43$) compared with the stressor without harassment [$M = 12.6$, $SD = 8.56$; $t_{(25)} = 3.07$, $p = 0.005$, 95% *CI* [3.51, 17.8], $d = 1.19$]. DBP was significantly higher in the stressor with harassment ($M = 12.9$, $SD = 1.40$) compared with the stressor without harassment [$M = 8.98$, $SD = 4.26$; $t_{(26)} = 2.27$, $p = 0.032$, 95% *CI* [−2.13, 0.09], $d = 1.61$, respectively]. Furthermore, TPR was significantly lower in the stressor with harassment condition ($M = -1.44$, $SD = 0.42$), compared with the stressor without harassment [$M = -0.34$, $SD = 0.26$; $t_{(18.62)} = 3.07$, $p = 0.036$, 95% *CI* [−2.13, −0.08], $d = 1.16$, respectively]. No significant differences ($p > 0.10$) in HR ($d = 0.62$) and RMSSD ($d = 0.12$) were found between conditions. These findings were confirmed by Repeated Measures ANOVAs. Gender, body mass index (BMI) and smoking were not related to the outcome measures and were not included in the models.

Cardiovascular Reactivity and Affect

The association between implicit and explicit affect and CV reactivity was examined with a hierarchical regression analysis for each CV outcome measure resulting in five separate models. In all the models condition was added at step 1 and explicit affect at step 2. Since we expected that implicit affect would explain CV activity over and above explicit affect, we added INA and IPA in step 3. Even though ENA and EPA were highly correlated [$r_{(28)} = -0.83$, $p < 0.001$], VIF and tolerance were of acceptable levels in all tests and thus the assumption of multi-collinearity was not violated (Tabachnick and Fidell, 2007). The final models are displayed in **Table 7**.

SBP was not significantly associated with ENA and EPA. However, INA and IPA were marginal significantly associated and explained an additional 16.1% of the variance [$F_{(5,\ 19)} = 2.60$, $p = 0.059$, $\Delta F = 2.58$, $p = 0.104$]. The final model explained 40.7% of the variance, with condition [$t_{(24)} = 2.10$, $p = 0.049$] and INA [$t_{(24)} = 2.19$, $p = 0.041$] as significant univariate predictors. These results indicate that condition and a high level of INA were associated with an increased SBP. Regarding DBP, ENA and EPA, nor INA and IPA were significantly associated with the outcome measure. However, in the final model IPA was a marginal significant univariate predictor [$t_{(25)} = 1.76$, $p = 0.093$;

TABLE 6 | Cardiovascular activity during manipulation in Study 2.

Measure[b]	Total Sample[a]			Condition				
				Harassment		No Harassment		
	M	*SE*	*t*	*M*	*SE*	*M*	*SE*	*t*
SBP	144.1	2.92	−8.75***	153.4	4.53	137.2	3.14	−3.07**
DBP	78.7	1.71	−11.6***	78.9	2.67	77.7	2.31	−2.27^{+}
HR	85.2	1.89	−5.75***	82.8	3.36	86.8	2.51	−1.63
RMSSD	5.84	0.24	1.14	6.09	0.38	5.63	0.30	0.31
TPR	9.26	0.344	3.48**	8.67	0.497	9.74	0.478	2.33^{+}

All tests were performed one sided and corrected for multiple comparisons using the Benjamini-Hochberg procedure with the false discovery rate set at 10%. A square root transformation was applied to RMSSD. SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HR, Heart Rate; RMSSD, Root Mean Square of Successive Differences; TPR, Total Peripheral Resistance.

[a] *Compared with baseline.*

[b] *Stressor with harassment has two missing values for SBP and RMSSD and one for DBP and HR. Stressor without harassment has one missing value RMSSD and TPR.*

$^{+}p < 0.10$, $^{**}p < 0.01$, $^{***}p < 0.001$.

i.e., higher IPA, higher DBP]. The total variance explained was 33.2%. HR reactivity was not associated with ENA and EPA, nor INA and IPA. Total variance explained, by condition, was 25.2%. For RMSSD, ENA and EPA were not significantly associated. However, although INA and IPA did not significantly affect the model [$F_{(5,\ 19)} = 1.79$, $p = 0.16$, $\Delta R^2 = 0.30$, $\Delta F = 4.17$, $p = 0.032$] INA was a significant univariate predictor in the model $t_{(24)} = -2.67$, $p = 0.015$. The model explained 32.0% of the total variance and indicates thata higher INA was associated with a decrease in RMSSD during the stressor. Finally, reactivity of TPR was significantly associated with ENA and EPA at step 2 and explained 17.8% of the variance compared with step 1, $F_{(3,20)} = 4.86$, $p = 0.011$, $\Delta R^2 = 0.18$, $\Delta F = 3.08$, $p = 0.07$. In the final model INA and IPA showed a significant association [$F_{(5,\ 18)} = 5.27$, $p = 0.004$, $\Delta R^2 = 0.172$, $\Delta F = 3.82$, $p = 0.041$] and explained 58.4% of the total variance. INA was the only significant univariate predictor in the model, [$t_{(23)} = -2.63$, $p = 0.017$]. Again, a higher INA was related to a decrease in TPR during the stressor.

Cardiovascular Recovery and Affect

Multilevel modeling was applied to SBP and DBP. First, a growth model was fitted to the data to model the change over time, Model 1 (Lehman et al., 2015). Second, two separate models for the implicit (Model 2) and explicit (Model 3) scales were fitted that included the affect scales and their interaction with Time and Time^2, to examine the relation of the affect measures independently. Finally, a model was fitted that included both subscales (Model 4), to examine the hypothesis that implicit affect can explain CV activity over and beyond explicit affect. The models were evaluated with and without condition as a predictor, but adding condition did not improve the models. Models without condition are reported.

To model SBP recovery, a heterogeneous autoregressive covariance structure was applied to the error variance, as is appropriate for fitting growth models (see for example Singer and Willett, 2003). The slope of Time was allowed to vary randomly between participants. Results are displayed in **Table 8A**. There were significant associations of Time as well as Time^2, indicating that the recovery slope was composed of a linear decrease as well as a quadratic change (Model 1). The latter represented a trend with the fastest decrease at the beginning and a (small) increase in SBP toward the end of the recovery phase. Adding INA and IPA and their interactions with Time and Time^2 (Model 2) improved the model with a $\Delta AIC = 70.8$ and $\Delta BIC = 48.1$. IPA in interaction with Time and Time^2 showed marginal significance with $B = -1.13$, $t_{(58.2)} = -1.94$, $p = 0.057$ and $B = 0.06$, $t_{(43.6)} = 1.90$, $p = 0.098$, respectively, indicating that higher IPA was related to a stronger linear decrease of SBP and a stronger quadratic response. Thus, higher IPA was associated with a faster recovery of SBP, especially in the beginning of the recovery phase as displayed in **Figure 2**. By adding ENA and EPA (without implicit affect) and interactions with Time and Time^2 (Model 3), the fit also improved with $\Delta AIC = 68.1$ and $\Delta BIC = 45.5$. However, no individual predictors were found. Additionally, the *AIC* and *BIC* were higher than Model 2, with −2.72 and −2.56, respectively, indicating a better fit of Model 3. When both implicit and explicit affect and interactions with Time and Time^2 were added to the model (Model 4), it was a better fit to the data compared with Model 1 ($\Delta AIC = 141.1$ and $\Delta BIC = 96.2$), Model 2 ($\Delta AIC = 70.3$ and $\Delta BIC = 48.1$) and Model 3 ($\Delta AIC = 73.0$ and $\Delta BIC = 50.7$). The interactions of IPA and Time [$B = -1.54$, $t_{(55.2)} = 2.30$, $p = 0.025$] and Time^2 [$B = 0.08$, $t_{(44.2)} = 2.30$, $p = 0.026$] were significantly associated with recovery of SBP in the final model. INA, ENA and EPA were not associated with SBP.

To model DBP recovery, an autoregressive covariance structure was applied to the error variance, as is appropriate for fitting growth models (see for example Singer and Willett, 2003). The slope of Time was allowed to vary randomly between participants. Results are displayed in **Table 8B**. There was a significant association of Time and Time^2, indicating that the recovery slope was composed of a linear increase as well as a quadratic change representing an increase at the beginning and an decrease in DBP toward the end of the recovery phase (Model 1). Adding INA and IPA and interactions with Time and Time^2 (Model 2) improved the model with a $\Delta AIC = 80.0$ and ΔBIC = 56.5. Here, INA showed a positive significant

TABLE 7 | Summary of hierarchical multiple regressions for the CV change scores during the stressors in Study 2.

	SBP (mmHg)[a]			DBP (mmHg)[b]			HR (bpm)[b]			RMSSD (ms)[a,c]			TPR (mmHg.min/L)[d]		
	B	*SE*	β	*B*	*SE*	β	*B*	*SE*	β	*B*	*SE*	β	*B*	*SE*	β
Constant	−3.57	21.6		−3.57	9.78		−2.98	19.4		4.38*	1.82		3.33	1.98	
Condition	−10.0*	4.76	−0.49	−3.11	2.28	−0.34	−4.65	4.51	−0.27	0.13	0.48	0.07	−0.61	0.49	−0.24
Explicit NA	−0.09	0.10	−0.30	−0.08	0.05	−0.56	0.07	0.09	0.26	0.005	0.01	0.20	−0.004	0.01	−0.11
Explicit PA	−0.07	0.10	−0.22	−0.05	0.05	−0.49	0.03	0.09	0.16	0.009	0.01	0.39	0.01	0.01	0.38
Implicit NA	8.54*	3.89	0.40	2.10	1.86	0.21	6.33	3.68	0.34	−1.02*	0.38	−0.52	−1.05*	0.40	−0.41
Implicit PA	2.30	3.73	0.14	2.83+	1.61	0.38	1.40	3.18	0.10	−0.42	0.35	−0.27	−0.35	0.38	−0.17
F		2.60+			1.98			1.35			1.79			5.27	
R^2		0.41			0.33			0.25			0.32			0.34	
ΔR^2		0.16			0.15			0.12			0.30*			0.20+	

The table shows the associations between condition, affect and CV change scores as generated by the final model (step 3); condition was added at step 1, Explicit NA and PA at step 2 and Implicit NA and PA at step 3 to indicate the additional value of the implicit measure. The F statistic refers to that of the final model. ΔR^2 is the difference in explained variance between step 2 and step 3 the additional variance of the change scores explained by INA and IPA. NA, negative affect; PA, positive affect; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HR, Heart Rate; RMSSD, Root Mean Square of Successive Differences; TPR, Total Peripheral Resistance.

[a] *N = 25.*

[b] *N = 26.*

[c] *A square root transformation was applied.*

[d] *N = 24.*

*+p < 0.10, *p < 0.05.*

interaction with Time [$B = 0.50$, $t_{(89.0)} = 2.06$, $p = 0.043$] and a negative significant interaction with Time^2 [$B = -0.04$, $t_{(67.4)} = -2.26$, $p = 0.027$]. These associations indicate that higher INA was related to a smaller decrease in DBP with in fact a slight increase at first. Additionally, the IPA by Time interaction was significant with $B = -0.45$, $t_{(89.5)} = -2.46$, $p = 0.016$, indicating that higher IPA was related to a faster linear recovery of DBP over time. Adding EPA and ENA to the model did not substantially improve the model (Model 3). When both implicit and explicit affect and interactions with Time and Time^2 were added to the model (Model 4), the fit did not improve and the associations between implicit affect and DBP recovery remained. The results are illustrated in **Figure 3**. Separate models of both SBP and DBP were also run with gender, BMI and smoking as covariates. Adding these covariates to the models did not change the associations of implicit and explicit affect with SBP and DBP recovery.

As mentioned before recovery of the other outcome measures took place within 1 min after the stressors had ended and could therefore not be modeled over time using multilevel analysis. Alternatively, to test the association with the affect measures partial correlations were performed on the first minute after recovery of the means of HR, RMSSD and TPR, correcting for the preceding reactivity. HR, RMSSD and TPR were not significantly related to implicit or explicit affect. Results are displayed in **Table 9**.

Discussion

Study 2 examined whether affect measured at an implicit level, as measured with the IPANAT, was associated with CV reactivity to and CV recovery after a stressor with or without anger harassment. During both stressors participants showed increased SBP, DBP and HR, and lower TPR compared with baseline. When comparing the two conditions, these associations were more pronounced for SBP, DBP and TPR after the stressor with harassment compared with the stressor without harassment. HR and RMSSD responses were similar for both conditions. Taken together this suggests a more pronounced cardiac controlled vascular response during harassment in addition to a math stressor.

There were no differences between the conditions in implicit affect. In contrast, those in the stressor with harassment condition experienced more ENA and less EPA as expected. This indicates that the more negative affective component of the harassment stressor was only reflected in explicit affect and not in implicit affect. However, higher INA was related to higher SBP reactivity and lower RMSSD and TPR reactivity during the stressors independent of stressor type. No associations between implicit affect and DBP and HR levels were observed during the stressors. Unexpectedly, the pattern of recovery was similar for both conditions. Overall, BP recovered rather slowly after an initial somewhat faster decrease. Importantly, the slow recovery of BP over the course of the recovery was (partly) statistically explained by implicit affect, but not by explicit affect. More precisely, slow recovery of SBP was related to low IPA, but not to INA. Slow recovery of DBP was partly related to both high INA and low IPA. HR, RMSSD, and TPR seem to have recovered

TABLE 8A | Summary of multilevel analysis for recovery of SBP (mmHg).

Predictor	Model 1			Model 2			Model 3			Model 4		
	B	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	137.1	1.59	86.2***	137.0	1.60	85.6***	137.0	1.67	82.0***	136.8	1.70	80.4***
Time	−1.23	0.35	−3.54***	−1.34	0.34	−3.90***	−1.20	0.36	−3.37**	−1.35	0.35	−3.84***
Time^2	0.07	0.02	3.42**	0.07	0.02	3.81***	0.06	0.02	3.23**	0.07	0.02	3.70***
SBP task	0.74	0.08	9.26***	0.75	0.09	8.61***	0.69	0.08	8.39***	0.73	0.09	7.76***
Implicit NA				−2.55	3.42	−0.75				−2.76	3.56	−0.78
Implicit PA				0.26	2.66	0.10				1.32	3.24	0.41
Time*Implicit NA				0.22	0.73	0.30				0.14	0.74	0.85
Time*Implicit PA				−1.13	0.58	−1.84$^+$				−1.54	0.67	−2.30*
Time^2*Implicit NA				−0.03	0.04	−0.77				−0.03	0.04	−0.73
Time^2*Implicit PA				0.06	0.03	1.90$^+$				0.08	0.03	2.30*
Explicit NA							−0.06	0.09	−0.70	−0.05	0.09	−0.53
Explicit PA							−0.07	0.07	−0.94	−0.06	0.08	−0.76
Time*Explicit NA							0.01	0.02	0.76	0.02	0.02	0.93
Time*Explicit PA							0.005	0.02	0.29	0.02	0.02	1.28
Time^2*Explicit NA							−0.0003	0.001	−0.26	−0.0008	0.001	−0.73
Time^2*Explicit PA							−0.0003	0.0009	−0.36	−0.001	0.0009	−1.22
AIC		2347.5			2276.7			2279.4			2206.4	
BIC		2438.8			2390.7			2393.3			2342.6	
N		23			29			29			35	

Error at Level-1 was organized with a heterogeneous autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centered. SBP, systolic blood pressure; NA, Negative Affect; PA, Positive affect; N, number of parameters; AIC, Akaike information criterion; BIC, Bayesian information criterion.
*$^+p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.*

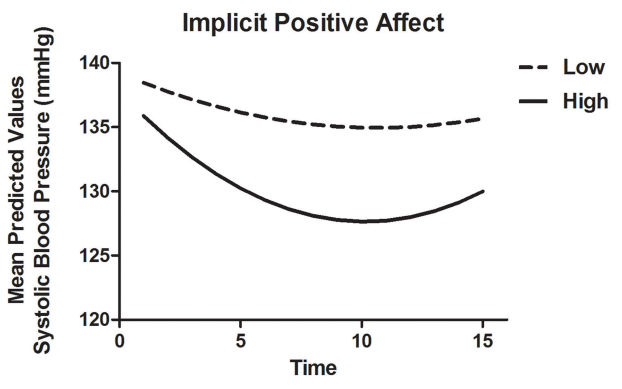


FIGURE 2 | Mean predictive values of SBP over each of the 15 min of recovery (Model 4) displayed for high and low implicit positive affect. For display purposes scores of implicit positive affect were dichotomized.

rather quickly, that is, within the first minute after the stressor. For these outcome measures no relationship with implicit affect measures was found. Remarkably, explicit affect was not related to any of the CV measures.

Taken together, the most salient result of Study 2 seems to be that not explicit, but implicit affect explained variance in reactivity and recovery, but that at the same time explicit, but not implicit affect, was influenced by the stressor types, and thus by the experimental increase in negative emotionality. One explanation of these contrasting results might be that self-reported (explicit) affect reflected mainly the experimental demand characteristic ("the experimenter made me angry so I think I am angry") while implicit affect reflected the core affective state induced by both stressors (Russell, 2003), which was not substantially influenced by the harassment, as will be discussed below.

GENERAL DISCUSSION

Traditional self-report measurements of stress, or explicit measures of affect, cannot fully explain CV activity. Hence, the relationship between affect as an indicator of psychological stress and CV health remains largely indeterminate, and the examination of a possible role for implicit measures of affect is warranted. In the present work the IPANAT, as a promising implicit measure of affect, was evaluated in two studies to examine its ability to assess changes in affective state and explain stress-related CV activity beyond explicit measures of affect. In Study 1 the IPANAT appeared to be able to measure affect-congruent changes in INA and IPA after anger and happiness inducing film clips. Of the multiple expected congruent effects only an effect on IPA, but not INA, after a fear inducing clip was found. Importantly, implicit affect changed independently from explicit affect. Thus, the IPANAT is able to measure changes in affect that are generally congruent with the valence of the presented stimuli and independent of explicit affect. We conclude that the differential responses of the IPANAT in response to the

TABLE 8B | Summary of multilevel analysis for recovery of DBP (mmHg).

Predictor	Model 1			Model 2			Model 3			Model 4		
	B	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*	*B*	*SE*	*t*
Constant	71.5	0.62	115.8***	71.3	0.56	126.0***	71.6	0.61	116.5***	71.3	0.58	122.8***
Time	0.25	0.12	2.46*	0.22	0.11	1.99*	0.29	0.11	2.55*	0.26	0.11	2.38*
Time^2	−0.02	0.008	−2.46**	−0.02	0.007	−2.30*	−0.02	0.007	−2.70**	−0.02	0.007	−2.60*
DBP Task	0.85	0.07	13.06***	0.94	0.07	14.1***	0.81	0.07	12.3***	0.91	0.07	12.5***
Implicit NA				0.69	1.24	−0.56				−0.76	1.27	−0.59
Implicit PA				−0.81	0.94	−0.86				−0.30	1.09	−0.28
Time*Implicit NA				0.50	0.24	2.06*				0.59	0.24	2.45*
Time*Implicit PA				−0.45	0.18	−2.46*				−0.41	0.19	−2.15*
Time^2*Implicit NA				−0.04	0.02	−2.26*				−0.04	0.02	−2.66**
Time^2*Implicit PA				0.01	0.01	1.21				0.02	0.01	1.22
Explicit NA							−0.04	0.03	−1.18	−0.02	0.03	−1.09
Explicit PA							−0.04	0.03	−0.04	−0.02	0.03	−0.85
Time*Explicit NA							−0.006	0.006	−0.93	−0.007	0.006	−1.09
Time*Explicit PA							−0.005	0.005	−1.12	−0.004	0.005	−0.75
Time^2*Explicit NA							0.0003	0.0004	0.73	0.0004	0.0004	1.11
Time^2*Explicit PA							0.0003	0.0003	0.41	0.001	0.0003	0.40
AIC		1732.7			1652.7			1669.6			1593.5	
BIC		1768.7			1712.3			1729.1			1676.0	
N		9			15			15			21	

Error at Level-1 was organized with an autoregressive first-order covariance structure. At Level-2 the covariance was unstructured. Predictors were grand mean centered. DBP, diastolic blood pressure; NA, Negative Affect; PA, Positive affect; N, number of parameters; AIC, Akaike information criterion; BIC, Bayesian information criterion.
$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

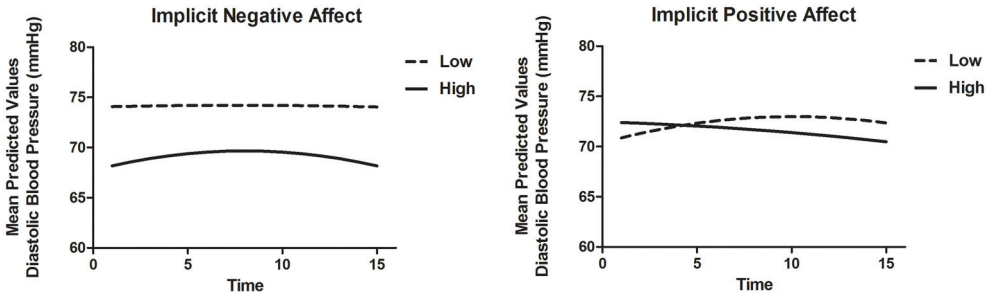
film clips form an important extension of the modest number of available validation studies of the IPANAT and add ecological validity to previously used methods (e.g., pictorial stimuli).

Study 2 employed a realistic stressor with and without an enhanced negative affective component and continuously measured CV activity. The affective component was reflected in differences in explicit affect, but not in implicit affect. Nevertheless, only the implicit affect measures, and not the explicit ones, were associated with the CV responses to both stressors and their recovery afterwards. Specifically, SBP increases and HRV and TPR decreases during the stressors were related to higher INA, but implicit affect did not clearly relate to DBP and HR reactivity. Slower recovery of SBP was associated with lower levels of IPA, and DBP recovery was associated with both IPA and INA in the expected direction. HR, HRV and TPR showed a very quick recovery that was not related to implicit or explicit affect. Thus, the IPANAT adds to the understanding of the CV response to stressors were explicit measure do not. These results and some unexpected findings, such as the prolonged physiological effects of the stressors on BP but not HR, HRV or TPR, are discussed in more detail below.

Stressors and CV Activity

We did not find a direct effect of the manipulation of the stressors on recovery, but the differences in recovery can be attributed to the differences in reactivity. The stressors yielded higher SBP and DBP and lower TPR, and for all CV measures the magnitude of reactivity contributed to speed of recovery. This suggests a role for the reactivity, not the stressor itself, in the effect of a stressor on the speed of CV recovery. Consequently, the notion of Brosschot et al. (2014) and Linden et al. (1997) that an emotional stressor would delay CV recovery compared with non-emotional stressor holds to the extent that it increases reactivity that, independent from condition, slows down recovery.

In general, the pattern of CV activity in Study 2, a vascular (i.e., BP) and myocardial (i.e., HR) increase during the stressor and a prolonged recovery that appeared to be mostly vascular under cardiac control, is comparable to other studies (e.g., Haynes et al., 1991; Gregg et al., 1999; Glynn et al., 2002; Mauss et al., 2007; Juster et al., 2012; Brindle et al., 2014). The quick recovery of HR is in line with the observation that an increase in HR can be seen as primarily reflecting task engagement or effort (e.g., Berntson et al., 1996), and less related to possible emotional aspects of the task that might linger on after its completion. Furthermore, the speech activity required in the current stress task (i.e., calculating loudly) might also have played a role. Sloan et al. (1991) found a smaller increase in HR during a mathematical task when vocalization of the response was not required. More specifically, changes in respiratory frequency due to speaking were found to increase HR. The neccesity to speak ended right after the task resulting in a quick decrease of HR. Sloan et al. (1991) also attributed the absence of changes in HRV to the effect of speaking on HRV. Thus, the findings regarding HR and HRV might not or to a lesser extent be related to the psychological component of the stressors but rather to the design characteristcs of the study.



FIGURES 3 | Mean predictive values of DBP over each of the 15 min of recovery (Model 4) displayed for high and low implicit negative affect and high and low implicit positive affect. For display purposes scores of implicit affect were dichotomized.

TABLE 9 | Pearson product-moment partial correlations between measures of affect and first minute of recovery of Study 2.

Affect	HR[a]	RMSSD[b]	TPR[b]
Implicit NA	−0.24	0.30	0.17
Implicit PA	−0.20	−0.14	0.25
Explicit NA	−0.18	−0.001	0.18
Explicit PA	0.16	−0.05	−23

Controlled for HR, RMSSD and TPR during the stressor. A square root transformation was applied to RMSSD. There were no significant correlations. NA, Negative affect; PA, Positive affect; HR, Heart Rate; RMSSD, Root Mean Square of Successive Differences; TPR, Total Peripheral Resistance.

[a] *N = 23.*

[b] *N = 22.*

In contrast to what is commonly found in threatening situations, namely an increase in TPR, we here found a decrease in TPR (Blascovich, 2008; Seery, 2011). It is possible that the stressors, a mathematical task with or without harassment, did not induce a threatened but a challenged state. Regarding our findings with TPR the stressors might not have been as straining as we had anticipated, for example because of lack of personal relevance of the stressors to the participants (Blascovich, 2008). The findings also suggests that the prolonged effects on SBP and DBP cannot be explained by TPR, that recovered within a minute after the stressor, but are due to other factors that we have not measured directly, such as stroke volume or cardiac output. Overall, the results support previous notions that researchers should include recovery in the laboratory models of stress, as the activity seen during reactivity does not necessarily reflect clinically relevant responses (Linden et al., 1997).

IPANAT and CV Activity

The findings of Study 1 add to the understanding of affect, measured at an implicit and explicit level, by addressing the ongoing nature of affect through presentation of film clips. Furthermore, the absence of changes in INA after the fear inducing clip is similar to the study of Quirin et al. (2011) in which they showed a threat-related film clip and measured INA and IPA but found no changes in implicit affect after a threat inducing film clip. This suggests that the INA subscale might not be sensitive or specific enough to detect fear. The construct validity, both convergent and discriminant, seem supported by Study 1: the scores on the IPANAT scales are reasonably congruent with the emotional content of the different emotional film clips. This was only partially the case for Study 2 where only convergent validity seems apparent from the expected correlations with physiological measurements stress responses. In line with previous research, we observed no association between INA and IPA, which explains why the results we found with INA did not always mirror those with IPA (Quirin et al., 2009a,b).

The stressors in Study 2 led to group specific changes in explicit but not implicit affect. This is even more surprising considering the independent relation we found between implicit affect and CV outcome measures. The increased ENA and decreased EPA can be explained by demand characteristics of the stressors. In the condition with harassment the affective component was quite obvious to the participants. They were told they were not doing a good job. In the stressor group without harassment there was no feedback which created an ambiguous setting. These differences might very well be what was measured with the explicit measures of affect; the ambiguous situation was not experienced as overtly negative. An alternative explanation is that in Study 2 that the IPANAT scores were in fact related to the trait component, and not the state component, of affect (Quirin et al., 2009a). As no baseline measure of the IPANAT was taken, the current study does not exclude this possibility; perhaps it is the trait part of affect captured by the IPANAT that is related to CV activity. However, it is likely that self-reported affect reflected what the participants thought they had to report and not necessarily how they were feeling, i.e., their core affect (Russell, 2003). Moreover, core affect might be best reflected on the IPANAT subscales; both stressors elicited discomfort which was overridden by demand characteristics of the experiment on the explicit level of affect but was displayed in both conditions on the implicit level. This explanation is further amplified by the finding that only implicitly measured affect contributed to CV activity during and after the stressors. If this interpretation is correct, implicit affect scores reflected core affect that was manifested in CV changes. This highlights the additional value of implicit measures, or the IPANAT in particular, in addressing the relation between stress and CV

diseases (Egloff and Schmukle, 2002; Egloff et al., 2002; Verkuil et al., 2014).

The role of positive affect in the development of disease has not been explicitly addressed in the unconscious perseverative cognition hypothesis, which emphasizes the health consequences of stress-related cognition beyond awareness (e.g., Brosschot et al., 2010). However, in the current study we found that a higher IPA is related to higher DBP reactivity and lower IPA is related to slow recovery of both SBP and DBP. This is consistent with the results of Quirin et al. (2009b, Study 1) who found that increased IPA, not INA, measured during 2 days, was related to a lower cortisol awakening response and total diurnal cortisol the following day in addition to EPA. The finding that IPA is related to CV activity and cortisol excretion provides new insights in the relation between the IPANAT and two biological mechanisms.

Overall, the prolonged BP responses were best explained by implicit affect more than any other variable measured. Together these results suggest that stress-related cognition beyond self-report is related to physiological effects of stress, but, importantly, reduced levels of IPA play an equally detrimental role.

Limitations

The results should be interpreted while considering some limitations. In Study 2 the sample sizes, particularly regarding the two conditions, were rather small which increases the risk for Type 2 error, i.e., the study may have been underpowered to reveal statistical significant findings. In this light we have interpreted marginal statistically significant findings in both studies as potentially relevant, which was supported by the effect sizes. Furthermore, in Study 2 there was no neutral condition, merely a mathematical task with and without anger harassment. No differences between conditions were found for affect measured at an implicit level and CV recovery. Adding a true neutral condition without a stressor might provide additional information about the ability of the IPANAT to detect INA induced by a psychological stressor and enabling inferences about the role of affect, measured implicitly and explicitly, in physiological recovery. Alternatively other methods of stress induction could be considered, such as a public speech stressor or the Trier Social Stress Test, which combines a public speech with the anger harassment used in Study 2 (e.g., Kirschbaum et al., 1993). Also, we cannot exclude the possibility that participants differed, despite randomization, in natural math-related abilities, which could have been a confounder. Finally, Study 1 and 2 did not use the same explicit measures and can therefore not be readily compared; it cannot be excluded, for example, that we would have found associations of explicit affect with CV activity in Study 2 if we had used the PANAS used in Study 1. To further clarify the relation between implicit stress-related cognition and CV health, future studies should not be limited to implicit measures of affect after experimentally induced stress, but should also apply the measures to daily life (Mossink et al., 2015) and/or in individuals with chronic stress. Finally, the current experiments focused on the assessment of implicit affect with the IPANAT. However, other measures of implicit constructs to assess other aspects of unconscious stress-related cognition, e.g., action tendencies or emotion recognition, could also provide more information to clarify the relation between psychological stress and CV health.

CONCLUSION

The IPANAT is the first specific measure of implicit affect. The current two studies suggest that it is able to measure differences not only between affective responses to pictorial stimuli, as reported previously, but also between fear (with its positive subscale), anger and happiness as elicited using film clips (Study 1). The findings suggest that the IPANAT is associated with CV activity during and after a stressor (Study 2). Importantly, all findings for the IPANAT were independent of those for explicit affect, which were mostly absent.

Notwithstanding the remaining questions and limitations, these findings offer support for the theory that stress beyond self-report measures, i.e., unconscious stress-related cognition, at least partly relates to CV responses, that, when prolonged in daily life, are related to the progress and development of CV diseases. Especially because of this relevance for health, further research is needed to clarify the explanatory value of the IPANAT and possible other implicit measures of stress-related cognition, and their applicability to stress research.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by a grant from ZON-MW (Netherlands Organization for Health Research and Development; TOP Grant) to JB, nr. 40-00812-98-11029 and a grant from the Netherlands Organization for Scientific Research (NWO) awarded to BV (Veni Grant 451-14-013).

REFERENCES

Bargh, J. A., and Morsella, E. (2008). The unconscious mind. *Perspect. Psychol. Sci.* 3, 73–79. doi: 10.1111/j.1745-6916.2008.00064.x

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57, 289–300.

Berntson, G. G., Cacioppo, J. T., and Fieldstone, A. (1996). Illusions, arithmetic, and the bidirectional modulation of vagal control of the heart. *Biol. Psychol.* 44, 1–17. doi: 10.1016/S0301-0511(96)05197-6

Berntson, G. G., Quigley, K. S., and Lozano, D. (2007). "Cardiovascular psychophysiology," in *Handbook of Psychophysiology, 3rd Edn.,* eds J. T. Cacioppo, L. G. Tassinary, and G. G. Berntson (New York, NY: Cambridge University Press), 182–210.

Blascovich, J. (2008). "Challenge and threat," in *Handbook of Approach and Avoidance Motivation*, ed A. J. Elliot (New York, NY: Psychology Press), 431–445.

Brindle, R. C., Ginty, A. T., Phillips, A. C., and Carroll, D. (2014). A tale of two mechanisms: a meta-analytic approach toward understanding the autonomic basis of cardiovascular reactivity to acute psychological stress. *Psychophysiology* 51, 964–976. doi: 10.1111/psyp.12248

Brosschot, J. F. (2010). Markers of chronic stress: prolonged physiological activation and (un)conscious perseverative cognition. *Neurosci. Biobehav. Rev.* 35, 46–50. doi: 10.1016/j.neubiorev.2010.01.004

Brosschot, J. F., Gerin, W., and Thayer, J. F. (2006). The perseverative cognition hypothesis: a review of worry, prolonged stress-related physiological activation, and health. *J. Psychosom. Res.* 60, 113–124. doi: 10.1016/j.jpsychores.2005.06.074

Brosschot, J. F., Geurts, S. A. E., Kruizinga, I., Radstaak, M., Verkuil, B., Quirin, M., et al. (2014). Does unconscious stress play a role in prolonged cardiovascular stress recovery? *Stress Health* 30, 179–187. doi: 10.1002/smi.2590

Brosschot, J. F., Van Dijk, E., and Thayer, J. F. (2007). Daily worry is related to low heart rate variability during waking and the subsequent nocturnal sleep period. *Int. J. Psychophysiol.* 63, 39–47. doi: 10.1016/j.ijpsycho.2006.07.016

Brosschot, J. F., Verkuil, B., and Thayer, J. F. (2010). Conscious and unconscious perseverative cognition: is a large part of prolonged physiological activity due to unconscious stress? *J. Psychosom. Res.* 69, 407–416. doi: 10.1016/j.jpsychores.2010.02.002

Brotman, D. J., Golden, S. H., and Wittstein, I. S. (2007). The cardiovascular toll of stress. *Lancet* 370, 1089–1100. doi: 10.1016/S0140-6736(07)61305-1

Chida, Y., and Hamer, M. (2008). Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. *Psychol. Bull.* 134, 829–885. doi: 10.1037/a0013342

Crawford, J. R., and Henry, J. D. (2004). The Positive and Negative Affect Schedule (PANAS): construct validity, measurement properties and normative data in a large non-clinical sample. *Br. J. Clin. Psychol.* 43, 245–265. doi: 10.1348/0144665031752934

De Houwer, J., Teige-Mocigemba, S., Spruyt, A., and Moors, A. (2009). Implicit measures: a normative analysis and review. *Psychol. Bull.* 135, 347–368. doi: 10.1037/a0014211

Dijksterhuis, A., Bos, M. W., Nordgren, L. F., and Van Baaren, R. B. (2006). On making the right choice: the deliberation-without-attention effect. *Science* 311, 1005–1007. doi: 10.1126/science.1121629

Dimsdale, J. E. (2008). Psychological stress and cardiovascular disease. *J. Am. Coll. Cardiol.* 51, 1237–1246. doi: 10.1016/j.jacc.2007.12.024

Egloff, B., and Schmukle, S. C. (2002). Predictive validity of an implicit association test for assessing anxiety. *J. Pers. Soc. Psychol.* 83, 1441–1455. doi: 10.1037/0022-3514.83.6.1441

Egloff, B., Wilhelm, F. H., Neubauer, D. H., Mauss, I. B., and Gross, J. J. (2002). Implicit anxiety measure predicts cardiovascular reactivity to an evaluated speaking task. *Emotion* 2, 3–11. doi: 10.1037/1528-3542.2.1.3

Fazio, R. H., and Olson, M. A. (2003). Implicit measures in social cognition research: their meaning and use. *Annu. Rev. Psychol.* 54, 297–327. doi: 10.1146/annurev.psych.54.101601.145225

Forgas, J. P. (1995). Mood and judgment: the affect infusion model (AIM). *Psychol. Bull.* 117, 39. doi: 10.1037/0033-2909.117.1.39

Gerin, W. (2011). "Acute stress responses in the psychophysiological laboratory," in *The Handbook of Stress Science: Biology, Psychology, and Health*, eds R. J. Contrada and A. Baum (New York, NY: Springer Publishing Company, LLC), 501–513.

Gerin, W., Davidson, K. W., Christenfeld, N. J., Goyal, T., and Schwartz, J. E. (2006). The role of angry rumination and distraction in blood pressure recovery from emotional arousal. *Psychosom. Med.* 68, 64–72. doi: 10.1097/01.psy.0000195747.12404.aa

Glynn, L. M., Christenfeld, N., and Gerin, W. (2002). The role of rumination in recovery from reactivity: cardiovascular consequences of emotional states. *Psychosom. Med.* 64, 714–726. doi: 10.1097/01.PSY.0000031574.42041.23

Gregg, M. E., James, J. E., Matyas, T. A., and Thornsteinsson, E. B. (1999). Hemodynamic profile of stress-induced anticipation and recovery. *Int. J. Psychophysiol.* 34, 147–162. doi: 10.1016/S0167-8760(99)00074-4

Gross, J. J., and Levenson, R. W. (1995). Emotion elicitation using films. *Cogn. Emot.* 9, 87–108. doi: 10.1080/02699939508408966

Gyurak, A., Gross, J. J., and Etkin, A. (2011). Explicit and implicit emotion regulation: a dual-process framework. *Cogn. Emot.* 25, 400–412. doi: 10.1080/02699931.2010.544160

Haynes, S. N., Gannon, L. R., Orimoto, L., O'Brien, W. H., and Brandt, M. (1991). Psychophysiological assessment of poststress recovery. *Psychol. Assess.* 3, 356–365. doi: 10.1037/1040-3590.3.3.356

Hill, L., Sollers, I. J., and Thayer, J. (2011). Evaluation of a simple estimation method for the derivation of cardiac output from arterial blood pressure and heart rate. *Biomed. Sci. Instrum.* 48, 165–170.

Hill, L., Sollers, I. J., and Thayer, J. (2012). Resistance reconstructed estimation of total peripheral resistance from computationally derived cardiac output-biomed 2013. *Biomed. Sci. Instrum.* 49, 216–223.

Juster, R. P., Perna, A., Marin, M. F., Sindi, S., and Lupien, S. J. (2012). Timing is everything: anticipatory stress dynamics among cortisol and blood pressure reactivity and recovery in healthy adults. *Stress* 15, 569–577. doi: 10.3109/10253890.2012.661494

Key, B. L., Campbell, T. S., Bacon, S. L., and Gerin, W. (2008). The influence of trait and state rumination on cardiovascular recovery from a negative emotional stressor. *J. Behav. Med.* 31, 237–248. doi: 10.1007/s10865-008-9152-9

Kihlstrom, J. F. (1987). The cognitive unconscious. *Science* 237, 1445–1452. doi: 10.1126/science.3629249

Kirschbaum, C., Pirke, K. M., and Hellhammer, D. H. (1993). The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28, 76–81. doi: 10.1159/000119004

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flugge, G., Korte, S. M., et al. (2011). Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.* 35, 1291–1301. doi: 10.1016/j.neubiorev.2011.02.003

Lehman, B. J., Kirsch, J. A., and Jones, D. R. (2015). Effectively analyzing change over time in laboratory research on stress and health: a multilevel modeling approach. *Soc. Personal. Psychol. Compass* 9, 551–566. doi: 10.1111/spc3.12202

Linden, W., Earle, T. L., Gerin, W., and Christenfeld, N. (1997). Physiological stress reactivity and recovery: conceptual siblings separated at birth? *J. Psychosom. Res.* 42, 117–135. doi: 10.1016/S0022-3999(96)00240-1

Llabre, M. M., Spitzer, S. B., Saab, P. G., Ironson, G. H., and Schneiderman, N. (1991). The reliability and specificity of delta versus residualized change as measures of cardiovascular reactivity to behavioral challenges. *Psychophysiology* 28, 701–711. doi: 10.1111/j.1469-8986.1991.tb01017.x

Llabre, M. M., Spitzer, S. B., Saab, P. G., and Schneiderman, N. (2001). Piecewise latent growth curve modeling of systolic blood pressure reactivity and recovery from the cold pressor test. *Psychophysiology* 38, 951–960. doi: 10.1111/1469-8986.3860951

Lu, X. T., Zhao, Y. X., Zhang, Y., and Jiang, F. (2013). Psychological stress, vascular inflammation, and atherogenesis: potential roles of circulating cytokines. *J. Cardiovasc. Pharmacol.* 62, 6–12. doi: 10.1097/FJC.0b013e3182858fac

Ludbrook, J. (2013). Should we use one-sided or two-sided P values in tests of significance? *Clin. Exp. Pharmacol. Physiol.* 40, 357–361. doi: 10.1111/1440-1681.12086

Mauss, I. B., Cook, C. L., and Gross, J. J. (2007). Automatic emotion regulation during anger provocation. *J. Exp. Soc. Psychol.* 43, 698–711. doi: 10.1016/j.jesp.2006.07.003

McDonald, J. H. (2014). "Multiple comparisons [spreadsheet]," in *Handbook of Biological Statistics, 3rd Edn.* Retrieved from: http://www.biostathandbook.com/multiplecomparisons.html

McEwen, B. S. (1998). Stress, adaptation, and disease. *Allostasis and allostatic load. Ann. N.Y. Acad. Sci.* 840, 33–44. doi: 10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (2003). Mood disorders and allostatic load. *Biol. Psychiatry* 54, 200–207. doi: 10.1016/S0006-3223(03)00177-X

Mossink, J. C. L., Verkuil, B., Burger, A. M., Tollenaar, M. S., and Brosschot, J. F. (2015). Ambulatory assessed implicit affect is associated with salivary cortisol. *Front. Psychol.* 6:111. doi: 10.3389/fpsyg.2015.00111

Munoz, M. L., van Roon, A., Riese, H., Thio, C., Oostenbroek, E., Westrik, I., et al. (2015). Validity of (Ultra-) short recordings for heart rate variability measurements. *PLoS ONE* 10:e0138921. doi: 10.1371/journal.pone.0138921

Nussinovitch, U., Elishkevitz, K. P., Katz, K., Nussinovitch, M., Segev, S., Volovitz, B., et al. (2011). Reliability of ultra-short ECG indices for heart rate variability. *Ann. Noninvasive Electrocardiol.* 16, 117–122. doi: 10.1111/j.1542-474X.2011.00417.x

Ottaviani, C., Thayer, J. F., Verkuil, B., Lonigro, A., Medea, B., Couyoumdjian, A., et al. (2016). Physiological concomitants of perseverative cognition: a systematic review and meta-analysis. *Psychol. Bull.* 142, 231–259. doi: 10.1037/bul0000036

Panaite, V., Salomon, K., Jin, A., and Rottenberg, J. (2015). Cardiovascular recovery from psychological and physiological challenge and risk for adverse cardiovascular outcomes and all-cause mortality. *Psychosom. Med.* 77, 215–226. doi: 10.1097/PSY.0000000000000171

Payne, B. K., Cheng, C. M., Govorun, O., and Stewart, B. D. (2005). An inkblot for attitudes: affect misattribution as implicit measurement. *J. Pers. Soc. Psychol.* 89, 277–293. doi: 10.1037/0022-3514.89.3.277

Pieper, S., and Brosschot, J. F. (2005). Prolonged stress-related cardiovascular activation: is there any? *Ann. Behav. Med.* 30, 91–103. doi: 10.1207/s15324796abm3002_1

Pieper, S., Brosschot, J. F., Van der Leeden, R., and Thayer, J. F. (2007). Cardiac effects of momentary assessed worry episodes and stressful events. *Psychosom. Med.* 69, 901–909. doi: 10.1097/PSY.0b013e31815a9230

Pieper, S., Brosschot, J. F., Van der Leeden, R., and Thayer, J. F. (2010). Prolonged cardiac effects of momentary assessed stressful events and worry episodes. *Psychosom. Med.* 72, 570–577. doi: 10.1097/PSY.0b013e3181dbc0e9

Quintana, D. S., and Heathers, J. A. J. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Front. Psychol.* 5:805. doi: 10.3389/fpsyg.2014.00805

Quirin, M., and Bode, R. C. (2014). An alternative to self-reports of trait and state affect. *Eur. J. Psychol. Assess.* 1, 1–7. doi: 10.1027/1015-5759/a000190

Quirin, M., Bode, R. C., and Kuhl, J. (2011). Recovering from negative events by boosting implicit positive affect. *Cogn. Emot.* 25, 559–570. doi: 10.1080/02699931.2010.536418

Quirin, M., Kazen, M., and Kuhl, J. (2009a). When nonsense sounds happy or helpless: the Implicit Positive and Negative Affect Test (IPANAT). *J. Pers. Soc. Psychol.* 97, 500–516. doi: 10.1037/a0016063

Quirin, M., Kazen, M., Rohrmann, S., and Kuhl, J. (2009b). Implicit but not explicit affectivity predicts circadian and reactive cortisol: using the implicit positive and negative affect test. *J. Pers.* 77, 401–425. doi: 10.1111/j.1467-6494.2008.00552.x

Quirin, M., and Lane, R. D. (2012). The construction of emotional experience requires the integration of implicit and explicit emotional processes. *Behav. Brain Sci.* 35, 159–160. doi: 10.1017/S0140525X11001737

Radstaak, M., Geurts, S. A. E., Brosschot, J. F., Cillessen, A. H. N., and Kompier, M. A. J. (2011). The role of affect and rumination in cardiovascular recovery from stress. *Int. J. Psychophysiol.* 81, 237–244. doi: 10.1016/j.ijpsycho.2011.06.017

Rosengren, A., Hawken, S., Ounpuu, S., Sliwa, K., Zubaid, M., Almahmeed, W. A., et al. (2004). Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet* 364, 953–962. doi: 10.1016/S0140-6736(04)17019-0

Rottenberg, J., Ray, R. D., and Gross, J. J. (2007). "Emotion elicitation using films," in *The Handbook of Emotion Elicitation and Assessment,* eds J. A. Coan and J. J. B. Allen (London: Oxford University Press), 9–28.

Rozanski, A., Blumenthal, J. A., and Kaplan, J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 99, 2192–2217. doi: 10.1161/01.CIR.99.16.2192

Russell, J. A. (2003). Core affect and the psychological construction of emotion. *Psychol. Rev.* 110, 145–172. doi: 10.1037/0033-295X.110.1.145

Schaefer, A., Nils, F., Sanchez, X., and Philippot, P. (2010). Assessing the effectiveness of a large database of emotion-eliciting films: a new tool for emotion researchers. *Cogn. Emot.* 24, 1153–1172. doi: 10.1080/02699930903274322

Seery, M. D. (2011). Challenge or threat? *Cardiovascular indexes of resilience and vulnerability to potential stress in humans. Neurosci. Biobehav. Rev.* 35, 1603–1610. doi: 10.1016/j.neubiorev.2011.03.003

Sherwood, A., Allen, M. T., Fahrenberg, J., Kelsey, R. M., Lovallo, W. R., and Vandoornen, L. J. P. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology* 27, 1–23. doi: 10.1111/j.1469-8986.1990.tb02171.x

Simes, R. J. (1986). An improved Bonferroni procedure for multiple tests of significance. *Biometrika* 73, 751–754. doi: 10.1093/biomet/73.3.751

Singer, J. D., and Willett, J. B. (2003). *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. London: Oxford University Press.

Sloan, R. P., Korten, J. B., and Myers, M. M. (1991). Components of heart rate reactivity during mental arithmetic with and without speaking. *Physiol. Behav.* 50, 1039–1045. doi: 10.1016/0031-9384(91)90434-P

Smith, A. L., Owen, H., and Reynolds, K. J. (2013). Heart rate variability indices for very short-term (30 beat) analysis. Part 2: validation. *J. Clin. Monit. Comput.* 27, 577–585. doi: 10.1007/s10877-013-9473-2

Strike, P. C., and Steptoe, A. (2004). Psychosocial factors in the development of coronary artery disease. *Prog. Cardiovasc. Dis.* 46, 337–347. doi: 10.1016/j.pcad.2003.09.001

Sun, J. X., Reisner, A. T., Saeed, M., and Mark, R. G. (2005). Estimating cardiac output from arterial blood pressure waveforms: a critical evaluation using the MIMIC II database. *Comput. Cardiol.* 32, 295–298. doi: 10.1109/CIC.2005.1588095

Tabachnick, B. G., and Fidell, L. S. (2007). *Using Multivariate Statistics, 5th Edn.* New York, NY: Allyn and Bacon.

Ursin, H., and Eriksen, H. R. (2004). The cognitive activation theory of stress. *Psychoneuroendocrinology* 29, 567–592. doi: 10.1016/S0306-4530(03)00091-X

Verkuil, B., Brosschot, J. F., Gebhardt, W. A., and Thayer, J. F. (2010). When worries make you sick: a review of perseverative cognition, the default stress response and somatic health. *J. Exp. Psychopathol.* 1, 87–118. doi: 10.5127/jep.009110

Verkuil, B., Brosschot, J. F., Meerman, E. E., and Thayer, J. F. (2012). Effects of momentary assessed stressful events and worry episodes on somatic health complaints. *Psychol. Health* 27, 141–158. doi: 10.1080/08870441003653470

Verkuil, B., Brosschot, J. F., and Thayer, J. F. (2014). Cardiac reactivity to and recovery from acute stress: temporal associations with implicit anxiety. *Int. J. Psychophysiol.* 92, 85–91. doi: 10.1016/j.ijpsycho.2014.03.002

Watson, D., Clark, L. A., and Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* 54, 1063–1070. doi: 10.1037/0022-3514.54.6.1063

Zajonc, R. B. (1980). Feeling and thinking: preferences need no inferences. *Am. Psychol.* 35, 151–175. doi: 10.1037/0003-066X.35.2.151

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
PSYCHOLOGY

ORIGINAL RESEARCH ARTICLE
published: 10 February 2015
doi: 10.3389/fpsyg.2015.00111

Ambulatory assessed implicit affect is associated with salivary cortisol

Joram C. L. Mossink[1], Bart Verkuil[1,2]*, Andreas M. Burger[1], Marieke S. Tollenaar[1] and Jos F. Brosschot[2]

[1] Clinical Psychology, Faculty of Social Sciences, Leiden University, Leiden, Netherlands
[2] Health Psychology, Faculty of Social Sciences, Leiden University, Leiden, Netherlands

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Mario F. Juruena, University of Sao Paulo, Brazil
Tamlin Conner, University of Otago, New Zealand

***Correspondence:**
Bart Verkuil, Clinical Psychology, Faculty of Social Sciences, Leiden University, Wassenaarseweg 52, 2333 AK Leiden, Netherlands
e-mail: bverkuil@fsw.leidenuniv.nl

One of the presumed pathways linking negative emotions to adverse somatic health is an overactive HPA-axis, usually indicated by elevated cortisol levels. Traditionally, research has focused on consciously reported negative emotions. Yet, given that the majority of information processing occurs without conscious awareness, stress physiology might also be influenced by affective processes that people are not aware of. In a 24-h ambulatory study we examined whether cortisol levels were associated with two implicit measures. Implicit affect was assessed using the Implicit Positive and Negative Affect Test, and implicit negative memory bias was assessed with the word fragment completion tasks. In 55 healthy participants, we measured subjective stress levels, worries, implicit, and explicit affect each hour during waking hours. Also, saliva samples were collected at three fixed times during the day, as well as upon waking and 30 min thereafter (cortisol awakening response). Multilevel analyses of the daytime cortisol levels revealed that the presence of an implicit negative memory bias was associated with increased cortisol levels. Additionally, implicit PA and, unexpectedly, implicit NA were negatively associated with cortisol levels. Finally, participants demonstrating higher levels of implicit sadness during the first measurement day, had a stronger cortisol rise upon awakening at the next day. Contrary to previous research, no associations between explicit affect and cortisol were apparent. The current study was the first to examine the concurrent relation between implicit measures and stress physiology in daily life. The results suggest that the traditional focus on consciously reported feelings and emotions is limited, and that implicit measures can add to our understanding of how stress and emotions contribute to daily physiological activity and, in the long term, health problems.

Keywords: unconscious stress, implicit affect, sadness, cortisol, daily life, IPANAT, cortisol awakening response

INTRODUCTION

In our daily lives we are constantly facing many forms of stress, including social demands, time constraints, and pressure to achieve (e.g., work, family, friends, and deadlines). Yet, stress is so ingrained into our daily lives that we may not always be consciously aware of and/or be able to accurately report that we have elevated stress levels until we feel a sense of relief when we're not stressed anymore. This inability to perceive and report stress has been termed as 'unconscious stress,' which can be defined as the cognitive representation of stressful events when attention is directed elsewhere (Brosschot et al., 2010). While we can easily relate to the notion of unconscious stress, few studies have attempted to study it.

Importantly, chronic stress has serious health consequences as it causes wear and tear on the body and it has been linked to psychological and physiological problems such as chronic fatigue, cardiovascular disease, depression, abdominal obesity as well as specific neuronal changes in the prefrontal cortex, hippocampus, and amygdala (Sapolsky, 1996; Smyth et al., 1998; Rosengren et al., 2004; McEwen, 2005). One of the physiological factors contributing to this wear and tear of the human body is prolonged hyperactivity of the HPA-axis, as indicated by high ultradian levels of cortisol (Lupien et al., 2009). Prolonged enhanced levels of cortisol have been shown to be a risk factor for cardiovascular disease (Manenschijn et al., 2013). Furthermore, a bulk of research is available that reports the associations between a wide variety of stressors and elevated cortisol levels. Documented stressors associated with elevated cortisol levels vary in intensity such as trauma (e.g., Howard et al., 1955; Resnick et al., 1995) daily hassles (e.g., Sher, 2004), life events (e.g., van Eck et al., 1996) as well as stressors that only occur 'in one's head,' such as those worried about (e.g., Schlotz et al., 2004; Zoccola et al., 2008 – for a review see: Verkuil et al., 2010). In addition, in several studies cortisol has been associated with one's affective state (e.g., Smyth et al., 1998; Jacobs et al., 2007; Chida and Steptoe, 2009). Given that frequently experienced but relatively minor stressors may even lead to elevated cortisol levels, it can be suggested that our body may also respond to stressors that we are not consciously aware of but are nonetheless cognitively represented in the brain. However, only little research has been directed at this topic.

Whereas triggering the physiological stress response through worrying or explicit affect takes place in the absence of an impending stressor, it still involves our conscious attention (Brosschot et al., 2006). Yet, it is believed that most cognitive processes

take place rather implicitly, that is, while we are not consciously aware of this (e.g., Bargh and Morsella, 2008), and emotional cognitive processes are not unlikely to be an exception (Winkielman and Berridge, 2004; Brosschot et al., 2010). Brosschot et al. (2010) suggest that stress may affect us implicitly through cognitive representations of stressful events that people are not aware of, that is, unconscious stress. Preliminary – indirect – evidence for this idea comes from studies showing that stress and worries predict cardiovascular activity during sleep (while we are not consciously worrying; Brosschot et al., 2007; Yoshino and Matsuoka, 2009). Furthermore, an ambulatory study showed that after a worry episode has ended, cardiovascular activity can remain high for up to 2 h – a prolonged effect of worry itself that could not be explained by new worry episodes, mood, and lifestyle factors (Pieper et al., 2010). In addition, several experimental studies showed that subliminal negative emotional primes may increase cardiovascular activity (Levy et al., 2000; Hull et al., 2002), and this strongly suggests unconscious NA (stress) to be a mediator. However, thus far, evidence directly linking unconscious stress to physiological activity is scarce.

The fact that unconscious stress' physiological effects have been under-investigated could have to do with the scarcity of available measures of unconscious affect. One of the measures of implicit affect that has been used for decades is the word fragment completion task, which assesses the extent to which affective information is accessible from memory (e.g., Denny and Hunt, 1992; Anderson et al., 2003). When people complete word fragments in a negative way – i.e., "p – – son" can become "poison" or "person" – this indicates an enhanced accessibility of negative information (implicit negative memory bias), and potentially captures the extent to which people are currently experiencing unconscious stress. However, it is unknown whether implicit negative memory biases are associated with stress-related physiology. We here examined for the first time whether implicit negative memory bias – as assessed with the word fragment completion task – is associated with enhanced cortisol levels assessed at several moments during the day.

Additionally, a new instrument has recently been developed and validated that may make the assessment of unconscious stress possible via the indirect measurement of implicit affect. The Implicit Positive and Negative Affect Test (IPANAT), developed by Quirin et al. (2009a), uses people's spontaneous emotional judgments on adjectives paired with pseudo-words to assess levels of both positive (PA) and negative (NA) implicit affect. The IPANAT is a test of 'automatic activation of cognitive representations of affective experiences' (Quirin et al., 2009a, p. 501), which is very close to the definition of unconscious stress provided above. Importantly, the implicit NA subscale of the IPANAT was found to predict the cortisol response when subjects were confronted with acute stress (Quirin et al., 2009b). Moreover, a parallel study showed that the IPANAT scores for implicit PA are associated with cortisol measures in daily life, i.e., a reduced total circadian cortisol release, specifically due to the association of implicit PA with reduced morning levels of cortisol (Quirin et al., 2009b). In both these studies, no associations were found with explicit PA and NA, suggesting that implicit measures can have independent effects, and thus can really add to our understanding of how and when the physiological stress response is triggered.

In the present study, we aimed to replicate and extend these findings with the IPANAT. Quirin et al. (2009b) only administered the IPANAT once, and thus did not examine whether momentary state fluctuations in implicit NA and PA were associated with momentary fluctuations in cortisol levels. Their results suggests that at least *trait* variance in implicit affect is associated with daily cortisol levels. It is the main aim of the current study to assess whether *state* variance in implicit affect is associated with *state* (momentary) cortisol levels. Specifically, assessing ultradian cortisol secretion, we hypothesized that momentary implicit PA would show a negative association with ultradian cortisol levels, and inversely, that momentary implicit NA is positively associated with ultradian cortisol levels. We examined this association for fixed assessment moments during the day, while controlling for explicit affect and bio-behavioral variables that may influence cortisol levels.

In addition to examining ultradian cortisol secretion, we tested the relationship between implicit affect and the cortisol awakening response (CAR). The CAR is defined as the increase in cortisol secretion that is observed within the first hour after awakening (Chida and Steptoe, 2009). The magnitude of this increase is prospectively associated with increased risk for anxiety and depressive disorders (Adam et al., 2014). In addition, the CAR is lower in the offspring from long-lived families (Noordam et al., 2012) and heightened levels of the CAR have been associated with impaired endothelial function in women (Violanti et al., 2009). Furthermore, the CAR has proven to be a relatively robust phenomenon and evidence suggests that it is associated with explicit measures of NA (Wüst et al., 2000; Adam et al., 2006). For example, Adam et al. (2006) observed that higher levels of NA (feelings of sadness, loneliness, and feeling overwhelmed) across the day predicted higher CAR levels the next morning. In addition, – as mentioned above – lower levels of implicit PA have already been linked to higher morning levels of cortisol (Quirin et al., 2009b). Thus, in line with previous research by Adam et al. (2006) on explicit NA, we expected that prior-day mean implicit negative memory bias and implicit NA would be significantly positively associated with the CAR the next day. In addition, we predicted a negative relationship between implicit PA and the CAR the next day, indicating that higher levels of implicit PA are associated with reductions in the CAR. Lastly, we hypothesized that implicit memory bias and implicit affect – assessed directly after awakening – were significantly associated with the CAR on the day itself.

MATERIALS AND METHODS

PARTICIPANTS

Participants were undergraduates from the social science faculty of Leiden University. The study was approved by the local ethics committee. Participants were told that they were participating in a study on emotions in daily life to keep the hypotheses with regard to unconscious stress/emotions unknown. Participants were rewarded with research participation credits or 20 €.

Based on the effect that Quirin et al. (2009b) found [$r(30) = -0.46$ for PA and $r(42) = 0.40$ for NA] we calculated that to achieve the same effect size in our study at least 35 participants (power = 0.80, alpha = 0.05) would be required. Fifty-six participants signed up by registering themselves in scheduled time slots online. Individuals who smoked, used drugs, used chronic medication known to affect cortisol levels, or anyone who currently had a pulmonary-, cardiovascular- or mental health problem were excluded because of its known effect on cortisol (Kirschbaum and Hellhammer, 1989). Due to these recruitment demands, we had to exclude one participant, because of smoking. Our sample consisted primarily of psychology students with a mean age of 20.8 years (SD = 2.4, Range 17–27 years) of which 19 were men and 36 women. Forty-eight participants were of Caucasian descent, four of mixed descent, one African, one Hindu, and one Arabian.

PROCEDURE

Participants signed up for a specific time slot, starting at 9 am, 11 am, or 12.30 pm. After being given a brief overview of the study-procedures participants gave written informed consent. Thereafter, we assessed basic demographic variables as well as the exclusion criteria. The current study was part of a larger study, including a laboratory part (with several tests assessing emotion regulation; not reported here) and an ambulatory part, which required participants to wear an ECG belt (not reported here), keep an electronic diary, and to sample saliva at set times, the latter being the focus of our research. After the laboratory part participants were informed of the ambulatory procedure. They were explained how the ECG apparatus and the salivettes worked and given a manual containing instructions for cortisol sampling as well as information on the state questionnaires, favorable eating times and information related to the other parallel studies. With regard to the implicit measures (word fragment completion and IPANAT), participants were told that we wanted them to play several "language or word-games" that could best be played if they used their intuition to complete these games, and that there were no right or wrong answers.

During the study we made use of ecological momentary assessments to assess the variables of interest (salivary cortisol, implicit affect, explicit affect, and bio-behavioral variables). For 24 h, excluding any time between 10 pm and 10 am (to ensure no interference during sleep), participants were triggered by an alarm on a smartphone to answer several questions each full hour. Considering their return to the lab 24 h later this resulted in about 12 triggered alarms per person. During three of these times (11 am, 3 pm, and 9 pm), participants also had to sample saliva for later cortisol analysis. Time slots for these assessments were chosen in such a way that we could examine stress-related changes in the cortisol profile throughout the day and compare them taking their temporal relationship in consideration (Kirschbaum and Hellhammer, 1989). On top of these measurements at fixed intervals, participants were required to complete a morning assessment directly after waking up which consisted of first taking a saliva sample, then filling in the IPANAT and several questions about sleep, and finally, after 30 min, another saliva sample to measure the CAR.

INSTRUMENTS

Cortisol sampling

Participants were instructed to sample their saliva by chewing on a cotton dental role, consequently absorbing the saliva. Thereafter, the saturated cotton role is placed in a plastic tube with a lid (Salivette®, Sarstedt, Essen, Belgium). Upon reception we stored the tubes at −20° celsius until further analysis. Importantly, we instructed participants on how to take the samples. During this we emphasized that any smoking or consumption beforehand, or touching of the dental role during the sampling would affect the results and should therefore be avoided as much as possible. The tubes were sent to the bio-psychological lab of Prof. Dr. C. Kirschbaum (TU Dresden, Dresden, Germany) where unbound (free) cortisol (in nmol/l) in saliva was determined using a commercially available chemiluminescence immunoassay (CLIA; IBL-International, Hamburg, Germany).

To obtain the CAR we simply subtracted the second morning measurement (waking + 30 min) from the baseline morning measurement (waking). Previous research has shown that this sampling procedure is sufficient to estimate the CAR (e.g., Adam et al., 2006; Chida and Steptoe, 2009) and that difference scores from waking to 30 min yield similar scores to more intrusive methods using more samples (Williams et al., 2005).

In addition, cortisol samples that were flawed (e.g., saliva not sampled within a 10 min interval after the alarm went off (for daytime cortisol) or when the second sample in the morning was not taken within 40 min after awakening) were coded using a separate variable (e.g., no compliance). Furthermore, we added a second variable indicating any food intake in the 30 min before the sample was taken.

Word fragment completion task

A word fragment completion task was constructed to assess implicit negative memory biases (Graf et al., 1982; Quirin et al., 2009a). At each assessment, participants were required to complete four word fragments as fast as they could. The fragments were constructed using the following rules: (1) each fragment could be completed as negative word, (2) Each word consisted of four letters, (3) each of these fragments already contained two letters (e.g., "B_O_), and (4) dictionaries listed at least four alternatives for completing each fragment to form a word of four letters, and (5) negative words were never the most common completion of the fragment.

To obtain negative bias scores, the words were finally rated by three raters (including one of the authors; BV) on whether the valence of the words was negative or not (1 or 0). To estimate interrater reliability, intraclass correlations were calculated which showed high agreement between the raters (all ICC[3,k] > 0.89). Finally, total scores were dichotomized to obtain a measure of the presence of a negative memory bias (minimally one negative word coded as '1') or the absence of such a bias ('0').

Implicit affect

Unconscious stress was measured with a modified version of the IPANAT (Quirin et al., 2009a). The IPANAT originally is a paper and pencil test that asks participants to rate to what extent certain artificial or pseudo-words express certain moods. It uses a

cover story telling participants that "*The following words are from an artificial language. They are intended to express various moods. In all languages, there are words that help to express their meanings by the way they sound (for example, the word 'rattle' almost sounds like something that rattles). In poetry and literature, this is known as onomatopoeia.*" (cited from: Quirin et al., 2009a, p. 503). Thus, participants are led to believe that the test is about finding onomatopoeias, whereas it is assumed that the ratings that they make actually express the amount of implicit affect.

The original IPANAT consists of six pseudo-words (e.g., 'segam,' 'haswi') and participants are asked to rate on a scale from one to four how much this pseudo-word is expressing a certain affect (Dutch: blij, geërgerd, energiek, gespannen, bedroefd, goedgehumeurd; English: happy, irritated, energetic, tense, sad, merry). For our ambulatory study we created an ambulatory version of the IPANAT, thereby slightly modifying it. Instead of providing the participants with all pseudo-words and all affect words at once, participant in our ambulatory study were presented one pseudo-word per hour. They subsequently rated the extent to which this pseudo-word expressed the six affective states, with one combination of a pseudo and an affective word per screen. To create a Dutch version of the IPANAT we selected 12 pseudo-words from a validated list of 30 neutral pseudo-words. Though it might have had implications for the internal consistency of the test, the reasoning behind this modification of the IPANAT is that because we administered the IPANAT each hour we believed that using a reduced version of the IPANAT would form less of an interruption in daily life, but would still provide us with accurate data to assess state implicit affect. Other than Quirin et al. (2009a) we changed the scales from a '1 to 4' format to scales that ran from '1 (not at all)' to '6 (very much)' to add response variability in order to measure minor fluctuations. The implicit NA subscale was computed as the mean of the ratings for the negative adjectives (i.e., irritated, tense, sad) at each assessment and the implicit PA subscale as the mean for all positive adjectives (i.e., happy, energetic, merry) at each assessment.

Cronbach alphas for implicit PA and NA – for the original version of the IPANAT – are each 0.81, with 1-week test-retest reliabilities of 0.72 for implicit PA and 0.76 for implicit NA (Quirin et al., 2009a). The original IPANAT further shows adequate internal validity with primary loadings on congruent components between 0.8 and 0.9 and all cross-loadings lower than 0.10. Moreover, the positive and negative scale were uncorrelated indicating the presence of independent constructs, $r(203) = 0.03$, n.s. (Quirin et al., 2009a). Because the psychometric properties of the ambulatory IPANAT are unknown, we decided to report these properties in the results section.

Explicit affect

To examine explicit affect as the conscious counterpart of implicit affect we formulated four basic emotional questions that cover the width of emotions (see also Pieper et al., 2007). Participant were asked to rate if they have felt this emotion (angry/irritated, happy/joyful, let down/sad, restless/tense) during the past hour. Much like the IPANAT, these answers were also scored on a scale from 1 (not at all) to 6 (very much). Like the IPANAT we also combined the explicit affect scores into a subscale for explicit PA and a subscale for explicit NA (see above).

Stress and worries

Each hour, participants were asked whether they had experienced any stressful event (yes or no) or any episodes of worry (yes or no) or whether participants were anticipating a stressful event in the next couple of hours (yes or no). Yes-answers to either of these questions also led to questions on intensity and duration of the event (see also Pieper et al., 2007).

Bio-behavioral data

Lastly, we finished each assessment with a small behavioral questionnaire. These questions comprise bio-behavioral assessments on physical activity and intensity, whether they smoked (yes/no), and how much coffee or alcohol they consumed (NB, Although being a smoker was an exclusion criterion, it was still possible that 'recreational smokers' entered the study; two participants indeed indicated to have smoked one cigarette during the evening, excluding these participants did not alter the results).

Materials

The apparatus used for momentary assessment is the android-operated Motorola Razr Smartphone. The use of a digital device in a momentary study provides many advantages over a paper-based study (e.g., usability, portability, data transfer, and data export). The application MovisensXS (https://xs.movisens.com/) was used to trigger the forms for assessment on the phones.

STATISTICAL ANALYSIS

Firstly, we explored whether our modified version of the IPANAT showed psychometric properties that were similar to the original one (Quirin et al., 2009a). Therefore we conducted a principal components analysis on the adjectives ratings using varimax rotation. Furthermore, we looked at the reliability of the subscales and the cross-correlations of the subscales with the other psychological variables, the latter to obtain insight into the validity of the implicit measures. With respect to the reliability, we used the approach put forward by Cranford et al. (2006) and Shrout and Lane (2012), who use a generalizability theory approach to estimating reliability coefficients. Thereafter we tested our main hypotheses.

Multilevel growth curve modeling was used to examine the relationship between implicit negative memory bias, the IPANAT subscales and cortisol levels (Singer and Willett, 2003). Because the raw cortisol data were non-normally distributed, we successfully performed a natural log transformation to meet the model's basic assumptions. We chose multilevel analysis because it allows for data to have a hierarchical structure, and takes into account that intra-individual measurements are correlated. In this study affect measures are gathered at time points which are nested within persons. Therefore, our model contains two levels: an "episode level" with measurements that vary across the hourly assessments and a "person level" with demographic and stable person level variables (i.e., age, gender, BMI, use of oral contraceptives, and ethnicity). Episode level variables were time of day (coded as: 0 = 11 am, 1 = 3 pm, 2 = 9 pm), the implicit measures (IPANAT subscales and the implicit negative memory test), explicit affect measures,

occurrence of stress-events and worry episodes, as well as the bio-behavioral variables general activity, smoking, coffee, alcohol, and food intake. Continuous episode level variables (except for time of the day) were initially grand mean centered. When using grand mean centered time-varying variables, the slopes estimated in the analyses represent a composite of both within and between subjects effects (e.g., Hoffman and Stawski, 2009; Peugh, 2010).

For the ultradian cortisol data we first assessed the bivariate associations between the predicting variables (implicit negative memory bias, implicit PA, implicit NA) and cortisol levels, while controlling for the effects of time of the day. Thereafter, we assessed the independent contribution of the variables by entering them all as predictors in an overall model. Finally, we added the explicit measures and the bio-behavioral variables to examine whether the observed effects were not explained by these latter variables. Error covariance was best modeled using the diagonal structure.

To assess the second set of hypotheses pertaining to the CAR, we aggregated the implicit memory bias and IPANAT data from the first measurement day into a prior-day variable. To examine the relation between the CAR and current and prior-day implicit measures, we used bivariate Pearson correlations.

Analyses were performed in SPSS version 20.0 (MIXED and GENLIN MIXED) and R (lme4 and qgraph packages) with a two-tailed alpha set at 0.05.

RESULTS

Fifty-five participants were, in total, prompted to answer hourly forms 904 times, yet ignored the forms 110 times, dismissed them in 34 occasions and they were incomplete 11 times. Here we will focus on the 227 prompts that occurred simultaneously with the saliva sampling. From these 227 prompts, 35 were missed and 8 assessments were incomplete. Two participants were excluded from all analyses because they responded too fast to the questions and had too many identical responses (e.g., 1-1-1-1-1-1 responses on the IPANAT). With regards to the CAR measurements, 3 participants did not sample the CAR (i.e., forgot to take a second sample after 30 min).

As a way of ascertaining the validity of the implicit assessments with the IPANAT, during debriefing participant were asked to list what they thought we intended to measure with this test. Because our research was entitled "emotions in daily life" about half of the 55 people listed the obvious link they thought the study had with emotions. Only four participants mentioned associations with stress or unconscious emotions, excluding these participants from subsequent analyses did not significantly change the results.

Descriptive statistics for the measured variables are given in **Table 1**. In comparison with most similar ambulatory cortisol studies (van Eck et al., 1996; Smyth et al., 1998; Jacobs et al., 2007) our sample was relatively young. In line with data found by Quirin et al. (2009a; See Table 1), participants showed a bias toward both implicit PA and explicit PA (i.e., higher scores for positive than for NA). With respect to the negative memory bias, assessed with the word fragment completion task, 50% of all assessments were associated with at least one word that was completed negatively. Additionally, in 7.9% of the assessments a stressful event was reported and in 18.7% of the assessments an episode of worry was reported. Calculated alternatively, participants reported a mean of 0.21 (SD = 0.46) stress-events and a mean of 0.49 (SD = 0.78) worry episodes, which is lower compared to previous ambulatory studies by our group (e.g., Ms > 1; Verkuil et al., 2012).

Figure 1 shows that the cortisol levels followed the expected ultradian pattern, with a clear increase in cortisol directly after waking up and an overall decrease of cortisol during the day.

PROPERTIES OF THE MODIFIED IPANAT AND ASSOCIATIONS WITH OTHER STRESS-RELATED VARIABLES

A principal components analysis using varimax rotation was conducted on the IPANAT ratings to examine whether our modified version of the IPANAT would possess a similar factor structure as the original version. Based on eigenvalues being bigger than one,

Table 1 | Descriptive statistics.

	N	*M*	SD	Minimum	Maximum	%
Person level						
Gender	53					64.2% women
Age	53	20.70	2.37	17	27	
BMI	53	22.05	2.36	18.19	28.41	
Ethnicity	53					88.5% Caucasian
Contraceptive use	34					67.6% of all women
Episode level						
Implicit PA	174	3.35	1.20	1	6	
Implicit NA	173	2.84	1.02	1	5.67	
Implicit negative memory bias	170					50%
Explicit PA	132	3.95	1.23	1	6	
Explicit NA	132	2.01	0.83	1	5	
Worry episode	139					18.7%
Stressful event	139					7.9%

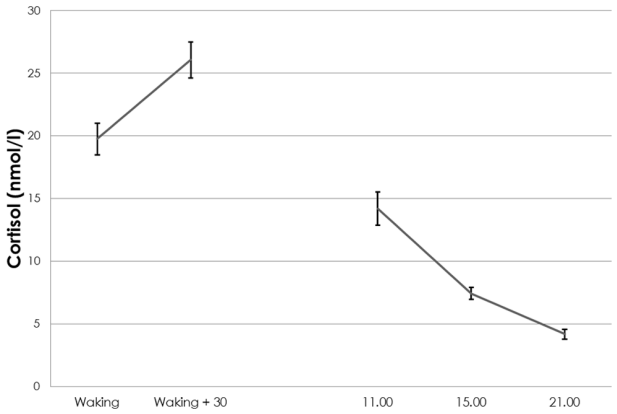


FIGURE 1 | Ultradian cortisol levels (±1 SE).

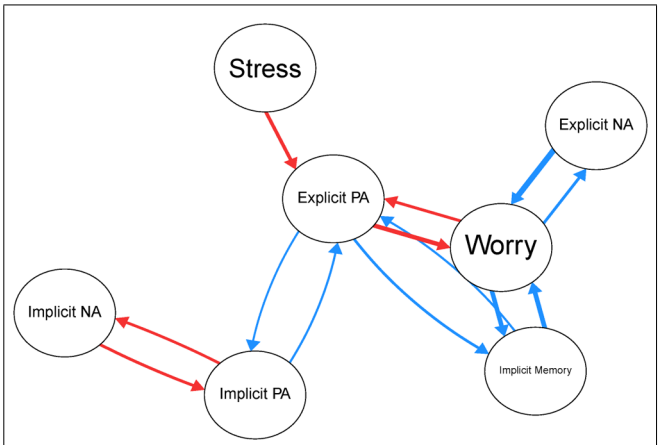


FIGURE 2 | Significant associations between the psychological variables. Blue lines indicate positive associations and red lines indicate negative associations. Thicker lines represent stronger associations.

two components were extracted, explaining 64% of the variance. The results from **Table 2** clearly provide evidence for the existence of the two proposed subscales: implicit PA and implicit NA, and thereby supports the construct validity of this modified IPANAT version.

We additionally calculated the reliability of the subscales. We calculated the reliability pertaining to individual differences in the average ratings on the IPANAT subscales (aggregated across the four assessments). Results showed that between person reliability of the average ratings was satisfactory for implicit PA, $R_{kf} = 0.84$ and for implicit NA, $R_{kf} = 0.81$. That is, when averaging the ratings across all four assessments, the means are quite stable and seem to reflect individual differences.

Multilevel analyses showed that momentary assessed implicit PA was negatively associated with implicit NA, $B = -0.667$, $p < 0.001$, 95% CI [−0.830, −0.504]. This association remained significant when controlling for explicit affect, stress, and worry. Next, we examined the association between the implicit affect and explicit affect, stress and worries. **Figure 2** shows the results of multilevel analyses wherein each psychological variable is predicted by the other variables. This figure makes clear that while controlling for the other variables in the model, there were significant associations between implicit PA and explicit PA and implicit NA and explicit NA. Additionally, worry and stress were not independently associated with implicit affect. Stressful events were associated with reduced explicit PA and worry episodes were associated with reduced explicit PA, increased explicit NA and increased implicit memory bias.

Table 2 | Varimax rotated loadings of ratings on the IPANAT items.

IPANAT item	Factor 1	Factor 2
Happy	0.845	−0.278
Energetic	0.802	0.130
Merry	0.809	−0.181
Tense	−0.08	0.841
Irritated	−0.205	0.629
Sad	−0.230	0.729

ASSOCIATION OF IMPLICIT AFFECT WITH CORTISOL

Using natural log transformed cortisol levels assessed at 11 am ($n = 45$), 3 pm ($n = 48$), and 9 pm ($n = 46$) as our dependent variable, we found that time of the day had a significant fixed effect on cortisol: $B = -0.548$, $p < 0.001$, 95% CI [−0.637, −0.459]. This baseline model showed that during the day, cortisol levels decreased. Next, we added the three implicit measures in three separate models, which led to three better fitting models [all $\chi^2(1) > 26$, $ps < 0.001$] compared to the baseline model. While controlling for time, implicit negative affective memory bias was positively associated with cortisol ($B = 0.179$, $p = 0.012$). The associations between cortisol and implicit PA ($B = -0.041$, $p = 0.161$) and implicit NA ($B = 0.002$, $p = 0.940$) were not significant (tested two-tailed). Subsequently, we examined the independent associations between the implicit measures (the IPANAT subscales and implicit memory bias) and cortisol levels. When adding all three implicit measures to the baseline model, a better fit was obtained [$\chi^2(3) = 57.07$, $p < 0.0001$]. **Table 3** shows that cortisol levels were negatively associated with implicit PA [$B = -0.134$, 95% CI (−0.229, −0.039)], but were – unexpectedly – also negatively associated with implicit NA [$B = -0.106$, 95% CI (−0.201, −0.011)]. In addition, cortisol levels were positively associated higher levels of implicit memory bias [$B = 0.196$, 95% CI (0.060, 0.333)].

We additionally explored whether the associations were driven by either between subjects effects or by within subjects effects. To do so, we entered the ratings averaged per person, across the assessments (the person mean, representing between subjects effects) as well as the ratings centered on the personal mean (person mean centered, representing within subjects effects; cf. Hoffman and Stawski, 2009). Results (not reported here) showed that only between subjects effects accounted for the associations between the implicit measures and cortisol levels.

In a final step, the explicit measures (affect, stressful events, and worries) were added to the model, but this did not improve model

Table 3 | Estimates of fixed effects predicting log-transformed cortisol levels.

Parameter	*B*	SE	*df*	*t*	*p*	Interpretation[†]
Intercept	2.565	0.078	84.75	32.61	0.000	12.93 nmol/l
Time	−0.621	0.056	64.54	−11.00	0.000	46% decrease per period
Implicit PA	−0.111	0.039	82.83	−2.81	0.006	10% decrease
Implicit NA	−0.098	0.044	80.16	−2.21	0.030	9% decrease
Implicit memory bias	0.196	0.068	67.13	2.87	0.005	21% increase

[†] *Cortisol values were transformed using the natural log transformation, coefficients (B) were transformed to percentage change in cortisol per unit change in the predictor, using the formula: percentage change* $= 100(e^B - 1)$ *(cf. Adam et al., 2006).*

fit [$\chi^2(4) = 2.956$, $p = 0.565$]. Furthermore, none of the explicit measures was significantly associated with cortisol levels. Interestingly, this was also the case when only including time of the day and the explicit measures as predictors of cortisol, showing that explicit affect measures were not related to cortisol. Inclusion of bio-behavioral variables (caffeine intake, physical activity, smoking, alcohol intake, BMI, use of contraceptives) and personal characteristics (gender, age, ethnicity) did not lead to different results.

In order to specify the association between implicit affect and cortisol, we explored the associations between cortisol and the separate implicit affect variables (merry, happy, energetic, irritated, tense, sad), while controlling for implicit negative memory bias and time of the day. Cortisol was not significantly associated with any of these specific affect variables, although a trend appeared for implicitly feeling energetic ($B = -0.045$, $p = 0.076$).

CURRENT-DAY AND PRIOR-DAY IMPLICIT AFFECT AND THE CORTISOL AWAKENING RESPONSE (CAR)

Measured over 50 participants we found that within the first 30 min of waking, baseline cortisol increased by about 73% showing an increase of 7.00 nmol/l (SD = 9.17). Maintaining the criterion for defining a cortisol response (2.5 nmol/l increase; Wüst et al., 2000) we observed a CAR in 64% of the subjects.

Prior-day implicit measures and the CAR

Pearson correlations showed that the CAR was not related to the aggregated prior-day measures of implicit PA [$r(48) = -0.159$, $p = 0.271$], implicit NA [$r(48) = 0.208$, $p = 0.148$], or implicit memory bias [$r(46) = 0.038$, $p = 0.797$]. Inspection of whether the CAR was related to any specific prior-day affect revealed a significant association between implicit sadness and the CAR, $r(48) = 0.291$, $p = 0.041$. This association is depicted in **Figure 3**, indicating that increased levels of implicit sadness are associated with increases in the CAR. The CAR was not associated with explicit affect, stress, or worries during the previous day ($p > 0.05$).

Current-day implicit measures and the CAR

Nine participants missed the morning assessment of the IPANAT and 13 did not fill in the word fragment completion task. Pearson correlations showed that the CAR was not related to the morning assessments of implicit PA [$r(39) = 0.068$, $p = 0.673$], implicit NA [$r(39) = -0.031$, $p = 0.847$], or implicit memory bias [$r(35) = -0.232$, $p = 0.167$]. In addition, none of the specific implicit affect variables was associated with the CAR.

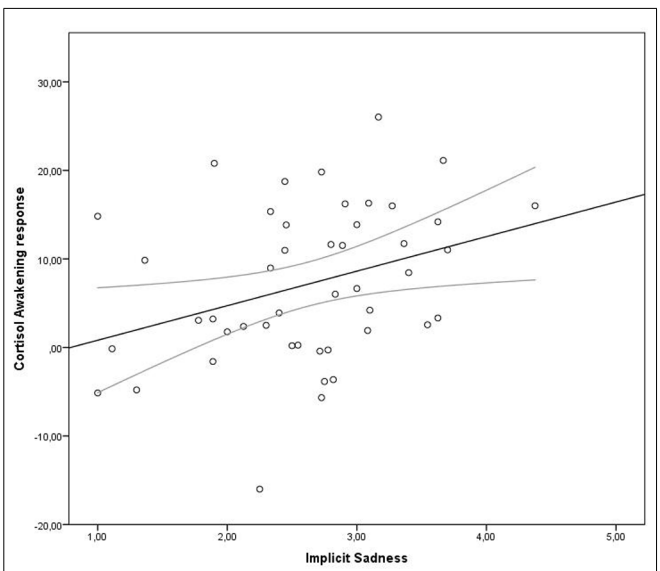


FIGURE 3 | Scatterplot of the association between prior-day implicit sadness and the cortisol awakening response (CAR).

DISCUSSION

The purpose of this study was to examine whether unconscious stress, assessed using measures of implicit affect (IPANAT) and implicit memory biases (word fragment completion task), is related to cortisol secretion in daily life. Our main finding is that implicit memory bias, implicit NA, and implicit PA were associated with cortisol levels across the day, but only when they were simultaneously used to explain the variance in cortisol levels. Furthermore, we could not replicate the findings of previous studies showing that explicit PA and NA, worries and stressful events are associated with cortisol levels (van Eck et al., 1996; Smyth et al., 1998; Sher, 2004). Surprisingly, when assessing the implicit measures separately, only the implicit memory bias proved to be significantly associated with cortisol secretion. Furthermore, in reviewing the associations with the CAR, we found a significant association with prior-day implicit sadness. Below these results will be discussed in more detail.

This study was the first to examine the relation between implicit measures of affect and cortisol levels in daily life. The results are partly in line with the hypothesis that unconscious stress can have physiological effects (Brosschot et al., 2010). That is, we found that participants with on average higher levels of implicit negative memory bias showed enhanced cortisol levels. Thus, when people completed one or more word fragments in a negative manner, they showed higher cortisol levels than people who completed all word fragments in a neutral or positive manner. Yet, of the three implicit affect variables, only implicit memory bias, as assessed with the word fragment completion task, was associated with cortisol levels. However, we did find that both IPANAT subscales (PA and NA) were associated with reduced ultradian cortisol levels but only when controlling for the implicit negative memory bias. These associations may have become significant due to suppression, which occurs when the association between an independent variable and the dependent variable becomes stronger through the addition of a third variable; in this case, when all three implicit measures are added to the model (MacKinnon et al., 2000). This would mean that the cortisol effect of implicit negative memory bias would suppress the effect of implicit affect. Such a process is difficult to understand, and is not easy to reconcile with the assumption that the two tests would measure, at least partially, the same phenomenon. Moreover, interpretations of these results have to be regarded with caution given the absence of direct relations between cortisol and the IPANAT subscales. That high implicit PA was associated with reduced cortisol levels seems consistent with Quirin et al. (2009b), who found that high trait implicit PA was associated with reduced total circadian cortisol secretion. In contrast to our expectation, we also found that implicit NA was associated with reduced ultradian cortisol levels. Furthermore, we also did not observe that state fluctuations of implicit affect or implicit negative memory biases were associated with fluctuations in cortisol levels within participants. That is, our findings demonstrate that trait differences between participants in these variables accounted for variation in cortisol. Longer assessment periods, i.e., longer than 24-h, might be more suited to capture the proposed within subject associations, which are possibly more subtle and only visible when measuring multiple days per person.

In addition, in our analysis of prior-day and current-day affect and its relationship with the CAR, we found that – in line with our expectation – prior-day implicit sadness was positively associated with the CAR. Taking all cortisol assessments together, the results provide a somewhat paradoxical picture. Implicit NA seemed negatively associated with cortisol during the day – albeit only after controlling for the other implicit measures, yet prior-day implicit sadness (a part of implicit NA) was positively associated with morning cortisol levels. First of all it has to be acknowledged that these results were obtained from exploratory analyses, without controlling for family wise error rate. Replication studies are therefore warranted to examine the robustness of the current findings. A possible – yet tentative – explanation for the current findings is that the discrepancy in the results can be explained by the fact that the CAR is considered an adaptive response that, based on the experiences of the day before, proactively secretes more or less cortisol secretion to anticipate on today's stressors (Adam et al., 2006). Put in perspective, because momentary implicit sadness does not require an immediate action tendency in the current moment, there is no immediate need for heightened cortisol levels (Roseman et al., 1994); however, implicit sadness from the day before would increase your cortisol the next morning in order to be better prepared for stressors than you were the day before (which most likely would have caused the implicit sadness the day before).

Finally, in examining the relation between implicit PA and the CAR, we were not able to replicate the results found by Quirin et al. (2009b). Quirin et al. (2009b) found that implicit PA was related to cortisol specifically to the moments after awakening, although they did not compute a CAR. In our study, the aggregated measure of prior-day implicit PA and the momentary assessment of implicit PA in the morning were both not associated with the CAR. All in all, although we did find some associations between the IPANAT and cortisol levels, future studies are clearly warranted that address whether the IPANAT is associated with stress-related physiology.

CONCLUSION

The study took place in a small homogenous group of largely female psychology students. Therefore the results found in this study may not be accurately generalized to other populations. In addition, the word fragment completion task that was used in the current study was devised to capture negative memory biases (all fragments could be completed using negative words). It might be worthwhile to examine positive memory biases as well, as such biases might buffer the negative effects of negative biases. Furthermore, although our ambulatory study was suited to address moment-to-moment associations between implicit processes and cortisol in daily life, the direction of these observed associations remains to be addressed in future studies. It might be worthwhile to use training procedures such as attentional bias modification to change implicit processes and examine changes in stress-related physiology in daily life. At least in one study by Dandeneau et al. (2007) it was demonstrated that reducing vigilance for threat was associated with reduced levels of cortisol (Dandeneau et al., 2007). Another limitation is that we were not able to randomize the order in which the word fragments and the IPANAT words were presented to the participants. Every participant reacted to the same stimuli (fragments/non-sense words) in the same order. This introduces the problem that, for example, no associations between cortisol and the morning assessments of the IPANAT and the word fragment completion task were observed, because the stimulus material at this assessment was less suited to capture implicit affect. Therefore, we recommend that future studies use a sampling method wherein the stimuli are presented to the participants in randomized order.

All things considered, this was the first study to examine the association between cortisol and implicit affect measures in daily life. The finding that implicit negative memory bias (assessed with a word fragment completion task) was associated with higher levels of cortisol in daily life, supports the recently formulated hypothesis that unconscious stress influences stress-related physiology (Brosschot et al., 2010). Yet, the results pertaining to the other instrument to assess implicit affect (IPANAT) were less consistent, although they suggest that implicit sadness predicts a stronger cortisol increase at awakening. This preliminary evidence that in daily

life our stress physiology may be responsive to unconscious stress, opens up new venues for future studies and suggest that by focusing on explicit affect and conscious stress – we might have been studying just the tip of the iceberg.

ACKNOWLEDGMENT

Funding for this study was provided by the ZonMW TOP grant provided to Dr. Jos F. Brosschot, Leiden University.

REFERENCES

Adam, E. K., Hawkley, L. C., Kudielka, B. M., and Cacioppo, J. T. (2006). Day-to-day dynamics of experience-cortisol associations in a population-based sample of older adults. *Proc. Natl. Acad. Sci. U.S.A.* 103, 17058–17063. doi: 10.1073/pnas.0605053103

Adam, E. K., Vrshek-Schallhorn, S., Kendall, A. D., Mineka, S., Zinbarg, R. E., and Craske, M. G. (2014). Prospective associations between the cortisol awakening response and first onsets of anxiety disorders over a six-year follow-up – 2013 Curt Richter Award Winner. *Psychoneuroendocrinology* 44, 47–59. doi: 10.1016/j.psyneuen.2014.02.014

Anderson, C. A., Carnagey, N. L., and Eubanks, J. (2003). Exposure to violent media: the effects of songs with violent lyrics on aggressive thoughts and feelings. *J. Pers. Soc. Psychol.* 84, 960–971. doi: 10.1037/0022-3514.84.5.960

Bargh, J. A., and Morsella, E. (2008). The unconscious mind. *Perspect. Psychol. Sci.* 3, 73–79. doi: 10.1111/j.1745-6916.2008.00064.x

Brosschot, J. F., Gerin, W., and Thayer, J. F. (2006). The perseverative cognition hypothesis: a review of worry, prolonged stress-related physiological activation, and health. *J. Psychosom. Res.* 60, 113–124. doi: 10.1016/j.jpsychores.2005.06.074

Brosschot, J. F., Van Dijk, E., and Thayer, J. F. (2007). Daily worry is related to low heart rate variability during waking and the subsequent nocturnal sleep period. *Int. J. Psychophysiol.* 63, 39–47. doi: 10.1016/j.ijpsycho.2006.07.016

Brosschot, J. F., Verkuil, B., and Thayer, J. F. (2010). Conscious and unconscious perseverative cognition: is a large part of prolonged physiological cognition due to unconscious stress? *J. Psychosom. Res.* 69, 407–416. doi: 10.1016/j.jpsychores.2010.02.002

Chida, Y., and Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. *Biol. Psychol.* 80, 265–278. doi: 10.1016/j.biopsycho.2008.10.004

Cranford, J. A., Shrout, P. E., Iida, M., Rafaeli, E., Yip, T., and Bolger, N. (2006). A procedure for evaluating sensitivity to within-person change: can mood measures in diary studies detect change reliably? *Pers. Soc. Psychol. Bull.* 32, 917–929. doi: 10.1177/0146167206287721

Dandeneau, S. D., Baldwin, M. W., Baccus, J. R., Sakellaropoulo, M., and Pruessner, J. C. (2007). Cutting stress off at the pass: reducing vigilance and responsiveness to social threat by manipulating attention. *J. Pers. Soc. Psychol.* 93, 651–666. doi: 10.1037/0022-3514.93.4.651

Denny, E. B., and Hunt, R. R. (1992). Affective valence and memory in depression: dissociation of recall and fragment completion. *J. Abnorm. Psychol.* 101, 575–580. doi: 10.1037/0021-843X.101.3.575

Graf, P., Mandler, G., and Haden, P. E. (1982). Simulating amnesic symptoms in normal subjects. *Science* 218, 1243–1244. doi: 10.1126/science.7146909

Hoffman, L., and Stawski, R. S. (2009). Persons as contexts: evaluating between-person and within-person effects in longitudinal analysis. *Res. Hum. Dev.* 6, 97–120. doi: 10.1080/15427600902911189

Howard, J. M., Olney, J. M., Frawley, J. P., Peterson, R. E., Smith, L. H., Davis, J. H., et al. (1955). Studies of adrenal function in combat and wounded soldiers. *Ann. Surg.* 141, 314–320. doi: 10.1097/00000658-195503000-00004

Hull, J. G., Slone, L. B., Meteyer, K. B., and Matthews, A. R. (2002) The nonconsciousness of self-consciousness. *J. Pers. Soc. Psychol.* 83, 406–424. doi: 10.1037//0022-3514.83.2.406

Jacobs, N., Myin-Germeys, I., Derom, C., Delespaul, P., van Os, J., and Nicolson, N. A. (2007). A momentary assessment of the relationship between affective and adrenocortical stress responses in daily life. *Biol. Psychol.* 74, 60–66. doi: 10.1016/j.biopsycho.2006.07.002

Kirschbaum, C., and Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 22, 150–169. doi: 10.1159/000118611

Levy, B. R., Hausdorff, J. M., Hencke, R., and Wiel, J. Y. (2000). Reducing cardiovascular stress with positive self-stereotypes of aging. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 55, P205–P213. doi: 10.1093/geronb/55.4.P205

Lupien, S. J., McEwen, B. S., Gunnar, M. R., and Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behavior and cognition. *Nat. Rev. Neurosci.* 10, 434–445. doi: 10.1038/nrn2639

MacKinnon, D. P., Krull, J. L., and Lockwood, C. M. (2000). Equivalence of the mediation, confounding and suppression effect. *Prev. Sci.* 1, 173–181. doi: 10.1023/A:1026595011371

Manenschijn, L., Schaap, L., van Schoor, N. M., van der Pas, S., Peeters, G. M., Lips, P., et al. (2013). High long-term cortisol levels, measured in scalp hair, are associated with a history of cardiovascular disease. *J. Clin. Endocrinol. Metab.* 98, 2078–2083. doi: 10.1210/jc.2012-3663

McEwen, B. S. (2005). Stressed or stressed out: what is the difference? *J. Psychiatry Neurosci.* 30, 315–318.

Noordam, R., Jansen, S. W. M., Akintola, A. A., Oei, N. Y. L., Maier, A. B., Pijl, H., et al. (2012). Familial longevity is marked by lower diurnal salivary cortisol levels: the Leiden Longevity Study. *PLoS ONE* 7:e31166. doi: 10.1371/journal.pone.0031166

Peugh, J. L. (2010). A practical guide to multilevel modeling. *J. Sch. Psychol.* 48, 85–112. doi: 10.1016/j.jsp.2009.09.002

Pieper, S., Brosschot, J. F., van der Leeden, R., and Thayer, J. F. (2007). Cardiac effects of momentary assessed worry episodes and stressful events. *Psychosom. Med.* 69, 901–909. doi: 10.1097/PSY.0b013e31815a9230

Pieper, S., Brosschot, J. F., van der Leeden, R., and Thayer, J. F. (2010). Prolonged cardiac effects of momentary assessed stressful events and worry episodes. *Psychosom. Med.* 72, 570–577. doi: 10.1097/PSY.0b013e3181dbc0e9

Quirin, M., Kazén, M., and Kuhl, J. (2009a). When nonsense sounds happy or helpless: the implicit positive and negative affect test (IPANAT). *J. Pers. Soc. Psychol.* 97, 500–516. doi: 10.1037/a0016063

Quirin, M., Kazén, M., Rohrmann, S., and Kuhl, J. (2009b). Implicit but not explicit affectivity predicts circadian and reactive cortisol: using the implicit positive and negative affect test. *J. Pers.* 77, 401–425. doi: 10.1111/j.1467-6494.2008.00552.x

Resnick, H. S., Yehuda, R., Kitman, R. K., and Foy, D. W. (1995). Effect of previous trauma on acute plasma cortisol level following rape. *Am. J. Psychiatry* 152, 1675–1677. doi: 10.1176/ajp.152.11.1675

Roseman, I. J., Wiest, C., and Swartz, T. S. (1994). Phenomology, behaviors, and goals differentiate discrete emotions. *J. Pers. Soc. Psychol.* 67, 206–221. doi: 10.1037/0022-3514.67.2.206

Rosengren, A., Hawken, S., Ôunpoo, S., Sliwa, K., Zubaid, M., Almaheed, W. A., et al. (2004). Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet* 364, 953–962. doi: 10.1016/S0140-6736(04)17019-0

Sapolsky, R. M. (1996). Why stress is bad for your brain. *Science* 273, 749–750. doi: 10.1126/science.273.5276.749

Schlotz, W., Hellhammer, J., Schulz, P., and Stone, A. (2004). Perceived work overload and chronic worrying predict weekend–weekday differences in the cortisol awakening response. *Psychosom. Med.* 66, 207–214. doi: 10.1097/01.psy.0000116715.78238.56

Sher, L. (2004). Daily hassles, cortisol, and the pathogenesis of depression. *Med. Hypotheses* 62, 198–202. doi: 10.1016/S0306-9877(03)00320-7

Shrout, P. E., and Lane, S. P. (2012). "Psychometrics," in *Handbook of Research Methods for Studying Daily Life*, eds M. R. Mehl and T. S. Conner (New York, NY: The Guilford Press), 302–320.

Singer, J. D., and Willett, J. B. (2003). *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. New York: Oxford University Press.

Smyth, J., Ockenfels, M. C., Porter, L., Kirschbaum, C., Hellhammer, D. H., and Stone, A. A. (1998). Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology* 24, 353–370. doi: 10.1016/S0306-4530(98)00008-0

van Eck, M., Berkhof, H., Nicolson, N., and Sulon, J. (1996). The effects of perceived stress, traits, mood states, and stressful daily life events on salivary cortisol. *Psychosom. Med.* 58, 447–458. doi: 10.1097/00006842-199609000-00007

Verkuil, B., Brosschot, J. F., Gebhardt, W. A., and Thayer, J. F. (2010). When Worries make you sick: a review of perseverative cognition, the default stress response and somatic health. *J. Exp. Psychopathol.* 1, 87–118. doi: 10.5127/jep.009110

Verkuil, B., Brosschot, J. F., Meerman, E. E., and Thayer, J. F. (2012). Effects of momentary assessed stressful events and worry episodes on somatic health complaints. *Psychol. Health* 27, 141–158. doi: 10.1080/08870441003653470

Violanti, J. M., Burchfiel, C. M., Fekedulegn, D., Andrew, M. E., Dorn, J., Hartley, T. A., et al. (2009). Cortisol patterns and brachial artery reactivity in a high stress environment. *Psychiatry Res.* 169, 75–81. doi: 10.1016/j.psychres.2008.06.012

Williams, E., Magid, K., and Steptoe, A. (2005). The impact of time of waking and concurrent subjective stress on the cortisol response to awakening. *Psychoneuroendocrinology* 30, 139–148. doi: 10.1016/j.psyneuen.2004.06.006

Winkielman, P., and Berridge, K. C. (2004). Unconscious emotion. *Am. Psychol. Soc.* 13, 120–123. doi: 10.1111/j.0963-7214.2004.00288.x

Wüst, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., and Kirschbaum, C. (2000). The cortisol awakening response – normal values and confounds. *Noise Health* 7, 79–88.

Yoshino, K., and Matsuoka, K. (2009). Effect of mood during daily life on autonomic nervous activity balance during subsequent sleep. *Auton. Neurosci.* 150, 147–149. doi: 10.1016/j.autneu.2009.03.013

Zoccola, P. M., Dickerson, S. S., and Zaldivar, F. P. (2008). Rumination and cortisol responses to laboratory stressors. *Psychosom. Med.* 70, 661–667. doi: 10.1097/PSY.0b013e31817bbc77

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 September 2014; accepted: 21 January 2015; published online: 10 February 2015.

Citation: Mossink JCL, Verkuil B, Burger AM, Tollenaar MS and Brosschot JF (2015) Ambulatory assessed implicit affect is associated with salivary cortisol. Front. Psychol. ***6****:111. doi: 10.3389/fpsyg.2015.00111*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in
PSYCHOLOGY

ORIGINAL RESEARCH ARTICLE
published: 24 February 2015
doi: 10.3389/fpsyg.2015.00019

Individual differences in vagal regulation are related to testosterone responses to observed violence

Eric C. Porges[1]*, Karen E. Smith[2] and Jean Decety[2,3]

[1] *Institute of Aging, Cognitive Aging and Memory Clinical Translational Research Program, University of Florida, Gainesville, FL, USA*
[2] *Department of Psychology, University of Chicago, Chicago, IL, USA*
[3] *Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL, USA*

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Daniel S. Quintana, University of Oslo, Norway
Gail A. Alvares, The University of Western Australia, Australia
Linda Booij, Queen's University, Canada

****Correspondence:***
Eric C. Porges, Institute of Aging, Cognitive Aging and Memory Clinical Translational Research Program, University of Florida, 2004 Mowry Road, Room 3117, Gainesville, FL, USA
e-mail: eporges@ufl.edu

Observing violent content has been hypothesized to facilitate antisocial behaviors including interpersonal violence. Testosterone is released in response to perceived challenges of social status, often followed by an increase in aggressive behaviors and physiological activation. Prior investigations evaluating the impact of observing violence on autonomic function have focused on sympathetic measures of arousal. Measurement of parasympathetic nervous system (PNS) activity has been neglected, although reduced PNS activity has been associated with antisocial behavior. Consistent with a hierarchical model of the autonomic nervous system (i.e., polyvagal theory), individual differences in PNS activity reflected in respiratory sinus arrhythmia (RSA) were hypothesized to have an inhibitory impact on sympathetic and hormonal reactivity in subjects who were observing a violent video. Autonomic data (i.e., electrodermal activity (EDA), heart rate, and RSA) were collected from forty adult males prior to and while viewing violent sports or a control video. Pre- and post-video saliva samples were assayed for cortisol and testosterone. Participants who viewed the violent video showed increased sympathetic activity compared to controls. In contrast to the sympathetic reactivity to the violent video, there were no significant RSA changes in response to the stimuli, suggesting that viewing violent sports selectively increases sympathetic activity without eliciting PNS withdrawal. However, within the group viewing the violent video, participants with lower RSA during baseline and the observation of violent videos, responded with greater increases in salivary testosterone, suggesting that high parasympathetic tone dampens testosterone reactivity. These individual differences in response to observed violence, associated with higher RSA, may account for some of the improved health, growth, and restoration outcomes across the lifespan, that this segment of the population benefits from.

Keywords: pain, respiratory sinus arrhythmia, testosterone, violence, challenge hypothesis, parasympathetic, autonomic nervous system

INTRODUCTION

The link between observing violence and the expression of violent behavior has a long history dating to the Victorian era (Paik and Comstock, 1994). The prevalence and ubiquity of violence in the modern media has spurred considerable interest, debate and public concern about its potential harm in forums ranging from scientific literature to Supreme Court and congressional hearings (Anderson and Bushman, 2001; Hall et al., 2011). Despite these concerns and mixed results from empirical research, it seems clear that there is a large range of individual differences in responses to observed violence, with the great majority of individuals not committing violent acts after observing violence. Given such individual variation, investigating individual differences in physiological responses to violent media may help characterize vulnerabilities that could manifest as antisocial behavior.

Testosterone has been the focus of research because of its putative role in male aggression. However, the exact role testosterone plays in facilitating aggressive behavior has been widely debated (Eisenegger et al., 2011; Josephs et al., 2011; van Honk et al., 2011b). Prior work has linked testosterone associated aggressive behaviors with functions that support reproductive/mating opportunities, such as gaining territory or improving social status. However, testosterone release is not without cost in the form of increased metabolic demands and immune system suppression (Boonekamp et al., 2008). The challenge hypothesis proposes that, because testosterone release can be so metabolically costly, an organism must balance the physiological and behavioral consequences of high testosterone levels to meet their environmental demands (Archer, 2006). As a result of this cost, when a direct, proxied, or symbolic opportunity to improve reproductive standing is not at stake, testosterone levels should not be responsive to a given situation. This hypothesis was originally used to describe the behavior of avian species, which exhibit slow seasonal changes in testosterone levels. Spikes in testosterone were detected when direct breeding opportunities or social instability occurred (Wingfield et al., 1990). The theory has since been tested in primates and used to predict behavior in humans (Archer, 2006),

with research suggesting that aggression related to territoriality and dominance releases testosterone, while defensive aggression does not result in a similar release of testosterone. This led to the hypothesis that social elicitors of testosterone release in human males are either threats to social status (Josephs et al., 2011) or anticipation of reproductive opportunities (Wingfield et al., 1990). Behaviors that influence human social status or present a challenge to social status may take many direct and indirect forms. For example, a social interaction in which status is challenged may range from active participation in a physical sport, to competing for desirable jobs, to a game of chess (Mazur et al., 1992). The consequences of these challenges (i.e., winning or losing) on testosterone levels are less clear, with some studies reporting a positive relationship while others reporting no relationship at all, and the consequences often being depending on individuals' own subjective perceptions and evaluations of what constitutes a win. For example, a technical loss may be considered a win if the individual expected a worse outcome (Costa and Salvador, 2012; Jiménez et al., 2012; van der Meij et al., 2012).

Testosterone influences individual targets and networks in the central nervous system that are known to be involved in the perception of threat related social stimuli. Both endogenous and exogenous testosterone appear to modulate amygdala response to fear and anger expression (Derntl et al., 2009), a neural structure involved in emotion perception and processing with a particularly sensitivity to threat related stimuli (Ohman, 2005; LeDoux, 2007). Additionally, endogenous testosterone levels are associated with prefrontal cortex and amygdala functional connectivity in response to affective social stimuli (Volman et al., 2011). Alterations in medial prefrontal cortex (mPFC) activity have been proposed to be one of the primary neurobiological mechanisms relating endogenous testosterone levels to aggressive behaviors (Mehta and Beer, 2010). For example, previous studies have found that circulating testosterone levels are related to ventromedial prefrontal cortex responses to anger in males (e.g., Stanton et al., 2009).

Recent work has demonstrated that the dorsal aspect of the mPFC (Napadow et al., 2008) is correlated with parasympathetic activity, and the mPFC generally may serve as a final common pathway linking affect with autonomic system response (Lane et al., 2009). The parasympathetic branch of the autonomic nervous system, via the myelinated vagus nerve, exerts inhibitory control of both behavior and cardiac activity (Porges, 2007) and has been shown to interact with the HPA axis (Ulrich-Lai and Herman, 2009), with stimulation of the vagus reducing HPA axis reactivity to corticotrophin releasing hormone (O'Keane et al., 2005). Throughout the present experiment, we measured heart rate and respiratory sinus arrhythmia (RSA), an index of vagal modulation of the parasympathetic system (Porges, 2007). Research suggests that reduced resting RSA may result in an inability to down regulate threat detection, potentially leading to inappropriate interpretations of social cues (Thayer and Lane, 2000). Atypical regulation of RSA is associated with many psychiatric disorders including major depressive disorder and generalized anxiety disorder (Kemp et al., 2012), populations with high levels of aggression, such as perpetrators of violent domestic abuse (Umhau et al., 2002) and delayed recovery of RSA after a moderate exercise has been reported in women with a history of trauma (Dale et al., 2009).

In the present study, we collected measures of heart rate, RSA, electrodermal activity (EDA; a measure of sympathetic reactivity), salivary testosterone, and salivary cortisol while participants viewed a violent sport competition to examine individual differences in patterns of physiological responding to observed violence. We restricted our study sample to males because the challenge hypothesis, which shaped our research question, is based on male testosterone responses and because the past literature on testosterone responses are more consistent in males than in females (Josephs et al., 2011). In keeping with previous work on viewing acts of violence (Palomba et al., 2000), we predicted that participants would exhibit increased heart rate and EDA, and, possibly, increased cortisol. Bridging research on vagal regulation and challenge hypothesis (Wingfield et al., 1990), we predicted that testosterone release should not occur in those participants with relatively higher parasympathetic tone (as indexed by RSA) as they should not perceive a violent video as posing a threat to their social status. However, given the more reactive and defensive state associated with reduced parasympathetic tone (Porges, 2007), relatively lower RSA should increase the tendency to perceive social status threat and this should in turn should result in increased testosterone release. Finally, we examined whether individual differences in RSA reactivity to observing a violent competition were associated with differential patterns of neuroendocrine (cortisol, testosterone) activation.

MATERIALS AND METHODS

PARTICIPANTS

Males aged 18–35 ($n = 43$) were recruited from the University of Chicago campus and compensated with course credit or payment for their participation in the study. Participants were not screened for current health status, but were instructed to avoid alcohol for 12 h prior and nicotine for 1 h prior to participation. Heart rate and RSA were within normative range for this population (Byrne et al., 1996). Participants' written informed consent was obtained, and this study was approved by the Institutional Review Board at the University of Chicago. Three subjects were dropped from analysis due to equipment problems, leaving 40 participants, 20 each randomly assigned to control or experimental conditions.

STIMULI

Participants were randomly assigned to one of two groups. The experimental group was shown a 28-min video of a complete Mixed Martial Arts (MMA) fight (UFC 86 Forrest Griffin vs. Quinton "Rampage" Jackson, 2008). MMA is a full contact combat sport similar to boxing that involves the use of martial arts techniques. The selected fight had closely matched contestants and was judged as being extremely close, continuing for a full five rounds with no clear winner in contemporary press reports (Brookhouse, 2008) and the outcome being decided by a judge's decision that was not shown to subjects. The selection of a fight without an obvious winner and with contestants that unfamiliar to the subjects was done to eliminate any hormonal changes induced by rooting for the winner or loser in a sporting event (Bernhardt et al.,

1998). The control group viewed a 28-min non-arousing video on environmental building methods selected to mimic baseline controls in similar studies (Wirth and Schultheiss, 2006).

PROCEDURE

Participants abstained from alcohol for 12 h and food, caffeine, and nicotine for 1 h prior to the experiment to prevent contamination of the saliva samples (Salimetrics©, 2009; Poll et al., 2007). Upon arrival, participants were brought to a private study room, briefed on the protocol, and informed consent was acquired. Electrodes were attached for EDA and electrocardiography (ECG), and participants were asked to give a practice saliva sample to expose them to the procedure prior to experimental sample collection. The experimenter then left the participants in the room to complete a series of questionnaires administered on a computer. Completion of these questionnaires was used as a non-arousing task for participants during 30 min of sitting in a still and quiet environment, necessary to bring physiological measures to baseline (Lam et al., 2009). Baseline measures were taken while completing non-arousing questionnaires, since it has been argued that having participants perform a low level cognitive task may provide a better baseline than sitting quietly as it leaves less room for variance in the activity they are engaged in (Jennings et al., 2007).

After the 30-min period, a baseline salivary hormone sample was collected and placed immediately into a −20°C freezer. Autonomic measures, which act on a more rapid time course of seconds and typically are left for ∼5 min to stabilize, had also stabilized at this point. Participants were then left alone to watch either the fight or documentary. After the video, a second saliva sample was collected, electrodes were removed, and participants in the experimental condition were asked to answer two questions regarding their rooting behavior ("which fighter were you rooting for?" and "which fighter did you think won the fight?"). These measures were used to confirm that participants' perceptions of who won and who they wanted to win did not influence their physiological responses to the fight by running correlations between participants' ratings and the physiological measures. These correlations were not significant (see **Table 1**).

AUTONOMIC NERVOUS SYSTEM MEASUREMENTS AND QUANTIFICATION PROCEDURES

All physiological measurements were recorded using AcqKnowledge data recording software (version 3.8.1), running on a Windows XP computer, connected to a set of Biopac © amplifiers connected to a Biopac © MP 100 A A/D digitization system (digitizing signals at 1 kHz; Biopac © Systems, Inc., Santa Barbara, CA, USA). Electrodes were left undisturbed for 5 min before data was collected to ensure they reached stable impedance. EDA, heart rate, and RSA were collected throughout the entire experiment. Data from the videos were split into 5.6 min segments to correspond with the five rounds of the fight and 5.6 min of data were extracted from the questionnaire period as baseline.

ELECTRODERMAL ACTIVITY

Electrodermal activity was collected using a pair of Ag/AgCl electrodes filled with Biopac © isotonic electrode gel from the distal phalanges of digits II and III of the left hand (Stern et al., 2001). AcqKnowledge was used to extract and count the skin conductance responses (SCRs), which reflect sympathetic nervous system arousal with a time course of 3–5 s, from the EDA recording using its automated SCR detector with an SCR threshold of 0.02 μmho (Dawson et al., 2000). This analysis was manually supervised to ensure proper SCR identification and the exclusion of artifacts.

HEART RATE AND RSA

A Biopac mp100 was used to collect the ECG signal. The ECG was sampled at 1 kHz and a 60 Hz notch filter and a 0.5–35 Hz finite impulse response (FIR) filter (Ruha and Nissila, 1997) were applied. Inter-beat intervals (IBIs) series, as defined by R–R intervals in milliseconds, were extracted using supervised R-wave peak detection in AcqKnowledge 4.1 (Biopac©, 2000). IBI data were edited as previously described (Lewis et al., 2012) in order to remove artifacts that might confound the quantification of periodic components. Editing and heart rate variability quantification were performed with CardioEdit and CardioBatch programs (Brain-Body Center, University of Illinois at Chicago, IL, USA). The IBI series were linearly interpolated and filtered to remove spontaneous (0.12–0.40 Hz) breathing for RSA. A lower threshold of 0.12 Hz was utilized, as reports of healthy participants exhibiting respiration rates slower than 0.15 Hz are not uncommon (Denver et al., 2007; Saboul et al., 2014). The result is mathematically equivalent during steady state conditions to spectral analysis. This method is not moderated by respiration and conforms to the assumptions necessary for parametric statistics (Lewis et al., 2012). Data for RSA were quantified for sequential 30 s epochs, and data for heart rate were quantified in IBIs within each 5.6 min condition (i.e., baseline or round). Measures of average heart rate and average RSA were quantified for each condition.

While there is debate over whether respiration rate should be controlled for accurate measurement of RSA (Quintana and Heathers, 2014), the relationship between heart rate and RSA implemented here, is not moderated by respiration (Lewis et al., 2012). Furthermore, subjects were given no instruction with regard to their respiration rate as there is evidence that paced breathing instruction could add an additional cognitive demand by inducing dual attentional demands (Quintana and Heathers, 2014), and as result influencing the processing of the stimuli.

HORMONAL SAMPLE COLLECTION

Salivary testosterone and cortisol were obtained through saliva samples collected using un-stimulated passive drool techniques (Roney et al., 2007; Fairchild et al., 2008). This involved participants pooling saliva in their mouth for a minute and then drooling passively through a straw into a 2 mL Cryovial (Salimetrics©, 2009) until the vial was full. Samples were frozen immediately in a −20°C freezer kept on site and then moved to a −80°C freezer until analysis. After completion of data collection, saliva samples were thawed and hormonal assays were performed on the saliva for both testosterone and cortisol using Salimetrics © enzyme immunoassay kits for testosterone and cortisol as instructed (Salimetrics© 2011). Inter-assay CV value for cortisol was 3.56% and for testosterone was 2.63%. Intra-assay CVs for

Table 1 | Non-significant correlations between physiological measures and winning and rooting ratings for participants who watched the fight.

Physiological measure		Rooting	Won
RSA baseline	Pearson correlation	–0.350	−0.167
	Sig (2-tailed)	0.130	0.482
	N	20	20
RSA Epoch 1	Pearson correlation	−0.252	–0.173
	Sig (2-tailed)	0.283	0.466
	N	20	20
RSA Epoch 2	Pearson correlation	–0.152	–0.312
	Sig (2-tailed)	0.522	0.181
	N	20	20
RSA Epoch 3	Pearson correlation	−0.226	−0.218
	Sig (2-tailed)	0.338	0.357
	N	20	20
RSA Epoch 4	Pearson correlation	−0.260	−0.124
	Sig (2-tailed)	0.268	0.573
	N	20	20
RSA Epoch 5	Pearson correlation	−0.276	−0.068
	Sig (2-tailed)	0.240	0.776
	N	20	20
Heart rate baseline	Pearson correlation	−0.446	0.057
	Sig (2-tailed)	0.049	0.811
	N	20	20
Heart rate Epoch 1	Pearson correlation	0.300	0.077
	Sig (2-tailed)	0.198	0.746
	N	20	20
Heart rate Epoch 2	Pearson correlation	0.295	0.158
	Sig (2-tailed)	0.207	0.506
	N	20	20
Heart rate Epoch 3	Pearson correlation	0.357	0.051
	Sig (2-tailed)	0.122	0.831
	N	20	20
Heart rate Epoch 4	Pearson correlation	0.311	0.051
	Sig (2-tailed)	0.181	0.831
	N	20	20
Heart rate Epoch 5	Pearson correlation	0.258	0.066
	Sig (2-tailed)	0.272	0.876
	N	20	20
EDA baseline	Pearson correlation	0.067	−0.102
	Sig (2-tailed)	0.779	0.668
	N	20	20
EDA Epoch 1	Pearson correlation	0.029	0.282
	Sig (2-tailed)	0.905	0.229
	N	20	20
EDA Epoch 2	Pearson correlation	−0.057	0.071
	Sig (2-tailed)	0.810	0.765
	N	20	20

Continued

Table 1 | Continued

Physiological measure		Rooting	Won
EDA Epoch 3	Pearson correlation	−0.144	0.157
	Sig (2-tailed)	0.544	0.508
	N	20	20
EDA Epoch 4	Pearson correlation	−0.012	0.170
	Sig (2-tailed)	0.961	0.766
	N	20	20
EDA Epoch 5	Pearson correlation	0.021	0.309
	Sig (2-tailed)	0.929	0.110
	N	20	20
Pre-video cortisol	Pearson correlation	0.181	−0.098
	Sig (2-tailed)	0.472	0.698
	N	18	18
Post-video cortisol	Pearson correlation	0.204	−0.159
	Sig (2-tailed)	0.417	0.530
	N	18	18
Residual change cortisol	Pearson correlation	0.093	−0.164
	Sig (2-tailed)	0.714	0.514
	N	18	18
Pre-video testosterone	Pearson correlation	0.108	−0.138
	Sig (2-tailed)	0.668	0.585
	N	18	18
Post-video testosterone	Pearson correlation	0.214	−0.159
	Sig (2-tailed)	0.394	0.528
	N	18	18
Residual change testosterone	Pearson correlation	0.239	−0.087
	Sig (2-tailed)	0.314	0.730
	N	18	18

cortisol were 3.92 and 5.84% for each plate and for testosterone were 6.10 and 6.04% for each plate. Both testosterone and cortisol levels were within already published ranges for similar populations (Burnham et al., 2003; Wirth and Schultheiss, 2006; Murphy et al., 2010; Pilgrim et al., 2010; Randler et al., 2012). Due to insufficient amounts of saliva, four participants' saliva samples were excluded. This left a total of 36 measurements each for testosterone level, measured in pg/ml, and cortisol level, measured in μg/dl, with 18 measurements in the control group and 18 in the fight group.

STATISTICAL ANALYSES

In order to examine the autonomic and hormonal effects of viewing violence, data were screened for outliers using the interquartile range multiplier approach (Tukey, 1977; Hoaglin, 1986), with a multiplier if 2.2 (Hoaglin and Iglewicz, 1987), using this approach, no outliers were identified. We first ran repeated measures ANOVAs for the autonomic measures (EDA, heart rate, RSA) and hormonal measures (cortisol and testosterone) to examine differences in autonomic and hormonal responses between the fight and control group over time. In addition, correlations were performed with pre and post-stimuli and change (using unstandardized residuals where appropriate) in hormonal measures and RSA in the fight group to assess any potential relationships between parasympathetic reactivity and hormonal responsivity to viewing violence. Effect sizes were also calculated for all the analyses using Cohen's d. SPSS (v. 18.0, SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

RESULTS

ELECTRODERMAL ACTIVITY

To analyze group differences in EDA, a repeated measures ANOVA was performed: 2 (group: experimental/control) × 6 (interval: 5.6 min from baseline and five 5.6 min segments from the fight corresponding with each round of the fight). There was a main effect for EDA [$F(1,19) = 5.91$, $p = 0.020$, $d = 0.44$], indicating that the group observing the violent sporting event had significantly more SCRs. Moreover, there was a significant group by interval effect [$F(3.53,19) = 4.59$, $p = 0.003$,

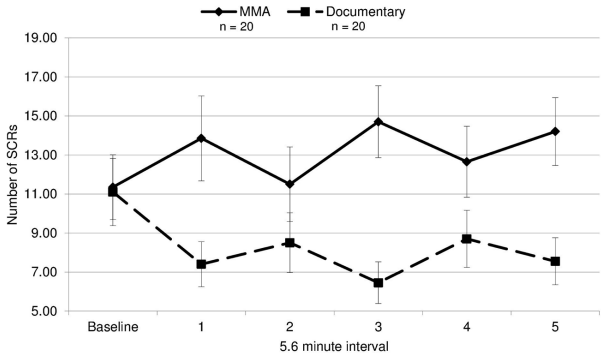


FIGURE 1 | Electrodermal activity. The mean number of skin conductance responses (SCRs) during each 5.6 min interval for the fight and the control documentary (error bars represent SD).

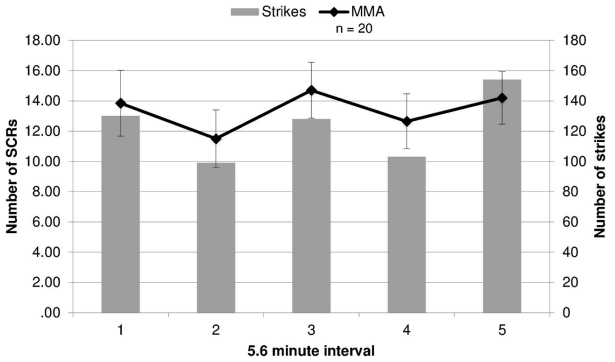


FIGURE 2 | Electrodermal activity responses compared to strikes during fights. The number of strikes and number of skin conductance responses (SCRs) during each round of the fight (error bars represent SD).

$\varepsilon = 0.71$[1]; **Figure 1**]. This interaction was characterized not only by the group observing violence having increased SCRs overall but also exhibiting round-specific differences compared to the group viewing the documentary. Uncorrected contrasts identified rounds 1 [$F(1,19) = 5.91$, $p = 0.020$], 3 [$F(1,19) = 12.40$, $p = 0.001$], and 5 [$F(1,19) = 8.98$, $p = 0.005$] as significantly different from baseline by group. This was not the case for rounds 2 [$F(1,19) = 1.13$, $p = 0.295$] and 4 [$F(1,19) = 2.71$, $p = 0.108$].

Because SCR response differed by round, we evaluated whether the round by round differences in SCRs were related to round specific quantitative differences in physical violence. Different amounts of violence between rounds might drive the differing EDA responses in viewers. This was tested by contrasting the number of strikes and maneuvers during each round of the MMA fight, quantified using an independent professional service which provides statistics for MMA and boxing events (www.compustrike.com, UFC 86: Jackson vs. Griffin), with the number of SCRs for each round. The rounds in which SCRs did not differ significantly from the baseline by group (rounds 2 and 4) contained the fewest strikes (**Figure 2**). This quantitative index of violence during each round mapped onto participants' EDA response to viewing the fight. Additionally, a correlation analysis was run between average SCRs for each round and total number of strikes, bordered on significance ($r = 0.82$, $p = 0.090$).

HEART RATE AND RSA

A 2 (condition: fight/control) × 6 (six 5.6 min intervals) repeated measures ANOVA was performed on both RSA and heart rate data to determine group differences and within-subject differences across time. For heart rate, a main effect of time [$F(3.30,19) = 14.321$, $p = 0.000$] and a near significant effect of group [$F(3.30,19) = 3.26$, $p = 0.079$] were identified. There was a significant group by condition interaction for heart rate [$F(3.30,19) = 5.04$, $p = 0.002$, $\varepsilon = 0.66$][1]. The differences between groups were amplified as the study progressed (**Figure 3**), with

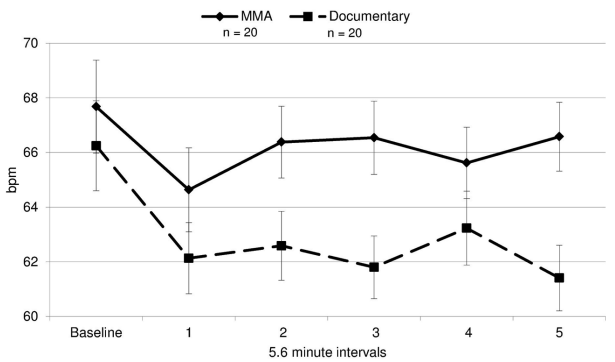


FIGURE 3 | Heart rate. Heart rate in beats per minute (bpm) during each time interval for each group for the fight and control group (error bars represent SD).

heart rate increasing in the fight group and decreasing in the control group. Uncorrected contrasts identified significant differences between groups during rounds one and 3 [$F(1,19) = 8.59$, $p = 0.006$], three and 4 [$F(1, 19) = 7.54$, $p = 0.009$], and 4 and 5 [$F(1,19) = 14.13$, $p = 0.001$]. In addition, uncorrected contrasts identified significant differences between baseline and rounds three by group [$F(1,19) = 6.07$, $p = 0.018$] and 5 [$F(1,19) = 11.43$, $p = 0.002$]. In contrast, as illustrated in **Figure 4**, RSA remained relatively stable through the experiment and did not exhibit group differences across baseline or the five rounds [$F(1,19) = 0.05$, $p = 0.834$, $d = 0.004$].

HORMONAL MEASURES

In order to discern if there were any significant group differences in changes in testosterone and cortisol, a 2 (group: MMA/control) × 2 (pre and post hormone levels) repeated measures ANOVA was performed for both testosterone and cortisol. There was a main effect of time for cortisol [$F(1,34) = 13.097$, $p = 0.001$] but not for testosterone [$F(1,34) = 1.192$, $p = 0.283$]. Neither testosterone [$F(1,17) = 0.45$, $p = 0.509$, $d = 0.17$] nor cortisol [$F(1,17) = 2.81$, $p = 0.103$, $d = 0.40$] exhibited any significant differences between groups, suggesting that there were no

[1]Degrees of freedom were adjusted using the Greenhouse-Geisser correction for violations of sphericity.

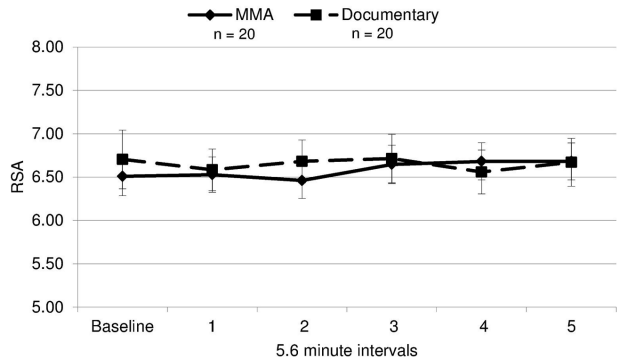


FIGURE 4 | RSA means. Respiratory sinus arrhythmia (RSA, i.e., a measure of parasympathetic nervous system activity) during each interval for each group (error bars represent SD).

significant increases in either measure in response to viewing the fight compared to the documentary. The medium effect size for cortisol ($d = 0.40$) suggests that the non-significant finding for cortisol may be a result of the low power ($n = 18$ per group) and should be investigated in future research.

For both testosterone and cortisol, change scores were calculated by taking post video level minus pre video level. Unstandardized residuals were then calculated by running a regression analysis using post-fight levels as the dependent variable and raw change score as the independent variable and saving the unstandardized residuals. While residual change is often calculated by regressing the post measure on pre measure, we used the more conservative method, regressing change from pre measures to post measures on pre measures. Using unstandardized residuals in place of raw change scores provides a more accurate depiction of change by accounting for variance in initial baseline levels between subjects, see **Table 2** (Mehta and Josephs, 2006). To examine whether there was a relationship between hormone responsivity to the fight and RSA, exploratory correlations between testosterone and cortisol unstandardized residuals and RSA for the fight and control group separately. Because RSA did not change in response to the fight, RSA was averaged over the six epochs (baseline and the five rounds) and this value was used in the correlations. A statistically significant correlation for the fight group was observed between the unstandardized residual for testosterone and RSA [$r(16) = -0.503$, $p = 0.033$; see **Figure 5**]. The correlation between the unstandardized residual for cortisol and RSA for the fight group was not statistically significant [$r(16) = -0.361$, $p = 0.142$], and there were no significant correlations between the unstandardized residuals for cortisol and testosterone and RSA in the control group [Testosterone: $r(16) = -0.266$, $p = 0.286$; Cortisol: $r(16) = 0.242$, $p = 0.333$]. The significant negative correlation between RSA and testosterone responses suggests that viewers with lower levels of RSA throughout the baseline condition and the fight exhibited greater increases in testosterone while viewing the fight.

DISCUSSION

This study investigated individual differences in autonomic and hormonal responses to viewing violence. Specifically, whether viewing violence results in a release of testosterone, and whether individuals' testosterone response to observed violence is modulated by individual differences in autonomic state. Research has previously demonstrated testosterone release in males in anticipation of conflicts that influence social status and reproductive opportunities (Archer, 1991). We predicted that violent stimuli without consequences for an individual's social status and/or reproductive opportunities generally should not elicit a testosterone response in a healthy population. If it did, it would be most likely in participants with lower parasympathetic tone (as indexed by RSA). The polyvagal theory (Porges, 1995) predicts that, as a physiological state, relatively lower RSA is associated with a more reactive, defensive state. A testosterone response may provide evidence that a physiologically defensive individual may have experienced this violent video as a challenge to their status (Archer, 2006; Eisenegger et al., 2011; Josephs et al., 2011; van Honk et al., 2011a).

The findings of this study support our predictions. Participants in the experimental condition displayed no significant increases in testosterone as compared to the control group. While participants did exhibit increased EDA and heart rate in response to viewing violent stimuli, interestingly, they did not demonstrate any change in vagal tone measured by RSA. In addition, there was also no significant increase overall in cortisol in participants after viewing the fight, indicating that the viewing the fight was not perceived as a stressor, as cortisol is also associated with increased reactivity and stress mobilization (Foley and Kirschbaum, 2010).

Table 2 | Summary of descriptive statistics for hormonal measures.

	Fight		Documentary	
	M	**SD**	***M***	**SD**
Pre-video cortisol	0.24 μg/dl	0.17 μg/dl	0.18 μg/dl	0.07 μg/dl
Post-video cortisol	0.20 μg/dl	0.12 μg/dl	0.13 μg/dl	0.05 μg/dl
Pre-video testosterone	150.8 pg/ml	54.1 pg/ml	141.3 pg/ml	52.6 pg/ml
Post-video testosterone	146.7 pg/ml	48.1 pg/ml	135.0 pg/ml	43.2 pg/ml
Residual cortisol	0.01 μg/dl	0.05 μg/dl	–0.01 μg/dl	0.04 μg/dl
Residual testosterone	2.4 pg/ml	25.2 pg/ml	–2.4 pg/ml	23.4 pg/ml

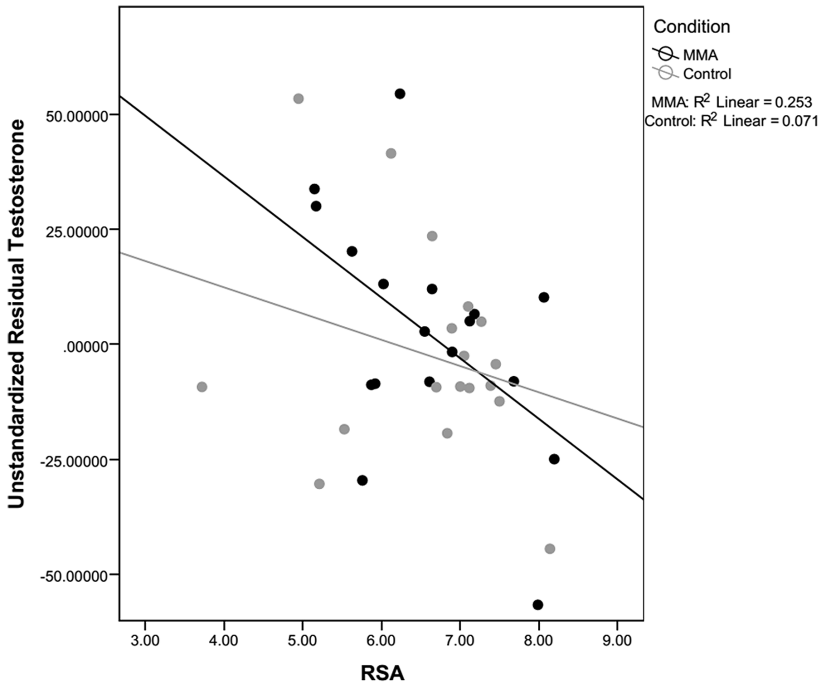


FIGURE 5 | Correlation between RSA and testosterone. Correlations between residual testosterone and respiratory sinus arrhythmia (RSA) level for the fight and control conditions. Only correlations for the fight were significant.

While this study did not find an overall increase in testosterone in individuals viewing the fight, there was a negative correlation between RSA and testosterone change to viewing the fight, indicating that individuals with lower levels of RSA throughout the study had greater increases in testosterone in response to viewing the fight. This finding suggests that individuals with low baseline vagal tone may be more vulnerable to perceiving observed violence as a challenge or threat to their status than those outside of the low range of RSA. It has been reported that low RSA is a risk factor for social and emotional regulatory disorders. Low levels of baseline RSA are associated with varying forms of social and emotional disorders. For instance, women with a history of abuse, borderline personality disorder patients, and patients on the spectrum for autistic disorders (Porges, 2011) have been associated with decreased RSA and atypical regulation of RSA during task demands. In addition, children with a history of abuse and lower baseline levels of RSA are at a greater risk for developing conduct problems than children with a history of abuse but higher baseline levels of RSA (Katz, 2007). Low baseline RSA in infants has been associated with increased regulatory problems during infancy and behavioral difficulties later in childhood (Porges, 2011). These findings indicate that while lower levels of cardiac vagal tone, an index of central nervous system regulation, are linked with increases in testosterone to viewing violence suggesting a possible misperception of the observed violence as a direct threat to their status. These individuals may represent an already susceptible population, with associated difficulties in social interactions and emotion regulation. It is important to note, that as this study focused on individual differences in physiological responses to viewing violence and did not measure behavior after viewing the fight, statements about possible behaviors as a result of physiological state are purely speculative and should be further tested.

This study is one of the first to examine the complex interaction among components of the autonomic nervous system while viewing violence, incorporating both an index of parasympathetic activity (RSA), and measures of sympathetic responses (EDA). In the population of young men studied here, the sympathetic arousal exhibited while viewing violence would not be likely to produce increased reactivity and increased mobilization because the observed increase in sympathetic nervous system activity was not coupled with the withdrawal of the vagal brake, which serves to inhibit physiological arousal (Porges, 2007). The complementary withdrawal of parasympathetic tone and increase of sympathetic is characteristic of mobilization in response to a threat and there was no parasympathetic withdrawal observed. In addition, the lack of overall increases in testosterone levels suggests that observing violence alone is not sufficient to elicit testosterone release.

Due to the exploratory nature of this study, there are several limitations that need to be acknowledged. The first, and most importantly, is the relatively small sample size for the correlation analyses performed on the fight group ($n = 18$). Additionally, the correlation analyses in this study were exploratory in nature and need to be replicated in future studies. The generalizability of the population is also limited as participants were all college students, a population exposed to greater levels of chronic stress, which could affect physiological responses (Pruessner et al., 1999; Wüst et al., 2000; Dickerson and Kemeny, 2004). While this study was

designed to investigate within-subject changes, the lack of normative responses to this novel paradigm limited the ability to interpret the findings. Future research should establish the reproducibility of these findings and account for attentional variance by utilizing a within-subjects rating of the stimuli. Additionally, the inclusion of "control" non-violent stimuli (e.g., tennis) that are comparably competitive and exciting would be informative.

Factors capable of influencing emotion regulation, including the role of viewing violence, and the physiological consequences of such experiences are only now becoming apparent. In the context of understanding the effects of observations of violence, future research should continue to investigate the possible role of changes in testosterone, as well as the potentially protective role of the parasympathetic nervous system (PNS). In addition, future studies should employ larger sample sizes and a broader range of individual differences and experiences, including gender differences in the effects of violent stimuli on endocrine and behavioral responses.

CONCLUSION

This study examined physiological and hormonal responses to viewing violent stimuli, and found that in an undergraduate population, individuals only show increases in sympathetic nervous system activity, with no parasympathetic changes. However, and more importantly, in individuals with low baseline levels of RSA, a physiology already associated with vulnerability to psychopathology, observing violence elicited a greater increase in testosterone compared to those with higher levels of RSA. This may suggest that this already vulnerable population may be at risk for antisocial and aggressive behaviors after viewing violence. Furthermore, the testosterone release shown here may have additional consequences with regard to their perception and evaluation of social stimuli. The consequences of this across the lifespan, could may be an increased likelihood of conflict and the associated socioemotional consequences. Additional research utilizing wider age range, larger populations and explicit measures of post viewing behaviors is important to confirm and expand on this finding.

ACKNOWLEDGMENTS

The study was supported by an NSF award (BCS-0718480) to Dr. Jean Decety. We are grateful for advice on data analyses and statistical analyses from Dr. Stephen Porges and his lab members at the University of Illinois at Chicago. Dr. Hossein Nazarloo of the University of Illinois at Chicago provided valuable assistance with the hormone analysis, and we are grateful to Dr. Greg Norman for his comments and help in the preparation of this manuscript.

REFERENCES

Anderson, C. A., and Bushman, B. J. (2001). Effects of violent video games on aggressive behavior, aggressive cognition, aggressive affect, physiological arousal, and prosocial behavior: a meta-analytic review. *Psychol. Sci.* 12, 353–359. doi: 10.1111/1467-9280.00366

Archer, J. (1991). The influence of testosterone on human aggression. *Br. J. Psychol.* 82, 1–28. doi: 10.1111/j.2044-8295.1991.tb02379.x

Archer, J. (2006). Testosterone and human aggression: an evaluation of the challenge hypothesis. *Neurosci. Biobehav. Rev.* 30, 319–345. doi: 10.1016/j.neubiorev.2004.12.007

Bernhardt, P. C., Dabbs, J. M., Fielden, J. A., and Lutter, C. D. (1998). Testosterone changes during vicarious experiences of winning and losing among fans at sporting events. *Physiol. Behav.* 65, 59–62. doi: 10.1016/S0031-9384(98)00147-4

Boonekamp, J. J. J., Ros, A. A. H. F., and Verhulst, S. (2008). Immune activation suppresses plasma testosterone level: a meta-analysis. *Biol. Lett.* 4, 741–744. doi: 10.1098/rsbl.2008.0347

Brookhouse, B. (2008). *UFC 86 Live Results–Forrest Griffin vs. Quinton "Rampage" Jackson*. Available at: BloodyElbow.com.

Burnham, T. C., Chapman, J. F., Gray, P. B., McIntyre, M. H., Lipson, S. F., and Ellison, P. T. (2003). Men in committed, romantic relationships have lower testosterone. *Horm. Behav.* 44, 119–122. doi: 10.1016/S0018-506X(03)00125-9

Byrne, E. A., Fleg, J. L., Vaitkevicius, P. V., Wright, J., and Porges, S. W. (1996). Role of aerobic capacity and body mass index in the age-associated decline in heart rate variability. *J. Appl. Physiol.* 81, 743–750.

Costa, R., and Salvador, A. (2012). Associations between success and failure in a face-to-face competition and psychobiological parameters in young women. *Psychoneuroendocrinology* 37, 1780–1790. doi: 10.1016/j.psyneuen.2012.03.012

Dale, L. P., Carroll, L. E., Galen, G., Hayes, J. A., Webb, K. W., and Porges, S. W. (2009). Abuse history is related to autonomic regulation to mild exercise and psychological wellbeing. *Appl. Psychophysiol. Biofeedback* 34, 299–308. doi: 10.1007/s10484-009-9111-4

Dawson, M., Schell, A., and Filion, D. (2000). "The electrodermal system," in *Handbook of Psychophysiology*, eds J. T. Cacioppo, L. Tassinary, and G. Berntston (Cambridge: Cambridge University Press), 200–223.

Denver, J. W., Reed, S. F., and Porges, S. W. (2007). Methodological issues in the quantification of respiratory sinus arrhythmia. *Biol. Psychol.* 74, 286–294. doi: 10.1016/j.biopsycho.2005.09.005

Derntl, B., Windischberger, C., Robinson, S., Kryspin-Exner, I., Gur, R. C., Moser, E., et al. (2009). Amygdala activity to fear and anger in healthy young males is associated with testosterone. *Psychoneuroendocrinology* 34, 687–693. doi: 10.1016/j.psyneuen.2008.11.007

Dickerson, S. S., and Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol. Bull.* 130, 355–391. doi: 10.1037/0033-2909.130.3.355

Eisenegger, C., Haushofer, J., and Fehr, E. (2011). The role of testosterone in social interaction. *Trends Cogn. Sci.* 15, 263–271. doi: 10.1016/j.tics.2011.04.008

Fairchild, G., van Goozen, S. H. M., Stollery, S. J., Brown, J., Gardiner, J., Herbert, J., et al. (2008). Cortisol diurnal rhythm and stress reactivity in male adolescents with early-onset or adolescence-onset conduct disorder. *Biol. Psychiatry* 64, 599–606. doi: 10.1016/j.biopsych.2008.05.022

Foley, P., and Kirschbaum, C. (2010). Human hypothalamus-pituitary-adrenal axis responses to acute psychosocial stress in laboratory settings. *Neurosci. Biobehav. Rev.* 35, 91–96. doi: 10.1016/j.neubiorev.2010.01.010

Hall, R. C., Day, T., and Hall, R. C. (2011). A plea for caution: violent video games, the supreme court, and the role of science. *Mayo Clin. Proc.* 86, 315–321. doi: 10.4065/mcp.2010.0762

Hoaglin, D. (1986). Performance of some resistant rules for outlier labeling. *J. Am. Stat. Assoc.* 81, 991–999. doi: 10.1080/01621459.1986.10478363

Hoaglin, D. C., and Iglewicz, B. (1987). Fine-tuning some resistant rules for outlier labeling. *J. Am. Stat. Assoc.* 82, 1147–1149. doi: 10.1080/01621459.1987.10478551

Jennings, J. R., Kamarck, T., Stewart, C., Eddy, M., and Johnson, P. (2007). Alternate cardiovascular baseline assessment techniques: vanilla or resting baseline. *Psychophysiology* 29, 742–750. doi: 10.1111/j.1469-8986.1992.tb02052.x

Jiménez, M., Aguilar, R., and Alvero-Cruz, J. (2012). Effects of victory and defeat on testosterone and cortisol response to competition: evidence for same response patterns in men and women. *Psychoneuroendocrinology* 37, 1577–1581. doi: 10.1016/j.psyneuen.2012.02.011

Josephs, R. R. A., Mehta, P. P. H., and Carré, J. M. J. (2011). Gender and social environment modulate the effects of testosterone on social behavior: comment on Eisenegger et al. *Trends Cogn. Sci.* 15, 509–510; author reply 510–511. doi: 10.1016/j.tics.2011.09.002

Katz, L. F. (2007). Domestic violence and vagal reactivity to peer provocation. *Biol. Psychol.* 74, 154–164. doi: 10.1016/j.biopsycho.2005.10.010

Kemp, A. H., Quintana, D. S., Felmingham, K. L., Matthews, S., and Jelinek, H. F. (2012). Depression, comorbid anxiety disorders, and heart rate variability in physically healthy, unmedicated patients: implications for cardiovascular risk. *PLoS ONE* 7:e30777. doi: 10.1371/journal.pone.0030777

Lam, S., Dickerson, S. S., Zoccola, P. M., and Zaldivar, F. (2009). Emotion regulation and cortisol reactivity to a social-evaluative speech task. *Psychoneuroendocrinology* 34, 1355–1362. doi: 10.1016/j.psyneuen.2009.04.006

Lane, R. R. D., McRae, K., Reiman, E. M. E., Chen, K., Ahern, G. L., and Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *Neuroimage* 44, 213–222. doi: 10.1016/j.neuroimage.2008.07.056

LeDoux, J. (2007). The amygdala. *Curr. Biol.* 17, R868–R874. doi: 10.1016/j.cub.2007.08.005

Lewis, G. F., Furman, S. A., McCool, M. F., and Porges, S. W. (2012). Statistical strategies to quantify respiratory sinus arrhythmia: are commonly used metrics equivalent? *Biol. Psychol.* 89, 349–364. doi: 10.1016/j.biopsycho.2011.11.009

Mazur, A., Booth, A., and Dabbs, J. M. Jr. (1992). Testosterone and chess competition. *Soc. Psychol. Q.* 55, 70–77. doi: 10.2307/2786687

Mehta, P. H., and Beer, J. (2010). Neural mechanisms of the testosterone-aggression relation: the role of orbitofrontal cortex. *J. Cogn. Neurosci.* 22, 2357–2368. doi: 10.1162/jocn.2009.21389

Mehta, P. H., and Josephs, R. A. (2006). Testosterone change after losing predicts the decision to compete again. *Horm. Behav.* 50, 684–692. doi: 10.1016/j.yhbeh.2006.07.001

Murphy, L., Denis, R., Ward, C. P., and Tartar, J. L. (2010). Academic stress differentially influences perceived stress, salivary cortisol, and immunoglobulin-A in undergraduate students. *Stress* 13, 365–370. doi: 10.3109/10253891003615473

Napadow, V., Dhond, R., Conti, G., Makris, N., Brown, E. N., and Barbieri, R. (2008). Brain correlates of autonomic modulation: combining heart rate variability with fMRI. *Neuroimage* 42, 169–177. doi: 10.1016/j.neuroimage.2008.04.238

Ohman, A. (2005). The role of the amygdala in human fear: automatic detection of threat. *Psychoneuroendocrinology* 30, 953–958. doi: 10.1016/j.psyneuen.2005.03.019

O'Keane, V., Dinan, T. G., Scott, L., and Corcoran, C. (2005). Changes in hypothalamic-pituitary-adrenal axis measures after vagus nerve stimulation therapy in chronic depression. *Biol. Psychiatry* 58, 963–968. doi: 10.1016/j.biopsych.2005.04.049

Paik, H., and Comstock, G. (1994). The effects of television violence on antisocial behavior: A Meta-Analysis1. *Commun. Res.* 21, 516–546. doi: 10.1177/009365094021004004

Palomba, D., Sarlo, M., Angrilli, A., Mini, A., and Stegagno, L. (2000). Cardiac responses associated with affective processing of unpleasant film stimuli. *Int. J. Psychophysiol.* 36, 45–57. doi: 10.1016/S0167-8760(99)00099-9

Pilgrim, K., Marin, M.-F., and Lupien, S. J. (2010). Attentional orienting toward social stress stimuli predicts increased cortisol responsivity to psychosocial stress irrespective of the early socioeconomic status. *Psychoneuroendocrinology* 35, 588–595. doi: 10.1016/j.psyneuen.2009.09.015

Poll, E.-M., Kreitschmann-Andermahr, I., Langejuergen, Y., Stanzel, S., Gilsbach, J. M., Gressner, A., et al. (2007). Saliva collection method affects predictability of serum cortisol. *Clin. Chim. Acta* 382, 15–19. doi: 10.1016/j.cca.2007.03.009

Porges, S. (2007). The polyvagal perspective. *Biol. Psychol.* 74, 116–143. doi: 10.1016/j.biopsycho.2006.06.009

Porges, S. (2011). *The Polyvagal Theory: Neurophysiological Foundations of Emotions, Attachment, Communication, and Self-Regulatoins.* New York: WW Norton.

Porges, S. W. (1995). Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A Polyvagal Theory. *Psychophysiology* 32, 301–318. doi: 10.1111/j.1469-8986.1995.tb01213.x

Pruessner, J. C., Hellhammer, D. H., and Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosom. Med.* 61, 197–204. doi: 10.1097/00006842-199903000-00012

Quintana, D. S., and Heathers, J. A. J. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Front. Psychol.* 5:805. doi: 10.3389/fpsyg.2014.00805

Randler, C., Ebenhöh, N., Fischer, A., Höchel, S., Schroff, C., Stoll, J. C., et al. (2012). Chronotype but not sleep length is related to salivary testosterone in young adult men. *Psychoneuroendocrinology* 37, 1740–1744. doi: 10.1016/j.psyneuen.2012.02.008

Roney, J. R., Lukaszewski, A. W., and Simmons, Z. L. (2007). Rapid endocrine responses of young men to social interactions with young women. *Horm. Behav.* 52, 326–333. doi: 10.1016/j.yhbeh.2007.05.008

Ruha, A., and Nissila, S. (1997). A real-time microprocessor QRS detector system with a 1-ms timing accuracy for the measurement of ambulatory HRV. *IEEE Trans. Biomed. Eng.* 44, 159–167. doi: 10.1109/10.554762

Saboul, D., Pialoux, V., and Hautier, C. (2014). The breathing effect of the LF/HF ratio in the heart rate variability measurements of athletes. *Eur. J. Sport Sci.* 14(Suppl. 1), S282–S288. doi: 10.1080/17461391.2012.691116

Stanton, S. J., Wirth, M. M., Waugh, C. E., and Schultheiss, O. C. (2009). Endogenous testosterone levels are associated with amygdala and ventromedial prefrontal cortex responses to anger faces in men but not women. *Biol. Psychol.* 81, 118–122. doi: 10.1016/j.biopsycho.2009.03.004

Stern, R. M., Ray, W. J., and Quigley, K. S. (2001). *Psychophysiological Recording.* 2nd Edn. Oxford: Oxford University Press.

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Tukey, J. W. (1977). *Exploratory Data Analysis.* Reading, PA: Addison-Wesley.

Ulrich-Lai, Y. M., and Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci.* 10, 397–409. doi: 10.1038/nrn2647

Umhau, J. C., George, D. T., Reed, S., Petrulis, S. G., Rawlings, R., and Porges, S. W. (2002). Atypical autonomic regulation in perpetrators of violent domestic abuse. *Psychophysiology* 39, 117–123. doi: 10.1111/1469-8986.3920117

van der Meij, L., Almela, M., Hidalgo, V., Villada, C., Ijzerman, H., van Lange, P. A. M., et al. (2012). Testosterone and cortisol release among Spanish soccer fans watching the 2010 World Cup final. *PLoS ONE* 7:e34814. doi: 10.1371/journal.pone.0034814

van Honk, J., Schutter, D. J., Bos, P. A., Kruijt, A.-W., Lentjes, E. G., and Baron-Cohen, S. (2011a). Testosterone administration impairs cognitive empathy in women depending on second-to-fourth digit ratio. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3448–3452. doi: 10.1073/pnas.1011891108

van Honk, J., Terburg, D., and Bos, P. A. (2011b). Further notes on testosterone as a social hormone. *Trends Cogn. Sci.* 15, 291–292. doi: 10.1016/j.tics.2011.05.003

Volman, I., Toni, I., Verhagen, L., and Roelofs, K. (2011). Endogenous testosterone modulates prefrontal-amygdala connectivity during social emotional behavior. *Cereb. Cortex* 21, 2282–2290. doi: 10.1093/cercor/bhr001

Wingfield, J. C., Hegner, R. E., Dufty, A. M., and Ball, G. F. (1990). The "Challenge Hypothesis": thoeretical implications for patterns of testosterone secrtion, mating systems, and breeding strategies. *Am. Nat.* 136, 829–846. doi: 10.1086/285134

Wirth, M. M., and Schultheiss, O. C. (2006). Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol. *Horm. Behav.* 50, 786–795. doi: 10.1016/j.yhbeh.2006.08.003

Wüst, S., Federenko, I., Hellhammer, D. H., and Kirschbaum, C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology* 25, 707–720. doi: 10.1016/S0306-4530(00)00021-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 17 July 2014; accepted: 07 January 2015; published online: 24 February 2015. Citation: Porges EC, Smith KE and Decety J (2015) Individual differences in vagal regulation are related to testosterone responses to observed violence. Front. Psychol. ***6****:19. doi: 10.3389/fpsyg.2015.00019*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in Psychology

ORIGINAL RESEARCH
published: 10 March 2015
doi: 10.3389/fpsyg.2015.00261

Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation

DeWayne P. Williams[1]*, *Claudia Cash*[1,2], *Cameron Rankin*[1], *Anthony Bernardi*[1], *Julian Koenig*[1] and *Julian F. Thayer*[1]

[1] Department of Psychology, The Ohio State University, Columbus, OH, USA, [2] The Rollins School of Public Health, Emory University, Atlanta, GA, USA

OPEN ACCESS

Edited by:
Andrew Kemp,
Universidade de São Paulo, Brazil

Reviewed by:
Stefan Sütterlin,
Lillehammer University College, Norway
Adrian Meule,
LWL University Hospital of the Ruhr University Bochum, Germany
Fay Geisler,
University of Greifswald, Germany

***Correspondence:**
DeWayne P. Williams,
Department of Psychology,
The Ohio State University,
1835 Neil Ave, Room 175, Columbus,
43210 OH, USA
williams.2917@buckeyemail.osu.edu

Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: 31 December 2014
Accepted: 22 February 2015
Published: 10 March 2015

Citation:
Williams DP, Cash C, Rankin C, Bernardi A, Koenig J and Thayer JF (2015) Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation. Front. Psychol. **6**:261. doi: 10.3389/fpsyg.2015.00261

The *Model of Neurovisceral Integration* suggests that vagally mediated heart rate variability (vmHRV) represents a psychophysiological index of inhibitory control and thus, is associated with emotion regulation capacity. Over the past decade, growing empirical evidence supports this notion, showing that those with higher resting vmHRV can regulate negative emotions more adequately. However, to our knowledge, no study has previously examined how resting vmHRV may relate to everyday perceived difficulties in emotion regulation. The present study attempts to examine such relationship in 183 undergraduate students (98 female, 60 minority, mean Age = 19.34). Resting vmHRV was collected during a 5-min resting baseline period, and everyday difficulties in emotion regulation were assessed using the Difficulties in Emotion Regulation Scale (DERS). Controlling for potential covariates (including both trait anxiety and rumination), results revealed a negative relationship between resting vmHRV and DERS such that lower resting vmHRV was associated with greater difficulties in emotional regulation, especially a lack of emotional clarity and impulse control, as indicated by the respective subscales of the DERS. These findings provide further evidence for the *Neurovisceral Integration Model*, suggesting that emotion regulation and autonomic regulation share neural networks within the brain. Moreover, the present study extends prior research by highlighting two distinct facets of emotion regulation (impulse control and emotional clarity) that should be of particular interest when investigating the link between emotion regulation, resting vmHRV, and related health outcomes including morbidity and mortality.

Keywords: heart rate variability, emotion regulation, rumination, anxiety, impulse control, emotional clarity, inhibition

Introduction

> "To act wisely, we must see clearly."
>
> (Easwaren, 2010, p. 184).

Emotion regulation (ER) is defined as a process by which individuals modify their emotional experiences, expressions, and subsequent physiological responses (Aldao, 2013).

Inhibitory control is a key mechanism for successful ER—individuals are required to inhibit prepotent emotional responses in service of more desirable and appropriate ones (Lane et al., 2009; Thayer et al., 2012). Emotional responses that are consistent with environmental demands represent adaptive ER and promote physical and mental health. In contrast, emotional responses that are inconsistent with environmental demands (e.g., impulsive emotional responses) represent maladaptive ER, and predict disease and mortality (Thayer and Lane, 2000; Thayer et al., 2012). To better understand psychophysiological mechanisms linking inhibition, ER, and overall health, Thayer and Lane (2000) proposed that the characteristic beat-to-beat variability in the heart rate (HR) time series—heart rate variability (HRV)—serves not only as an index of healthy heart function (Thayer and Lane, 2007), but as an readily available index and measure of inhibitory control, and more specifically, ER capacity.

The Neurovisceral Integration Model (NIM)

Executive brain areas, such as the prefrontal cortex, exert an inhibitory influence on sub-cortical structures, such as the amygdala, allowing the organism to adaptively respond to demands from the environment, and organize their emotional and behavioral responses effectively (Thayer et al., 2012). Thus, at rest, active cortical brain areas are indicative of greater inhibitory and ER (Thayer et al., 2012). Converging evidence suggests that these core sets of neural structures are not only responsible for inhibition, but also the regulation of the autonomic nervous system (ANS) (re)activity (Hansen et al., 2004). The heart (and other peripheral organs) is under tonic inhibitory control by the ANS. More specifically, this influence is characterized by a relative dominance of the parasympathetic nervous system (PNS) over influences of the sympathetic nervous system (SNS) (Thayer and Lane, 2009; Thayer et al., 2012). Vagal parasympathetic control represents the major descending inhibitory pathway (DIP), adaptively regulating physiological functions (i.e., immune, inflammatory, and cardiac function, Thayer and Sternberg, 2006; Weber et al., 2010) shaped by psychological processes such as ER (Thayer and Lane, 2000; Thayer and Sternberg, 2010). Therefore, the NIM posits that vagally mediated HRV (vmHRV) may be more than just a simple index of healthy heart function, but may in fact serve as readily available measure and index of the degree to which the brain's integrative system for adaptive regulation provides flexible control over the periphery. Overall, it is suggested that this common reciprocal inhibitory cortico-subcortical neural circuit serves as the structural link between psychological processes such as ER, and health-related physiological processes, and that this circuit can be indexed by vmHRV (Thayer et al., 2012).

Emotion Regulation and HRV

Adopting the NIM framework, researchers have explored various contexts where individuals with low resting vmHRV fail at and/or have difficulties with ER, in addition to downstream physical and mental consequences (as indexed by phasic or acute changes in vmHRV) (Carrico et al., 2006; Lane et al., 2009; Melzig et al., 2009; Geisler et al., 2010; Aldao et al., 2012). For example, there is evidence suggesting that those with high vmHRV, in comparison to those with lower levels, show appropriate emotional responses, as indexed by emotion-modulated startle responses, fear-potentiated startle responses, phasic heart rate responses, and behavioral emotional responses (Melzig et al., 2009). More recently, research showed that those with higher vmHRV are better able to suppress unwanted thoughts in comparison to individuals with low vmHRV (Gillie et al., 2014). While this substantial amount of evidence supports the link between vmHRV and ER, studies have not yet directly examined how resting vmHRV may predict individuals' perceived difficulties in ER, along with the strength of the association between resting HRV and varying facets of ER.

Multifaceted Conceptualization of Emotion Regulation

Indeed, some researchers propose that ER is not a unitary construct and instead, involves multiple components (Gratz and Roemer, 2004). Specifically, vmHRV has been shown to predict ER primarily in the context of strategies (Thayer and Lane, 2000; Aldao and Mennin, 2012), that is, people with higher vmHRV are often more successful at regulating emotions when told to use a particular ER strategy. However, this work may not capture other facets of ER, such as emotional clarity and acceptance of emotions, as such facets have been shown to undermine successful ER (Gratz and Roemer, 2004) and thus, may have subsequent effects on health. Given that little is known about the relationship between vmHRV and different facets of ER, the following investigation not only attempts to link vmHRV with self-reported ER, but it also aims to understand which facets of ER are most strongly associated with vmHRV.

Traits that Relate to Emotion Regulation Failure and Lower Resting Heart Rate Variability

It is proposed that both lower vmHRV and difficulties in ER are characteristic of certain psychopathological states (Thayer and Lane, 2000). For example, research has provided compelling evidence that trait anxiety is associated with both lower vmHRV and ER capabilities. Likewise, rumination has also been associated with lower vmHRV and ER capabilities. In fact, both higher trait anxiety and rumination, and lower vmHRV and difficulties in ER are characteristic of major psychopathological disorders, such as major depressive disorder, generalized anxiety disorder, and posttraumatic stress disorder (Friedman and Thayer, 1998; Thayer and Lane, 2000; Brosschot et al., 2006; Friedman, 2007). Thus, while many variables can influence the link between inhibitory control and ER, the following investigation takes both trait rumination and anxiety into consideration, as both may serve as "third variables" in the relationship between vmHRV and ER.

The Present Study

The present study aimed to explore the association of resting vmHRV and self-reported difficulties in ER. We hypothesized that those with lower resting vmHRV would report greater difficulties in everyday ER. However more specifically, the following investigation attempts to understand what day-to-day difficulties in ER resting vmHRV *best* predicts, beyond possible psychological confounds, including both trait rumination and anxiety. Overall, we sought to provide evidence of specific facets of

ER individuals with low vmHRV perceive most difficulties with, while providing recommendations for future research on ER, HRV, and well-being.

Methods and Materials

General Procedure

Data were pooled across five studies conducted within the Emotions and Quantitative Psychophysiology lab. Subjects were recruited from the Research Experience Program (REP) pool at The Ohio State University, allowing students to participate in research for partial class credit in an introductory level psychology course. Funding from The Ohio State University College of Social and Behavioral Sciences and College of Arts and Sciences also allowed us to recruit and compensate participants outside of the REP pool resulting in a diverse sample across the university (i.e., students from various majors and cohorts).

No individual participated in more than one of the five studies.

We asked all participants not to smoke, undergo vigorous physical activity, or drink caffeine 6 h prior to the experiment. Each study was approved by the institutional review board, and all participants signed written informed consent. Data from the five studies have not been submitted or accepted for publication elsewhere; however, results unrelated to the current data are publically available as Theses (Cash, 2014; Williams, 2014).

In all studies, participants were placed in a soundproof experimental room, equipped with a camera and microphone for safety and instructional reasons, and a high definition TV for stimuli presentation. Participants were given a detailed explanation of the procedures that would take place without indicating the specific hypothesis under the study or manipulations applied. Electrocardiogram (ECG) leads were attached to the subjects and while in a separate control room, the experimenter led the subjects to the initial phases of the experiment. All participants first completed a 5-min baseline-resting period, where participants, while spontaneously breathing, sat and viewed a blank, gray screen, and were instructed not to move or fall asleep. Participants either completed an experimental task[1] followed by a set of self-report questionnaires, or completed a set of self-report questionnaires followed by an experimental task. The total duration for each study was approximately 60 min.

Heart Rate Variability

Cardiac activity data was recorded continuously throughout each experiment via a 3-lead ECG at a 1000 Hz sampling rate using a Mindware™ 2000D (MW2000D) Impedance Cardiograph package. Resting vmHRV was assessed during a 5-min baseline (spontaneous breathing and resting state) period prior to any experimental task. Electrodes were placed (1) below the right clavicle, (2) on the left side of the abdomen (below the heart), and (3) on the right side of the abdomen. The variability between successive R-spikes (or variability within inter-beat-intervals, IBIs) was obtained from ECG recordings to calculate HRV. Participants' successive IBIs, in milliseconds, were extracted using HRV 2.51 Analysis software. IBIs were written in a text file and analyzed using Kubios HRV analysis package 2.0 (Tarvainen et al., 2014), allowing for the calculation of time- and frequency-domain indices of vmHRV. Artifacts within the R-to-R series were visually detected, and we applied an artifact correction level that would differentiate and remove artifacts (differing abnormal IBIs from the mean IBI) using a piecewise cubic spline interpolation method. The root mean square of successive differences (RMSSD), measured in milliseconds, was calculated and is considered to be a stable (Li et al., 2009), and valid (Thayer and Sternberg, 2010), time-domain measure of vmHRV. Studies have shown that in a spontaneous breathing and resting state, RMSSD has a trait specificity of 73% (Bertsch et al., 2012), suggesting that this one-time physiological assessment of RMSSD, particularly in a spontaneous breathing and resting state, primarily reflects trait influence (three times the state specificity) and thus, it is acceptable to construe resting RMSSD to be a physiological trait measure. PNN50 was also calculated, which is defined as the percentage of R-R intervals that differed by greater than 50 ms. Autoregressive estimates were also calculated, yielding high frequency power HRV (HF-HRV, 0.15–0.4 Hz) (Thayer and Sternberg, 2010). Whereas some have suggested that under certain circumstances RMSSD may reflect sympathetic influences (Berntson et al., 2005), in the present study RMSSD correlated highly with HF power ($r = 0.90$, $p < 0.001$), suggesting that RMSSD is primarily vagally-mediated and as such, we report vmHRV results using RMSSD. Results were identical using HF-HRV and PNN50 (results not shown).

Additionally, high-frequency peak values (HF peak) were obtained from the autoregressive analysis as a measure of respiration frequency to control for potential bias (Thayer et al., 2002). RMSSD values were natural log transformed (ln) to fit assumptions of linear analyses (Ellis et al., 2008). Mean (3.85) and standard deviation (0.49) values for RMSSD in the current study are comparable to average values (3.49) and standard deviations (0.26) reported elsewhere (Nunan et al., 2010).

Self-report Questionnaires

Perceived difficulties in ER were assessed using the *Difficulties in Emotion Regulation Scale* (DERS). The DERS is comprised of 36-items and six sub-scales designed to measure different facets of difficulties in ER (Gratz and Roemer, 2004). Participants are asked to respond on a scale from 1 (*almost never*) to 5 (*almost always*) (example item: "*When I'm upset, I acknowledge my emotions*"). Its subscales include (i) *non-acceptance of emotional responses* (NONACC; Cronbach's $\alpha = 0.87$); (ii) *difficulties engaging in goal-oriented behavior when experience negative emotions* (GOALS; $\alpha = 0.84$); (iii) *difficulties in controlling impulsive behavior when experiencing negative emotions* (IMPULSE; $\alpha = 0.80$); (iv) *lack of emotional awareness* (AWARE; $\alpha = 0.73$); (v) *lack of strategies to regulate emotions* (STRAT; $\alpha = 0.79$); (vi) *lack of emotional clarity* (CLARITY; $\alpha = 0.79$). The DERS showed excellent internal-consistency in the current investigation ($\alpha = 0.88$).

Rumination was assessed using the 22-item Ruminative Responses Scale (RRS; Conway et al., 2000). Participants answered on a scale from 1 (*almost never*) to 4 (*almost always*),

[1]Experimental tasks in each study were specific to the primary aims of each investigation. Presumably, these tasks did not alter influence the current results.

(sample item: *How often do you think* "*What am I doing to deserve this?*",) with higher numbers representing greater trait rumination. The RRS contains three subscales, including *brooding rumination* (*wallowing and sulking*), *dampening rumination* (*sadness and despair*), and *reflective rumination* (*problem solving and analyzing*). In the present analysis, only total RRS scores were used, and this scale showed excellent internal consistency (Cronbach's $\alpha = 0.91$).

Trait anxiety was assessed using the 20-item Spielberger Trait Anxiety Inventory (STAI-T; Spielberger, 1983). Participants answered on a scale from 1 (*almost never*) to 4 (*almost always*), (sample item: "*I feel pleasant*".) The STAI-T showed excellent internal consistency (Cronbach's $\alpha = 0.91$). Finally, height and weight were recorded and body mass index (BMI)– a measure that adjusts body weight for height–was calculated.

Statistical Analysis

All statistical tests were conducted using SPSS (ver. 19, IBM Chicago, IL, USA). To examine potential bias by pooling data across studies, two univariate analysis of variance (ANOVA) tests were conducted to examine differences in RMSSD and DERS scores across studies. Results showed that there were significant differences in mean DERS scores across studies [$F_{(4,\ 178)} = 2.54$, $r = 0.221$ $p < 0.041$] and marginal differences in lnRMSSD [$F_{(4,\ 178)} = 2.31$, $r = 0.232$ $p = 0.060$]. Thus, the five studies were given dummy codes (1–5) that were statistically controlled for in all analyses. All analyses contained additional covariates including gender (coded as 1 = male, 2 = female), ethnicity (coded as 1 = European American, 2 = Other), trait anxiety (STAI-T scores), trait rumination (RRS scores), respiration (as indexed by HFpeak values), age (in years), and BMI (Kg/M^2), all of which have been shown to be related to vmHRV (Dishman et al., 2000; Brosschot et al., 2006; Hu, 2011; Thayer et al., 2011; Koenig et al., 2014; Hill et al., 2015).

Multiple hierarchical regression tests were conducted to examine the relationship between lnRMSSD as a continuous variable and DERS (and subscales) scores controlling for aforementioned covariates. Step one included BMI, age, gender, ethnicity, and experiment as predictors. Step 2 added STAI-T and RRS scores, and step 3 added lnRMSSD and HF peak values as predictors of DERS total and subscale scores. Partial correlation coefficients and associated significance levels between lnRMSSD, covariates, and DERS (and subscales) scores are reported. In addition, a median split was performed on log-transformed RMSSD (RMSSD median value: 46.4586; lnRMSSD median value = 3.8386) to stratify subjects into groups with high and low resting vmHRV. Independent samples *t*-tests were conducted to explore potential differences between groups on all included variables. All tests were two-tailed and were analyzed using a set level of significance of $p > 0.05$.

Results

Across the five experiments, 199 participants were enrolled. Nine individuals were removed due to missing data. Seven individuals yielded DERS scores ± 2 standard deviations from the mean and were removed from the overall analyses, leaving data from a total of 183 undergraduate students (98 female, 60 minority[2], age range: 18–35, mean age = 19.34, standard deviation = 2.18) available for analyses. No outliers were removed due to abnormal lnRMSSD values (normal distribution). Independent sample *t*-tests showed that the low lnRMSSD group had higher STAI-T, RRS, DERS, and DERS subscale scores (each $p < 0.05$, with the exception of AWARE; see **Table 1**). Correlation results showed a significant negative relationship between lnRMSSD and RRS ($r = -0.180$, $p < 0.05$), STAI-T ($r = -0.261$, $p < 0.001$), and, as predicted, DERS total ($r = -0.325$, $p < 0.001$) scores (**Figure 1**), in addition to all DERS subscales with the exception of the AWARE subscale –**Table 2** contains a correlation matrix of all psychological variables and lnRMSSD.

Multiple hierarchical regression models showed that, after controlling for experiment type, age, ethnicity, gender, BMI, STAI-T scores, RRS scores, and HF peak (respiration) values, lnRMSSD was significantly associated with DERS total scores ($r_{partial} = -0.222$, $p < 0.01$). Moreover, lnRMSSD was significantly associated with scores obtained from the CLARITY ($r_{partial} = -0.175$, $p < 0.05$) and IMPULSE ($r_{partial} = -0.155$, $p < 0.05$) subscales (see **Table 3** for partial correlations between predictors and all DERS variables, see **Table 4** for a summary of regression models).

TABLE 1 | VmHRV group comparisons on all variables.

	Range of Data (min, max)	High vmHRV	Low vmHRV	Effect Sze (*r*)	*p*
n		91	92		
Age	18, 35	19.38 (1.97)	19.29 (2.38)	0.010	*0.778*
BMI	16.54, 47.51	24.30 (4.99)	23.64 (4.51)	0.070	*0.348*
RMSSD	15.01, 178.02	73.57 (24.04)	32.33 (7.97)	0.757	***0.000***
lnRMSSD	2.71, 5.18	4.26 (0.28)	3.44 (0.27)	0.829	***0.000***
HF Peak	0.16, 0.40	0.266 (0.047)	0.275 (0.046)	0.100	*0.183*
DERS	48, 120	77.84 (16.74)	86.27 (16.00)	0.250	***0.001***
NONACC	6, 29	12.09 (4.89)	13.85 (5.53)	0.167	***0.024***
GOALS	5, 25	12.96 (4.19)	14.72 (4.34)	0.202	***0.006***
IMPULSE	6, 25	9.59 (3.30)	11.05 (4.26)	0.189	***0.01***
AWARE	7, 30	16.91 (4.61)	17.15 (4.57)	0.031	*0.724*
STRAT	8, 31	15.41 (4.78)	17.33 (4.78)	0.184	***0.012***
CLARITY	5, 24	12.17 (3.76)	10.88 (3.76)	0.145	***0.021***
STAIT	23, 69	39.10 (9.84)	42.72 (9.12)	0.187	***0.011***
RRS	22, 73	41.03 (11.34)	45.30 (11.55)	0.184	***0.011***

This table shows the range of data and mean (standard deviation in brackets) values on baseline measures between high and low vmHRV groups, effect sizes (r) for each test, and p-values on the difference between groups (p-values italicized; bolded p-values, p < 0.05). HF peak, index of respiration; RMSSD, root mean of the squared successive differences—index of vmHRV; DERS, difficulties in regulation scale; RRS, ruminative responses scale; STAI-T, trait anxiety; NONACC, non-acceptance subscale; GOALS, goals subscale; IMPULSE, impulse control subscale; AWARE, emotional awareness subscale; STRAT, emotional regulation strategies subscale; CLARITY, emotional clarity subscale. It is important to note, only two participants were older than the age of 25.

[2]There were a total of 17 Asian Americans, 28 African Americans, 1 Brazilian, 12 Hispanic Americans, 2 Native Americans.

Discussion

The present study is the first study to report a significant negative association between resting vmHRV and self-reported difficulties in everyday ER, as indexed by the DERS. Both general anxiety and ruminative tendencies were correlated with vmHRV and difficulties in ER in the current investigation, such that greater anxiety and rumination were associated with lower vmHRV and greater difficulties in ER. However, after controlling for these psychological covariates, our results provide evidence that those with lower resting vmHRV have greater difficulties with everyday ER.

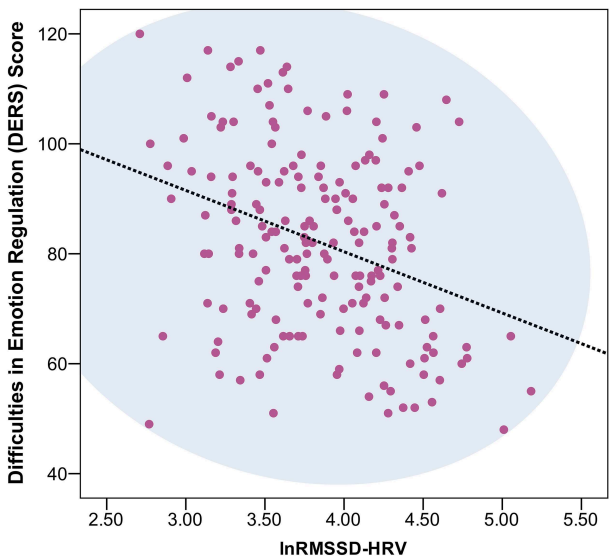


FIGURE 1 | Scatterplot of vmHRV and Difficulties in Emotion Regulation Scale (DERS) scores. This figure represents a scatterplot between RMSSD (in milliseconds and natural log (ln) transformed and difficulties in emotion regulation scale (DERS) total scale scores ($r = -0.325$, $p < 0.001$).

The DERS scale examines six different facets of ER difficulties. Thus, we were able to examine particular facets of ER and their relation to vmHRV. We found that those with lower vmHRV report greater difficulties with emotional clarity and emotional-impulse control. A lack of emotional clarity can be defined as lacking clarity or understanding of conscious or unconscious negative emotions (Gratz and Roemer, 2004). Thus, individuals with lower vmHRV may find it difficult to *identify* prepotent emotional responses and thus, are unable to inhibit and/or regulate them adaptively and consistently.

Additionally, we found vmHRV to be associated with difficulties in controlling impulsive behavior. Difficulty in impulse control is defined as having difficulties in controlling impulsive behaviors when experiencing negative emotions (Gratz and Roemer, 2004). Insomuch that individuals with low vmHRV show lesser inhibitory control in comparison to their counterparts that exhibit higher vmHRV, this relationship corresponds to prior theory—hypothesizing that those with lower vmHRV are unable to *inhibit* impulsive behaviors associated with negative emotions.

In addition, it is important to note that high and low HRV group analyses revealed the strongest difference between groups on the GOALS subscale of the DERS in comparison to the other subscales. The GOALS subscale represents difficulties in engaging in goal-oriented behavior—actions that are in accordance with present goals and motivations—when negative emotions are present. Therefore, our results suggest that at the group level, those with low HRV likely experience more difficulties in concentrating and accomplishing goal-oriented tasks when experiencing negative emotions in comparison to individuals with high HRV. These results are in line with previous work, suggesting a link between lower resting HRV and greater difficulties in both goal attainment and goal commitment (Verkuil et al., 2010).

Past research has examined the link between vmHRV and ER abilities using specific ER tasks and ER strategies (Melzig et al.,

TABLE 2 | Correlation matrix of HRV and psychological variables.

	lnRMSSD	RRS	STAI-T	DERS	GOALS	IMPULSE	AWARE	STRAT	CLARITY	NONACC
lnRMSSD	–									
RRS	**−0.180***	–								
STAI-T	**−0.261*****	**0.688*****	–							
DERS Total	**−0.325*****	**0.580*****	**0.688*****	–						
GOALS	**−0.219****	**0.416*****	**0.461*****	**0.668*****	–					
IMPULSE	**−0.244*****	**0.386*****	**0.459*****	**0.678*****	**0.415*****	–				
AWARE	−0.117	−0.111	0.099	**0.281****	−0.090	0.035	–			
STRAT	**−0.241****	**0.635*****	**0.698*****	**0.818*****	**0.580*****	**0.605*****	−0.016	–		
CLARITY	**−0.242****	**0.238****	**0.370*****	**0.589*****	**0.162***	**0.227****	**0.336*****	**0.272*****	–	
NONACC	**−0.167***	**0.528*****	**0.441*****	**0.675*****	**0.396*****	**0.303*****	**−0.146***	**0.525*****	**0.300*****	–

*This table shows zero-order correlation coefficients (statistically significant coefficients bolded) between all variables. HF peak, index of respiration; RMSSD, root mean of the squared successive differences—index of vagally-mediated HRV, RRS: ruminative responses scale; STAI-T, trait anxiety; DERS, difficulties in regulation scale; NONACC, non acceptance subscale; GOALS, goals subscale; IMPULSE, impulse control subscale; AWARE, emotional awareness subscale; STRAT, emotional regulation strategies subscale; CLARITY, emotional clarity subscale. *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.*

TABLE 3 | Partial correlations between predictors and DERS Scores.

	DERS	NONACC	GOALS	IMPULSE	AWARE	STRAT	CLARITY
LnRMSSD	**−0.222****	−0.036	−0.113	**−0.155***	−0.133	−0.088	**−0.175***
HF peak	−0.088	0.065	0.014	−0.007	**−0.158***	−0.104	−0.082
BMI	−0.069	0.018	−0.111	−0.054	−0.031	−0.088	0.051
Ethnicity	**−0.149***	**−0.158***	−0.002	−0.053	−0.042	0.029	**−0.215****
Gender	0.007	0.081	**0.165***	−0.048	**−0.253*****	0.111	−0.011
Age	**−0.204****	−0.038	−0.100	**−0.153***	**−0.153***	−0.020	**−0.170***
STAI-T	**0.469*****	0.122	**0.237****	**0.246*****	**0.225****	**0.456*****	**0.273*****
RRS	**0.212*****	**0.351*****	**0.157***	0.135	**−0.262*****	**0.299*****	−0.033
Experiment	0.078	−0.067	0.046	0.106	0.126	−0.025	0.061

*This table shows partial correlation coefficients (statistically significant coefficients bolded) between predictor variables and DERS total and subscale scored, yielded from the hierarchical regression model. Specifically, these partial correlation coefficients show the relationship between predictor variables (e.g., lnRMSSD) and DERS and subscale scores while partialing out the influence of the other predictor variables (e.g., HF peak, BMI, Ethnicity, Gender, Age, STAI-T, RRS, and experiment). HF peak, index of respiration; RMSSD: root mean of the squared successive differences—index of vagally-mediated HRV DERS, difficulties in regulation scale; RRS, ruminative responses scale; STAI-T, trait anxiety; NONACC, non acceptance subscale; GOALS, goals subscale; IMPULSE, impulse control subscale; AWARE, emotional awareness subscale; STRAT, emotional regulation strategies subscale; CLARITY, emotional clarity subscale. *p < 0.05 **p < 0.01 ***p < 0.001.*

TABLE 4 | Summary of hierarchical regression analysis for variables predicting DERS Scores.

Step	DERS total			IMPULSE			CLARITY		
	1	2	3	1	2	3	1	2	3
Age	−0.273***	−0.137*	−0.148***	−0.221***	−0.133**	−0.142***	−0.224***	−0.152*	−0.163*
Ethnicity	−0.032	−0.108*	−0.106**	−0.001	−0.050	−0.048*	−0.164*	−0.207**	−0.205**
Gender	−0.005	0.000	0.004*	−0.050	−0.047	−0.043	−0.020	−0.015	−0.010
Experiment	0.053	0.037	0.056	0.086	0.075	0.098	0.050	0.040	0.058
BMI	−0.032	−0.069	−0.050	−0.040	−0.064	−0.050	0.045	0.028	0.049
STAI-T		0.541***	0.507***		0.351***	0.312***		0.393***	0.359***
RRS		0.202**	0.119**		0.135	0.135		−0.037	−0.040
HF peak			−0.060			−0.006			−0.074
lnRMSSD			−0.161**			−0.143*			−0.167*
Constant	125.32	59.70	88.48	18.66	8.821	13.74	19.41	11.81	18.78
R2	0.075	0.535	0.559	0.049	0.246	0.264	0.069	0.198	0.226

*This table shows standardized beta (β) coefficients with associated significance levels at each step in the regression model. HF peak, index of respiration; RMSSD, root mean of the squared successive differences—index of vmHRV DERS, difficulties in regulation scale; RRS, ruminative responses scale; STAI-T, trait anxiety; IMPULSE, impulse control subscale; CLARITY, emotional clarity subscale. *p < 0.05 **p < 0.01 ***p < 0.001.*

2009; Aldao and Mennin, 2012). The present study adds to this literature by showing that resting vmHRV is associated with a self-report measure of *everyday perceived* difficulties in ER, and strongest with difficulties in emotional clarity, impulse control, and in some cases, goal-oriented behavior. One study found that those with low ER capabilities, as indexed by the DERS, did not show physiological recovery, as indexed by phasic changes in vmHRV, following a film-elicited emotion procedure (Berna et al., 2014). Our data are consistent with, but also extend these findings, suggesting that individuals with lower baseline-resting vmHRV experience difficulties/failures with everyday ER, and are able to report such difficulties.

Limitations and Future Directions

One limitation of the current investigation is that the sample consisted of college students and thus, the current results may not extend to other age ranges. While we are confident that difficulties in ER will be related to vmHRV in all age groups, we are not sure that difficulties in impulse control and emotional clarity will be closely associated with vmHRV as in the current sample. Thus, future research should attempt to examine the DERS and vmHRV relationship in individuals of various age ranges.

A second limitation of the current investigation is that, while we controlled for anxiety and rumination, other possible psychological covariates, such as perceived stress and depressive symptoms, were not statistically controlled for in the current analysis. Thus, future research should ensure that the current results remain strong and significant when considering other possible psychological covariates.

Moreover, while the relationship between resting vmHRV and difficulties in ER is strong, it is not perfect. Indeed, this could be due to the possible mediating role that rumination and/or anxiety plays on the link between vmHRV and difficulties in ER,

especially given the modest correlation between these variables. Nevertheless, within the current sample, there are individuals with adequate inhibitory abilities, as indexed by higher vmHRV, who perceive difficulties in ER. Conversely, there are individuals with lower vmHRV who do not perceive difficulties in ER, despite their lack of inhibitory abilities. This suggests a third variable at play—which could be as simple as the number of emotional encounters (lack of ER experiences) or as complex as individuals' emotional numbness, intelligence, or regulatory skills (lack of ER practice). In fact, recent research suggests that ER should be seen as a motivational process, such that the motivation to engage in ER, in addition to employing ER strategies, differs from person to person, independent of their actual ability (Aldao and Mennin, 2014). Thus, although an individual is equipped to deal with negative emotions (i.e., have high resting vmHRV), they may be unmotivated (or motivated differently) to regulate those emotions and as such, perceives more difficulties. Future researchers should attempt to identify these individuals, in addition to the variable accounting for the discrepancy between resting vmHRV and perceived difficulties in ER.

Finally, the current investigation is cross-sectional by nature and thus, causation cannot be determined in the present study. However according to the Model of Neurovisceral Integration, it is likely that vmHRV is influencing ER failure and success. On the other hand, other research proposes that maladaptive/adaptive ER decreases/increases acute vmHRV. Thus, it is also likely that those who are maladaptive in their emotional responding have subsequent maladaptive chronic physiological responses. Future research is warranted to explore such notions.

Conclusions

The present investigation shows that resting vmHRV is related to individuals' everyday perceptions of difficulties to regulate their emotions, especially difficulties with emotional clarity and impulse control. Thus, in order to increase adaptive ER success in those with lower vmHRV, these data suggest that individuals should: (1) work to increase vmHRV, as doing so may increase ER; (2) work to understand and identify negative emotions, so that they have the opportunity to adaptively regulate such distressing emotions and; (3) these individuals should work to inhibit impulsive behavior, as these responses may be undesirable. The current study both supports the NIM and extends prior research on ER and vmHRV, giving two facets of ER (impulse control and emotional clarity) that should be of particular focus when investigating the link between resting vmHRV and ER. Overall, the current data support the notion that vmHRV serves as a proxy of ER ability, especially when negative emotions are unclear and resulting behavior is impulsive. We live in a world where we often experience both simple and complex emotions—we hope that future research gives special attention to these facets of ER as doing so may assist in understanding the link between ER and negative physical and mental health outcomes and longevity.

Acknowledgments

Funding for Open Access provided by The Ohio State University Open Access Fund. Funding for participant compensation provided by The Ohio State University College of Social and Behavioral Sciences and College of Arts and Sciences.

References

Aldao, A., and Mennin, D. (2014). *Emotion Regulation. The Wiley Handbook of Anxiety Disorders*. Chichester, UK: John Wiley & Sons, Ltd.

Aldao, A., and Mennin, D. S. (2012). Paradoxical cardiovascular effects of implementing adaptive emotion regulation strategies in generalized anxiety disorder. *Behav. Res. Ther*. 50, 122–30. doi: 10.1016/j.brat.2011.12.004

Aldao, A., Mennin, D. S., and McLaughlin, K. A. (2012). Differentiating worry and rumination: evidence from heart rate variability during spontaneous regulation. *Cogn. Ther. Res*. 37, 613–619. doi: 10.1007/s10608-012-9485-0

Aldao, A. (2013). The future of emotion regulation research: capturing context. *Pers. Psychol. Sci*. 8, 155–172. doi: 10.1177/1745691612459518

Berna, G., Ott, L., and Nandrino, J. L. (2014). Effects of emotion regulation difficulties on the tonic and phasic cardiac autonomic response. *PLoS ONE* 9:e102971. doi: 10.1371/journal.pone.0102971

Berntson, G. G., Lozano, D. L., and Chen, Y. J. (2005). Filter properties of root mean square successive difference (RMSSD) for heart rate.*Psychophysiology* 42, 246–252. doi: 10.1111/j.1469-8986.2005.00277.x

Bertsch, K., Hagemann, D., Naumann, E., Schächinger, H., and Schulz, A. (2012). Stability of heart rate variability indices reflecting parasympathetic activity. *Psychophysiology* 49, 672–682. doi: 10.1111/j.1469-8986.2011.01341.x

Brosschot, J. F., Gerin, W., and Thayer, J. F. (2006). The perseverative cognition hypothesis: a review of worry, prolonged stress-related physiological activation, and health. *J. Psychosom. Res*. 60, 113–124. doi: 10.1016/j.jpsychores.2005.06.074

Carrico, A. W., Ironson, G., Antoni, M. H., Lechner, S. C., Durán, R. E., Kumar, M., et al. (2006). A path model of the effects of spirituality on depressive symptoms and 24-h urinary-free cortisol in HIV-positive persons. *J. Psychosom. Res*. 61, 51–58. doi: 10.1016/j.jpsychores.2006.04.005

Cash, C. S. (2014). *Think about your Heart The Effects of Cognitive Dissonance on cardiovascular functioning*. Undergraduate Thesis, The Ohio State University, Columbus, OH.

Conway, M., Csank, P. A., Holm, S. L., and Blake, C. K. (2000). On assessing individual differences in rumination on sadness. *J. Pers. Assess*. 75, 404–425. doi: 10.1207/S15327752JPA7503_04

Dishman, R. K., Nakamura, Y., Garcia, M. E., Thompson, R. W., Dunn, A. L., and Blair, S. N. (2000). Heart rate variability, trait anxiety, and perceived stress among physically fit men and women. *Int. J. Psychophysiol*. 37, 121–133. doi: 10.1016/S0167-8760(00)00085-4

Easwaren, E. (2010). *Conquest of Mind*. Tomales, CA: Nilgiri Press.

Ellis, R. J., Sollers, J. J. III, Edelstein, E. A., and Thayer, J. F. (2008). Data transforms for spectral analyses of heart rate variability. *Biomed. Sci. Instrum*. 44, 392–397.

Friedman, B. H., and Thayer, J. F. (1998). Autonomic balance revisited: panic anxiety and heart rate variability. *J. Psychosom. Res*. 44, 133–151. doi: 10.1016/S0022-3999(97)00202-X

Friedman, B. H. (2007). An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. *Biol. Psychol*. 74, 185–199. doi: 10.1016/j.biopsycho.2005.08.009

Geisler, F. C. M., Vennewald, N., Kubiak, T., and Weber, H. (2010). The impact of heart rate variability on subjective well-being is mediated by emotion regulation. *Pers. Indiv. Diff*. 49, 723–728. doi: 10.1016/j.paid.2010.06.015

Gillie, B. L., Vasey, M. W., and Thayer, J. F. (2014). Heart rate variability predicts control over memory retrieval. *Psychol. Sci*. 25, 458–465. doi: 10.1177/0956797613508789

Gratz, K. L., and Roemer, L. (2004). Multidimensional assessment of emotion regulation and dysregulation: development, factor structure, and initial validation of the difficulties in emotion regulation scale. *J. Psychopathol. Behav. Assess*. 26, 41–54. doi: 10.1023/B:JOBA.0000007455.08539.94

Hansen, A. L., Johnsen, B. H., Sollers, J. J., Stenvik, K., and Thayer, J. F. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur. J. Appl. Physiol*. 93, 263–272. doi: 10.1007/s00421-004-1208-0

Hill, L. K., Hu, D. D., Koenig, J., Sollers, I. I. I., J. J., Kapuku, G., Wang, X., et al. (2015). Ethnic differences in resting heart rate variability: a systematic review and meta-analysis. *Psychosom. Med*. 77, 16–25. doi: 10.1097/PSY.0000000000000133

Hu, D. D. (2011). *Ethnic, Sex, Age, and Socioeconomic Differences in Heart Rate Complexity and Variability: The Healthy Aging in Neighborhoods of Diversity across the Life Span [HANDLS] Study*. (Doctoral dissertation, The Ohio State University, Columbus, OH).

Koenig, J., Jarczok, M. N., Warth, M., Ellis, R. J., Bach, C., Hillecke, T. K., et al. (2014). Body mass index is related to autonomic nervous system activity as measured by heart rate variability–a replication using short term measurements. *J. Nutr. Health Aging* 18, 300–302. doi: 10.1007/s12603-014-0022-6

Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L., and Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *Neuroimage* 44, 213–222. doi: 10.1016/j.neuroimage.2008.07.056

Li, Z., Snieder, H., Su, S., Ding, X., Thayer, J. F., Treiber, F. A., et al. (2009). A longitudinal study in youth of heart rate variability at rest and in response to stress. *Int. J. Psychophysiol*. 73, 212–217. doi: 10.1016/j.ijpsycho.2009.03.002

Melzig, C. A., Weike, A. I., Hamm, A. O., and Thayer, J. F. (2009). Individual differences in fear-potentiated startle as a function of resting heart rate variability: implications for panic disorder. *Int. J. Psychophysiol*. 71, 109–117. doi: 10.1016/j.ijpsycho.2008.07.013

Nunan, D., Sandercock, G. R., and Brodie, D. A. (2010). A quantitative systematic review of normal values for Short−Term heart rate variability in healthy adults. *Pacing Clin. Electrophysiol*. 33, 1407–1417. doi: 10.1111/j.1540-8159.2010.02841.x

Spielberger, C. D. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologist Press.

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., and Karjalainen, P. A. (2014). Kubios HRV-heart rate variability analysis software. *Comput. Methods Programs Biomed.* 113, 210–220. doi: 10.1016/j.cmpb.2013.07.024

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev*. 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord*. 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biol. Psychol.*74, 224–242. doi: 10.1016/j.biopsycho.2005.11.013

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev*. 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Thayer, J. F., Loerbroks, A., and Sternberg, E. M. (2011). Inflammation and cardiorespiratory control: the role of the vagus nerve. *Respir. Physiol.Neurobiol*. 178, 387–394. doi: 10.1016/j.resp.2011.05.016

Thayer, J. F., Sollers, III, J. J., Ruiz-Padial, E., and Vila, J. (2002). Estimating respiratory frequency from autoregressive spectral analysis of heart period. *IEEE Eng. Med. Biol.* 21, 41–45.

Thayer, J. F., and Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Ann. N.Y. Acad. Sci*. 1088, 361–372. doi: 10.1196/annals.1366.014

Thayer, J. F., and Sternberg, E. M. (2010). Neural aspects of immunomodulation: focus on the vagus nerve. *Brain Behav. Immun*. 24, 1223–1228. doi: 10.1016/j.bbi.2010.07.247

Verkuil, B., Brosschot, J. F., Gebhardt, W. A., and Thayer, J. F. (2010). When worries make you sick: a review of perseverative cognition, the default stress response and somatic health. *J. Exp. Psychopathol.*1, 87–118. doi: 10.5127/jep.009110

Weber, C. S., Thayer, J. F., Rudat, M., Wirtz, P. H., Zimmermann-Viehoff, F., Thomas, A., et al. (2010). Low vagal tone is associated with impaired post stress recovery of cardiovascular, endocrine, and immune markers. *Eur. J. Appl. Physiol*. 109, 201–211. doi: 10.1007/s00421-009-1341-x

Williams, D. P. (2014). *The Relationship between Heart Rate Variability, Lay Theories of Self-Regulation, and Ego-Depletion: Evidence of Psychophysiological Pathways of Self-Regulation*. Master's Thesis, The Ohio State University, Columbus, OH.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
PSYCHOLOGY

ORIGINAL RESEARCH ARTICLE
published: 27 November 2014
doi: 10.3389/fpsyg.2014.01387

Major depressive disorder with melancholia displays robust alterations in resting state heart rate and its variability: implications for future morbidity and mortality

Andrew H. Kemp[1,2,3] *, Daniel S. Quintana[2,4,5], Candice R. Quinn[1], Patrick Hopkinson[1] and Anthony W. F. Harris[1,6]

[1] *Discipline of Psychiatry, Sydney Medical School, University of Sydney, Sydney, NSW, Australia*
[2] *School of Psychology, Faculty of Science, University of Sydney, Sydney, NSW, Australia*
[3] *Centro de Pesquisa Clínica e Epidemiológica, Hospital Universitário – Universidade de São Paulo, São Paulo, Brazil*
[4] *NORMENT, K.G. Jebsen Centre for Psychosis Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway*
[5] *Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway*
[6] *Brain Dynamics Centre, Westmead Millennium Institute, University of Sydney – Westmead Hospital, Sydney, NSW, Australia*

Edited by:
Lihong Wang, Duke University, USA

Reviewed by:
Jamie Lars Hanson, Duke University, USA
Linda Booij, Queen's University, Canada

****Correspondence:***
Andrew H. Kemp, Centro de Pesquisa Clínica e Epidemiológica, Hospital Universitário – Universidade de São Paulo, Avenida Lineu Prestes 2565, 05508-000 São Paulo, Brazil
e-mail: andrewhaddonkemp@gmail.com; andrew.kemp@sydney.edu.au; andrew.kemp@hu.usp.br

Background: Major depressive disorder (MDD) is associated with increased heart rate and reductions in its variability (heart rate variability, HRV) – markers of future morbidity and mortality – yet prior studies have reported contradictory effects. We hypothesized that increases in heart rate and reductions in HRV would be more robust in melancholia relative to controls, than in patients with non-melancholia.

Methods: A total of 72 patients with a primary diagnosis of MDD (age *M*: 36.26, SE: 1.34; 42 females) and 94 controls (age *M*: 35.69, SE: 1.16; 52 females) were included in this study. Heart rate and measures of its variability (HRV) were calculated from two 2-min electrocardiogram recordings during resting state. Propensity score matching controlled imbalance on potential confounds between patients with melancholia ($n = 40$) and non-melancholia ($n = 32$) including age, gender, disorder severity, and comorbid anxiety disorders.

Results: MDD patients with melancholia displayed significantly increased heart rate and lower resting-state HRV (including the square root of the mean squared differences between successive N–N intervals, the absolute power of high frequency and standard deviation of the Poincaré plot perpendicular to the line of identity measures of HRV) relative to controls, findings associated with a moderate effect size (Cohens *d*'s = 0.56–0.58). Patients with melancholia also displayed an increased heart rate relative to those with non-melancholia (Cohen's $d = 0.20$).

Conclusion: MDD patients with melancholia – but not non-melancholia – display robust increases in heart rate and decreases in HRV. These findings may underpin a variety of behavioral impairments in patients with melancholia including somatic symptoms, cognitive impairment, reduced responsiveness to the environment, and over the longer-term, morbidity and mortality.

Keywords: melancholia, non-melancholia, electrocardiogram, ECG, heart rate, heart rate variability, HRV, resting state

INTRODUCTION

> Unfortunately, most researchers in psychiatry and psychology express little interest in the mapping of autonomic regulation as a "vulnerability" dimension for various disorders and behavioral problems, although visceral features are often symptoms of the disorders they are treating.
> –Porges (2011)

Major depressive disorder (MDD) is associated with reduced resting-state heart rate variability (HRV; Kemp et al., 2010, 2012; Brunoni et al., 2013; see Kemp and Quintana, 2013 for review), and these reductions are inversely associated with disorder severity (Kemp et al., 2010). Heart rate and its variability (HRV) are determined by a variety of physiological factors, although the most prominent of these is the autonomic system. A high level of parasympathetic (vagal) function is both desirable and beneficial as it reflects the capacity for an individual to respond, adapt and regulate responses when required. One can only imagine the consequences of buildings in Tokyo not being sufficiently flexible to withstand an earthquake. The ability to adapt quickly to change in the environment requires flexibility. Researchers (Kashdan and Rottenberg, 2010) have argued that a fundamental component of health is psychological flexibility and have suggested that HRV may provide the psychophysiological foundation for such flexibility (Friedman and Thayer, 1998; Kashdan and Rottenberg, 2010). By contrast, chronic reductions in HRV

are associated with psychophysiological rigidity, dysregulation of a variety of allostatic systems, and increased risk for morbidity and mortality (see Kemp and Quintana, 2013 for review). The goal of the present paper is to determine whether specific subtypes of depression display more robust alterations in heart rate and commonly reported measures of HRV relative to controls.

In the resting state, the heart is under tonic inhibitory control by the vagus, yet measures of HRV are a more specific marker of vagal function than heart rate (Saul, 1990; Reyes Del Paso et al., 2013). That said, different measures of HRV provide information on different physiological control mechanisms (Reyes Del Paso et al., 2013). For instance, high frequency oscillations (0.15–0.4 Hz) relate to respiratory influences, while LF oscillations (0.04–0.15 Hz) reflect mechanisms relating to blood pressure control such as the baroreflex (Reyes Del Paso et al., 2013; see also Krygier et al., 2013).

Reduced HRV was first reported in depressed patients more than two-decades ago (Carney et al., 1988), and more recently, has been shown to predict adverse cardiovascular events over a follow-up period of 3–15 years (Hillebrand et al., 2013), highlighting the importance of continued research in this area. However, not all studies (Licht et al., 2008) – including our own (Kemp et al., 2014) – have reported reduced HRV in depressed patients highlighting the complexity of this issue. Recent debate has focused on whether the mood and anxiety disorders, or their treatments are associated with reductions in vagal function (Licht et al., 2011; Brunoni et al., 2012). Increases in heart rate are usually associated with decreases in HRV, and antidepressant medications clearly adversely affect heart rate and HRV (Licht et al., 2010; Kemp et al., 2014), yet uncertainty remains over whether unmedicated depressed patients display alterations in these psychophysiological markers (Kemp, 2011, 2012; Kemp et al., 2011, 2014; Licht et al., 2011; Brunoni et al., 2012).

One potential explanation for the contradictory findings is that distinct subtypes (e.g., melancholia; Gold and Chrousos, 2002; Malhi et al., 2005) may display more robust alterations in heart rate and HRV, yet studies are yet to determine whether this is the case. Melancholia is characterized by an over-active stress response, a loss of responsiveness to the environment, somatic symptoms (e.g., insomnia, loss of appetite), and worse depression in the morning (Gold and Chrousos, 2002). In addition, patients with melancholia display greater cognitive impairment relative to those without such features (Quinn et al., 2012a). There are several reasons to expect that HRV will be reduced in depression generally, and that these effects will be greatest in those patients with melancholic depression. These include the presence of somatic symptoms, cognitive impairment, increased disorder severity, and reduced responsiveness to the environment; all symptoms that have been previously associated with HRV reductions.

Firstly, somatic depressive symptoms appear to be strongly associated with reduced HRV, at least in patients with stable coronary heart disease (de Jonge et al., 2007). Secondly, there is a body of evidence linking cognitive function and executive function in particular to HRV (see Thayer et al., 2009 for review; Kemp et al., unpublished findings). Participants with high HRV have been shown to perform better on executive function tasks relative to those with low HRV. These effects have been observed in a variety of populations including young men (Hansen et al., 2004) and older women (Kim et al., 2006), while performance on cognitive tasks has been shown to improve with aerobic exercise (Hansen et al., 2004; Albinet et al., 2010). Thirdly, melancholic depression is generally a more severe form of depression – although differences between melancholia and non-melancholia are more than simple variation on severity (Quinn et al., 2012b) – and reductions in HRV are correlated with increasing depression severity (Kemp et al., 2010). Fourthly, the capacity to adequately respond to change in the environment requires flexibility – melancholic patients are less responsive – and HRV may provide the psychophysiological foundation for such flexibility (Thayer and Lane, 2000; Kashdan and Rottenberg, 2010). Finally, it is notable that stimulation of the left vagus nerve is a promising, alternative treatment for treatment resistant depression (Mayberg et al., 2006), further highlighting a role for impaired vagal function in MDD.

We have reported reductions in MDD patients across time-, frequency- and non-linear domain measures of HRV (Kemp et al., 2012; see also Brunoni et al., 2013), findings associated with a small-to-moderate effect size, replicating findings we reported in an earlier meta-analysis (Kemp et al., 2010). One of our previous studies (Kemp et al., 2012) further highlighted that reductions in HRV were greatest in MDD patients with comorbid generalized anxiety disorder, findings associated with a large effect size. Confirming these findings, we recently reported, in an independent Brazilian sample, that only those with generalized anxiety disorder display robust, though small, increases in heart rate and decreases in HRV after controlling for confounding variables using propensity scores (Kemp et al., 2014), a novel approach that allows for more appropriate control of confounders, and a technique we employ in the present study. Here we explore the impact of two major subtypes of depression – melancholia and non-melancholia – relative to controls, hypothesizing that patients with melancholic depression rather than non-melancholic depression will display robust decreases in HRV, relative to healthy controls, after controlling for major confounding variables including disorder severity (Kemp et al., 2010) and comorbid anxiety (Kemp et al., 2012; Alvares et al., 2013; Chalmers et al., 2014). Here we report on a variety of HRV measures to examine the which findings are robust across different measures. On the basis of parallel lines of evidence highlighting a major role for high heart rate in future morbidity and mortality (Fox et al., 2007; Lemogne et al., 2011; Åberg et al., 2014), we also examined the impact of depression on heart rate, expecting robust increases in heart rate in melancholia.

MATERIALS AND METHODS

PARTICIPANTS

A total of 72 patients with a primary diagnosis of MDD and 94 age- and sex-matched controls were included in this study. Participants in this study were recruited from the community as part of case-control study conducted in 2006 and 2007 (Kemp et al., 2012). Exclusion criteria included a history of brain injury (causing loss of consciousness for 10 min or more), neurological

disorder, other serious medical condition, or substance abuse or dependence for >1 year. All participants were medication free for at least five half-lives. Our study was approved by the University of Sydney, Sydney West Area Health Service, University of Adelaide and Flinders University human research ethics committees, and written informed consent was obtained from participants in accordance with National Health and Medical Research Council guidelines.

PROCEDURES

Depressed participants were diagnosed with MDD and categorized with or without melancholic symptoms by trained and supervised research officers using the Mini International Neuropsychological Interview (MINI; Sheehan et al., 1998), a structured psychiatric interview based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. Control participants were excluded if they self-reported a history or presence of psychiatric illness; they were also screened using the MINI. Depression severity was assessed using the 17-item structured interview guide for the Hamilton Depression Rating Scale (SIGH-D; Hamilton, 1960; Williams, 1988) and psychomotor disturbance was measured by the CORE assessment of psychomotor change (CORE; Hickie, 1996). The self-report, Depression, Anxiety and Stress Scale (DASS; Lovibond and Lovibond, 1995) was completed by participants at the completion of the clinical interview. The DASS depression subscale is compatible with DSM-IV criteria of mood disorders, the Anxiety scale, with symptom criteria of panic disorder and PTSD, and the Stress scale, with a diagnosis of generalized anxiety disorder.

Participants were seated in a sound and light controlled room at 24°C and two 2-min electrocardiogram (ECG) recordings were collected during resting state. The ECG recording disc was positioned on the inside of the left wrist, positioned at the radial pulse, relative to a common ground and referenced to two sites: Erbs point (located two thirds distant from midline on the clavicle) and C7 (the seventh Cervical vertebra; most pronounced transverse process). Both reference sites are positioned directly above bone and serve as relatively muscle-free references. Recordings were made under these conditions as part of a standardized, psychophysiological recording protocol (Gordon et al., 2005).

Data was sampled at 500 Hz, with 22-bit resolution digitization using a Compumedics Neuroscan Nuamps amplifier and SCAN software, version 4.3. ECG was analyzed using custom-developed software to perform semi-automated pre-processing to remove noise from the ECG, allowing for the identification of the R-peaks based on established methods (Pan and Tompkins, 1985). The cleaned, N-N time-series for each participant was then imported into Kubios (version 2.0, 2008, Biosignal Analysis and Medical Imaging Group, University of Kupio, Finland, MATLAB) from which measures of heart rate and HRV were calculated based on established guidelines (Electrophysiology TFotESoCtNASoP, 1996).

HEART RATE MEASURES

Heart rate and its variability during the resting-state are under tonic inhibitory control by the parasympathetic (vagal) nervous system (Thayer et al., 2009). It is in this regard that we refer to resting-state heart rate and HRV as surrogate measures of vagally mediated cardiac activity, although HRV measures are more pure (Saul, 1990), yet complex (Picard et al., 2009), indicators of vagal activity. HRV measures comprised time-domain estimates, including the standard deviation of N–N intervals (SDNN) and the square root of the mean squared differences between successive N–N intervals (RMSSD). SDNN is a commonly reported time-domain measure reflecting all the cyclic components responsible for variability in a recording (Electrophysiology TFotESoCtNASoP, 1996). RMSSD is a stable, time-domain index less affected by changes in breathing frequency (Penttilä et al., 2001). We also examined frequency-based estimates using the FFT method including the absolute power of high frequency (HF, 0.15–0.4 Hz) and low frequency (LF, 0.04–0.15 Hz). HF relates to respiratory influences, while LF provides information about baroreflex function (Goldstein et al., 2011). The standard deviation of the Poincaré plot perpendicular to the line of identity (PCSD1), a non-linear measure of HRV, was also calculated. We have previously reported that non-linear domain measures of HRV may be more sensitive to group differences (Kemp et al., 2010). Heart rate displays complex non-linear dynamic behavior, rather than regular, periodic oscillation (Billman, 2011), and the PCSD1 is a commonly reported, non-linear measure of short-term variability mainly caused by respiratory sinus arrhythmia (Tarvainen and Niskanen, 2008). All HRV measures were log transformed to normality.

DATA PROCESSING AND STATISTICAL ANALYSIS

All statistical analyses were performed using IBM SPSS Statistics, Version 21 with SPSS R Essentials plug-in and R statistics version 2.14.2. To avoid bias resulting from imbalance on disorder severity and comorbid anxiety – major confounding variables when seeking to compare patients with melancholia and non-melancholia – we conducted propensity score matching (PSM) using a custom designed plugin for IBM SPSS Statistics (Thoemmes, 2012). PSM involves producing a score (on the basis of entered covariates) for each participant that relates to the probability that the subject belongs to the melancholic versus non-melancholic grouping, and then matching patients in each grouping on this propensity score. If two participants have the same propensity score, then they are equally likely to have come from the same distribution (i.e., patient grouping). Therefore, selecting patients with non-melancholia that have the same propensity scores to those with melancholia, we avoid any bias resulting from an imbalance on covariates. The PSM procedure uses logistic regression to produce the propensity score, in which patient grouping is used as the outcome variable and selected covariates, as predictors. Covariates entered into PSM analysis included age, gender, depression, anxiety and stress DASS scores, SIGH-D, and MINI anxiety disorder status (yes, no). Cases with the closest score were then matched using a simple 1:1 nearest neighbor matching routine based on a 'greedy' matching algorithm. Balance statistics and associated graphs were inspected to confirm adequacy of the match.

As PSM requires a complete dataset without missing data, we first ran multiple imputation (MI) analysis (Schafer, 1999) in IBM SPSS Statistics to replace missing values using the automatic method. While a common approach to dealing with missing data

is deleting observations with missing values, and analyzing only those participants with a complete dataset, this listwise deletion approach is problematic for at least two reasons (Barzi and Woodward, 2004; van Ginkel and Kroonenberg, 2014). Firstly, it wastes data and reduces the power of analysis to determine an effect. Secondly, it may also produce biased estimates when loss of participants is systematic and not random. By contrast, MI yields estimates with good statistical properties. It uses all available data, makes less stringent assumptions about 'missingness' and pools plausible complete versions of an incomplete dataset into one analysis taking into account additional uncertainty due to missing data.

Data was missing for the following variables: depression (melancholia: 27.5%; non-melancholia: 37.5%), anxiety (melancholia: 22.5%; non-melancholia: 37.5%), and stress (melancholia: 22.5%; non-melancholia: 37.5%) from the DASS measure, MINI anxiety disorder status (yes, no; melancholia: no missing data; non-melancholia: 6.3%), and CORE total (melancholia: no missing data; non-melancholia: 3.1%) as predictors. MI procedures are appropriate for data in which up to 60% of values are missing (Barzi and Woodward, 2004). As recommended by others (Marshall et al., 2009), the imputation model used for missing data contained all variables to be subsequently analyzed including outcome variables (melancholic status in PSM, and heart rate and its variability in final analysis), variables to predict the missing data, and those variables to be imputed. Variables entered into MI analysis included: participant grouping, heart rate and HRV, depression, anxiety and stress from the DASS questionnaire, age, gender, SIGH-D, MINI anxiety disorder status and CORE total score.

Multiple imputation analysis produced 20 datasets relating to 72 MDD patients and PSM analysis was run on each of these datasets to obtain patients matched on propensity scores. The data for controls were then merged into each of the 20 datasets after which analyses were conducted on each dataset to examine group differences (MEL vs. NMEL vs. CTRL) on heart rate and HRV. As recommended previously (Hill, 2004), analysis of variance (ANOVA) analysis was carried out as a regression analysis using effect coding (Edwards, 1985; van Ginkel and Kroonenberg, 2014) so that results could then be combined according to Rubin's rules (van Ginkel and Kroonenberg, 2014). This approach has the advantage of averaging over the results from different groupings determined using PSM on the multiply imputed datasets (Hill, 2004). Guidelines and software for carrying out these procedures and combining results for pooled estimates and statistics are available here: http://www.socialsciences.leiden.edu/educationandchildstudies/childandfamilystudies/organisation/staffcfs/van-ginkel.html. One-tailed *t*-tests are reported given specific directional hypotheses. Effect size measures (Cohen's *d*) were also determined and Cohen's guidelines (Cohen, 1988) for interpreting Cohen's *d*'s (small, $d = 0.2$; medium, $d = 0.5$; large, $d = 0.8$) were followed. Cohen's *d* statistics were calculated using an online calculator available here: http://www.uccs.edu/~lbecker/

RESULTS

PARTICIPANT CHARACTERISTICS

Participant characteristics are reported in **Table 1**. No age or gender differences were observed, however, all groups differed on depression, anxiety and stress scales (Tukey's HSD $p < 0.05$), highlighting the need for PSM of patients in melancholia and non-melancholia patient groupings.

HEART RATE AND ITS VARIABILITY

After application of PSM and pooling results according to Rubin's rules, groups differed significantly on heart rate [$F(2,108.25) = 3.664$, $p = 0.029$], RMSSD [$F(2,107.86) = 3.21$, $p = 0.044$], HF (at trend levels) [$F(2,102.76) = 2.966$, $p = 0.056$], and PCSD1 [$F(2,107.87) = 3.199$, $p = 0.045$; **Figure 1**]. No significant differences on the combined overall test were observed for SDNN or LF. As hypothesized, heart rate was increased (by 7.85 beats per minute, BPM) in patients with melancholia – but not in those with non-melancholia – relative to controls ($p = 0.004$, one-tailed, $d = 0.58$). Also as hypothesized, HRV was decreased in patients with melancholia – but not in those with non-melancholia – relative to controls. More specifically, RMSSD ($p = 0.01$, one-tailed, $d = 0.56$), HF ($p = 0.014$, one-tailed, $d = 0.57$) and PCSD1 ($p = 0.01$, one-tailed, $d = 0.56$) were all decreased in patients with melancholia, relative to controls. No significant differences were observed between non-melancholia and controls. While patients with melancholia had higher heart rate than those with non-melancholia (by 6.75 BPM; $p = 0.046$, one-tailed, $d = 0.20$), no significant differences

Table 1 | Participant characteristics: mean (*M*) ± standard error (SD).[1]

	CTL (*M* ± SE)	NMEL (*M* ± SE)	MEL (*M* ± SE)	*p*-value[2]
Age	35.69 ± 1.16	34.98 ± 1.56	37.28 ± 2.07	0.657
Gender	52F/42M	17F/15M	25F/15M	0.675
SIGH-D	n/a	18.78 ± 0.68	21.50 ± 0.69	0.007
DASS-D	2.13 ± 0.25	21.70 ± 2.04	31.66 ± 1.39	<0.001[3]
DASS-A	1.19 ± 0.19	9.30 ± 1.79	15.23 ± 2.15	<0.001[3]
DASS-S	4.54 ± 0.47	19.00 ± 1.83	23.93 ± 1.88	<0.001[3]
CORE	n/a	3.45 ± 0.53	7.18 ± 0.86	<0.001[3]

[1] *M ± SE values based on original – not imputed data – data before propensity score matching of patient groupings;* [2] *p-value refers to that for omnibus ANOVA, or for SIGH-D and CORE, an independent-samples t-test;* [3] *post hoc tests revealed that all groups differ from each other, Tukey's HSD p < 0.05.*

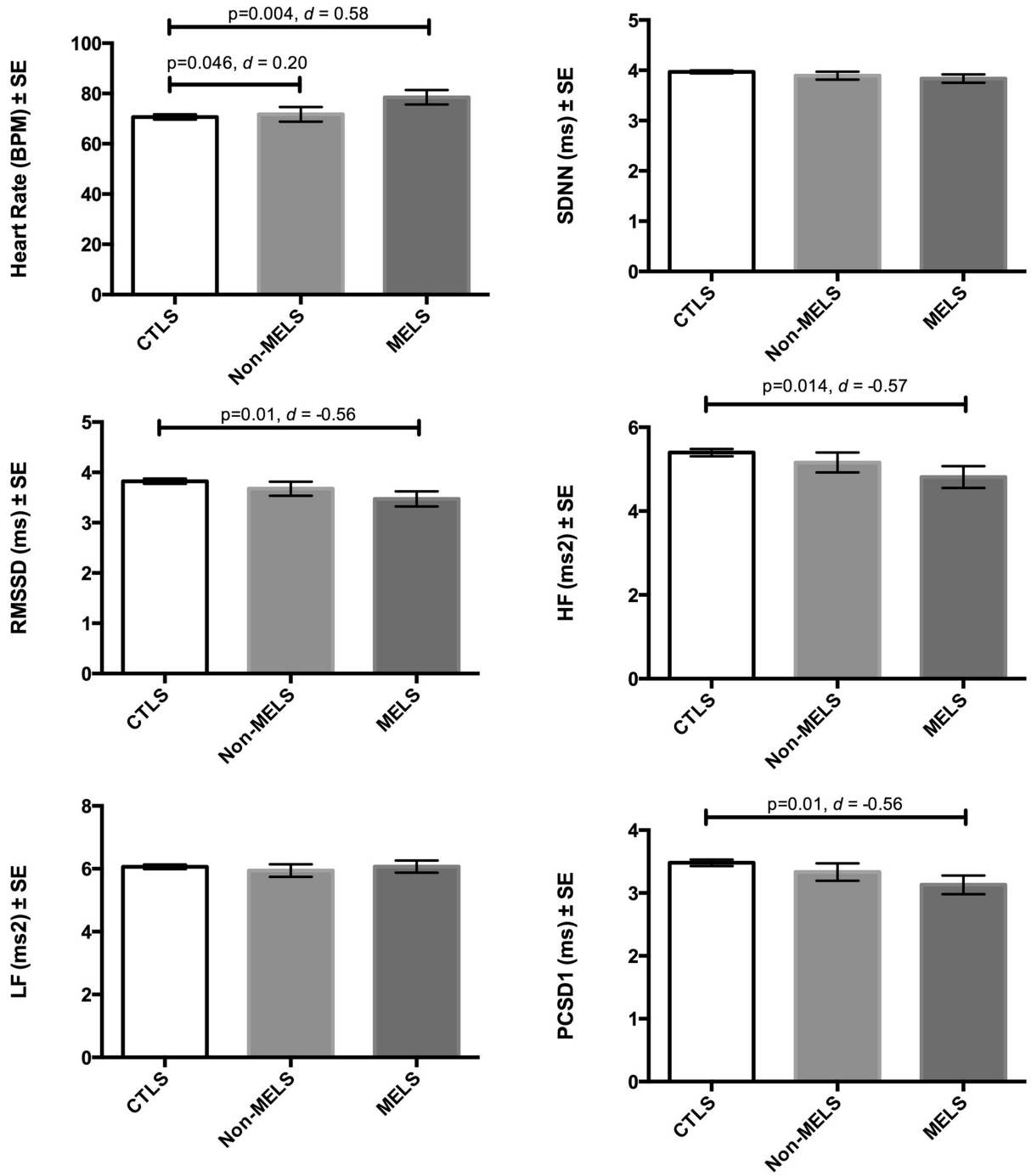


FIGURE 1 | Means and standard error for participant groupings (controls, non-melancholia, melancholia) across each of the dependent measures after controlling for age, gender, disorder severity, and comorbid anxiety disorders with propensity score matching.

on measures of HRV were observed between melancholia and non-melancholia.

DISCUSSION

The current study examined the impact of melancholia and non-melancholia on resting-state heart rate and HRV relative to controls, revealing robust alterations in patients with melancholia, but not in those with non-melancholia. These findings were associated with a moderate effect size across multiple measures (Cohens *d*'s = 0.56–0.58), providing strong evidence for an impact of melancholia on vagally mediated, cardiac function. We also observed patients with melancholia to display a higher

heart rate, relative to those with non-melancholia. We and others have demonstrated that HRV is inversely associated with disorder severity (Kemp et al., 2010) and that antidepressant medications – particularly tricyclic antidepressants and the serotonin and noradrenaline reuptake inhibitors often used in more severe depressions – have adverse effects on heart rate and HRV after controlling for disorder severity (Licht et al., 2010; Kemp et al., 2014). Together, these findings suggest that alterations in melancholic patients with severe depression treated with antidepressant medications would be stronger than those effects reported here, as our patients were all unmedicated.

Here we show that resting-state heart rate is increased – by almost eight beats per minute – and HRV, decreased in patients with melancholia relative to healthy controls, findings associated with a moderate effect size. An increased resting-state heart rate may reflect vagal withdrawal without necessarily, an increase in sympathetic nervous system activity (Porges, 2011; Kemp et al., 2014). This metabolically conservative response is usually observed during environmental challenge. We have suggested (Kemp et al., 2014) that this psychophysiological state may mirror the autonomic dysregulation observed in psychiatric illness during the resting-state, and the present study highlights that this may be the case for those with melancholia in particular. As noted above however, measures of HRV are more pure indicators of vagal activity (Saul, 1990) than heart rate, which also includes sympathetic input. Differences in heart rate were also observed between those with melancholia and non-melancholia, with those with melancholia displaying a higher heart rate, a difference of 6.75 BPM, a finding associated with a small effect size (Cohens $d = 0.20$). No differences however, were observed on measures of HRV. We suggest that this finding may reflect differences in a non-vagal (perhaps sympathetic) component of cardiac function. It is possible therefore that while vagal function distinguishes between patients and controls, non-vagal components of heart rate may further distinguish between disorder subtypes.

Critically, studies have reported strong evidence for a continuous increase in risk for cardiovascular and all-cause mortality in men and women with a resting heart rate above 60 beats/min, regardless of whether individuals have a history of cardiovascular disease (Fox et al., 2007; see also Cooney et al., 2010; Saxena et al., 2013). Here we observed patients with melancholia to display a higher resting, heart rate (78.48 BPM) relative to controls (70.63 BPM) and those with non-melancholia (71.73 BPM). Other studies (Lemogne et al., 2011; Åberg et al., 2014) have reported a relationship between high heart rate and suicide. In fact, 10 additional beats per minute has been shown to increase risk of suicide by 24 to 37% over a 9 year follow-up period in adjusted models (Lemogne et al., 2011). Another study on more than 1-million, 18-year-old participants with no prior mental illness (Åberg et al., 2014) reported that poor performance on cardiovascular fitness and cognitive tests was associated with a fivefold increased risk of suicide attempt or death over a 5- to 42-year follow-up period.

In addition to increases in heart rate, we also observed decreases in all measures of HRV except for SDNN and LF in patients with melancholia relative to controls. While SDNN reflects the 'ebb and flow' of a variety of factors including respiration, blood pressure control mechanisms, thermoregulation and kidney functioning, RMSSD is a more specific index of vagal function that correlates highly with the high-frequency component of HRV (Kleiger et al., 2005). Recent thinking also indicates that the low-frequency component of HRV reflects blood pressure control mechanisms such as the baroreflex (Goldstein et al., 2011; Reyes Del Paso et al., 2013). It is possible therefore that SDNN from short-term recordings is less sensitive than that extracted from 24-h long recordings, and that blood pressure control mechanisms are less involved in the differences observed here between patients with melancholia and controls.

A recent meta-analysis (Hillebrand et al., 2013) reported that HRV can predict the first cardiovascular event in individuals without known cardiovascular disease over a period of 3.5 to 15 years. Cardiovascular endpoints included hospitalization for angina pectoris, myocardial infarction, congestive heart failure, arterial peripheral vascular disease, coronary revascularization, stroke and cardiovascular death. This meta-analysis (Hillebrand et al., 2013) was based on eight studies with a total of 21,988 participants without known cardiovascular disease at baseline and reported pooled relative risks for a first cardiovascular event ranging from 1.35, 1.45, and 1.32 for SDNN, low-frequency or high-frequency HRV measures respectively. The authors proposed two possible mechanisms for their findings including vagal dysregulation activating inflammatory processes culminating in cardiovascular events. The other suggested mechanism was that individuals with low HRV already suffer from subclinical or silent CVD, highlighting that reduced HRV may be both a cause and consequence of ill health. Importantly, participants in the present study were free from serious medical conditions that may have otherwise impacted on the findings reported here.

It is important to note that our findings were obtained after accounting for a variety of confounding variables including severity of depression, anxiety and stress, and number of comorbid anxiety disorders. No significant differences were observed between patients with non-melancholia and controls, suggesting that heterogeneity in patient samples may underpin some of the past null findings that have been reported in the literature. Strengths of the present study include a medication free and physically healthy sample, and application of PSM to control for a variety of confounding variables across patient groupings. It is also important to acknowledge some limitations of our study. There are potential confounding factors that we did not control for here including physical activity (Rennie et al., 2003; Soares-Miranda et al., 2014), smoking status (Sjoberg and Saint, 2011; Harte and Meston, 2014), alcohol use (Quintana et al., 2013a,b), body mass index (Britton et al., 2007; Koenig et al., 2014), and biomarkers including fasting glucose (Stein et al., 2007) and cholesterol (Britton et al., 2007; Thayer and Fischer, 2013), all of which may impact on heart rate parameters. We refer interested readers to a recent review of the various issues that researchers should consider when collecting measures of HRV (Quintana and Heathers, 2014). Regardless, we note here that many of these factors known to impact on heart rate parameters are also observed in patients with mood disorders, highlighting the importance of cardiovascular risk reduction strategies in such patients, and on

the basis of the present study, those with melancholic symptoms in particular.

In conclusion, we report here that patients with melancholia – but not non-melancholia – display robust reductions in resting-state HRV relative to controls. This finding was observed even when applying PSM, a relatively novel technique to ensure that patient groupings did not differ on important confounds including age, gender, severity of depression, anxiety and stress, and comorbid anxiety disorders. Reduced vagal tone has important functional significance (e.g., impaired psychological flexibility; Thayer and Lane, 2000; Kashdan and Rottenberg, 2010), and over the longer-term may lead to significant morbidity and mortality from a host of conditions (Thayer et al., 2010; Kemp and Quintana, 2013). Our study therefore, has important implications for the health and wellbeing of patients with melancholic depression. Future studies are needed to further examine what particular behavioral features are most associated with the alterations in heart rate and HRV in patients with melancholia.

ACKNOWLEDGMENTS

Advice from Dr Felix Thoemmes at Cornell University and Joost van Ginkel at the University of Leiden are gratefully acknowledged for their assistance with propensity score matching and analysis of variance of multiply imputed data, respectively. We also acknowledge the data and support provided by BRAINnet; www.BRAINnet.net, under the governance of the BRAINnet Foundation. BRAINnet is the scientific network that coordinates access to the Brain Resource International Database for independent scientific purposes.

REFERENCES

Åberg, M. A., Nyberg, J., Torén, K., Sörberg, A., Kuhn, H. G., and Waern, M. (2014). Cardiovascular fitness in early adulthood and future suicidal behaviour in men followed for up to 42 years. *Psychol. Med.* 44, 779–788. doi: 10.1017/S0033291713001207

Albinet, C. T., Boucard, G., Bouquet, C. A., and Audiffren, M. (2010). Increased heart rate variability and executive performance after aerobic training in the elderly. *Eur. J. Appl. Physiol.* 109, 617–624. doi: 10.1007/s00421-010-1393-y

Alvares, G. A., Quintana, D. S., Kemp, A. H., Van Zwieten, A., Balleine, B. W., Hickie, I. B., et al. (2013). Reduced heart rate variability in social anxiety disorder: associations with gender and symptom severity. *PLoS ONE* 8:e70468. doi: 10.1371/journal.pone.0070468

Barzi, F., and Woodward, M. (2004). Imputations of missing values in practice: results from imputations of serum cholesterol in 28 cohort studies. *Am. J. Epidemiol.* 160, 34–45. doi: 10.1093/aje/kwh175

Billman, G. E. (2011). Heart rate variability – a historical perspective. *Front. Physiol.* 2:86. doi: 10.3389/fphys.2011.00086

Britton, A., Shipley, M., Malik, M., and Hnatkova, K. (2007). Changes in heart rate and heart rate variability over time in middle-aged men and women in the general population (from the Whitehall II Cohort Study). *Am. J. Cardiol.* 100, 524–527. doi: 10.1016/j.amjcard.2007.03.056

Brunoni, A. R., Kemp, A. H., Dantas, E. M., Goulart, A. C., Nunes, M. A., Boggio, P. S., et al. (2013). Heart rate variability is a trait marker of major depressive disorder: evidence from the sertraline vs. electric current therapy to treat depression clinical study. *Int. J. Neuropsychopharmacol.* 16, 1937–1949. doi: 10.1017/S1461145713000497

Brunoni, A. R., Lotufo, P. A., and Benseñor, I. M. (2012). Are antidepressants good for the soul but bad for the matter? Using noninvasive brain stimulation to detangle depression/antidepressants effects on heart rate variability and cardiovascular risk. *Biol. Psychiatry* 71, e27–e28; author reply e29–e30. doi: 10.1016/j.biopsych.2011.08.026

Carney, R. M., Rich, M. W., teVelde, A., Saini, J., Clark, K., and Freedland, K. E. (1988). The relationship between heart rate, heart rate variability and depression in patients with coronary artery disease. *J. Psychosom. Res.* 32, 159–164. doi: 10.1016/0022-3999(88)90050-5

Chalmers, J. A., Quintana, D. S., Abbott, M. J.-A., and Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. *Front. Psychiatry* 5:80. doi: 10.3389/fpsyt.2014.00080

Cohen, J. (1988). Set correlation and contingency tables. *Appl. Psychol. Meas.* 12, 425–434. doi: 10.1177/014662168801200410

Cooney, M. T., Vartiainen, E., Laakitainen, T., Juolevi, A., Dudina, A., and Graham, I. M. (2010). Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. *Am. Heart. J.* 159, 612–619.e3. doi: 10.1016/j.ahj.2009.12.029

de Jonge, P., Mangano, D., and Whooley, M. A. (2007). Differential association of cognitive and somatic depressive symptoms with heart rate variability in patients with stable coronary heart disease: findings from the Heart and Soul Study. *Psychosom. Med.* 69, 735–739. doi: 10.1097/PSY.0b013e31815743ca

Edwards, A. L. (1985). *Multiple Regression Analysis and the Analysis of Variance and Covariance*. New York, NY: Freeman.

Electrophysiology TFotESoCtNASoP. (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065.

Fox, K., Borer, J. S., Camm, A. J., Danchin, N., Ferrari, R., Sendon, J. L. L., et al. (2007). Resting heart rate in cardiovascular disease. *J. Am. Coll. Cardiol.* 50, 823–830. doi: 10.1016/j.jacc.2007.04.079

Friedman, B. H., and Thayer, J. (1998). Autonomic balance revisited: panic anxiety and heart rate variability. *J. Psychosom. Res.* 44, 133–151. doi: 10.1016/S0022-3999(97)00202-X

Gold, P. S., and Chrousos, G. P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol. Psychiatry* 7, 254–275. doi: 10.1038/sj.mp.4001032

Goldstein, D. S., Bentho, O., Park, M. Y., and Sharabi, Y. (2011). Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Gordon, E., Cooper, N., Rennie, C., Hermens, D., and Williams, L. M. (2005). Integrative neuroscience: the role of a standardized database. *Clin. EEG Neurosci.* 36, 64–75. doi: 10.1177/155005940503600205

Hamilton, M. (1960). A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* 23, 56–62. doi: 10.1136/jnnp.23.1.56

Hansen, A. L., Thayer, J., Johnsen, B. H., Sollers, J. J., and Stenvik, K. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur. J. Appl. Physiol.* 93, 263–272. doi: 10.1007/s00421-004-1208-0

Harte, C. B., and Meston, C. M. (2014). Effects of smoking cessation on heart rate variability among long-term male smokers. *Int. J. Behav. Med.* 21, 302–309. doi: 10.1007/s12529-013-9295-0

Hickie, I. (1996). "Validity of the CORE," in *Melancholia: A Disorder of Movement and Mood*, eds G. Parker and D. Hazdi-Pavlovic (New York: Cambridge University Press), 149–159.

Hill, J. (2004). *Reducing Bias in Treatment Effect Estimation in Observational Studies Suffering from Missing Data (No. ISERP Working Paper 04-01)*. New York: Institute for Social and Economic Research and Policy, Columbia University. Working Papers.

Hillebrand, S., Gast, K. B., de Mutsert, R., Swenne, C. A., Jukema, J. W., Middeldorp, S., et al. (2013). Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. *Europace* 15, 742–749. doi: 10.1093/europace/eus341

Kashdan, T., and Rottenberg, J. (2010). Psychological flexibility as a fundamental aspect of health. *Clin. Psychol. Rev.* 30, 865–878. doi: 10.1016/j.cpr.2010.03.001

Kemp, A. H. (2011). Depression, antidepressant treatment and the cardiovascular system. *Acta Neuropsychiatr.* 23, 82–83. doi: 10.1111/j.1601-5215.2011.00535.x

Kemp, A. H. (2012). Are antidepressants good for the soul but bad for the matter? using noninvasive brain stimulation to detangle depression/antidepressants effects on heart rate variability and cardiovascular risk. *Biol. Psychiatry* 71, e29–e30. doi: 10.1016/j.biopsych.2011.11.002

Kemp, A. H., Brunoni, A. R., Santos, I. S., Nunes, M. A., Dantas, E. M., Carvalho de Figueiredo, R., et al. (2014). Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil

cohort baseline study. *Am. J. Psychiatry* doi: 10.1176/appi.ajp.2014.13121605 [Epub ahead of print].

Kemp, A. H., and Quintana, D. S. (2013). The relationship between mental and physical health: insights from the study of heart rate variability. *Int. J. Psychophysiol.* 89, 288–296. doi: 10.1016/j.ijpsycho.2013.06.018

Kemp, A. H., Quintana, D. S., Felmingham, K. L., Matthews, S., and Jelinek, H. F. (2012). Depression, comorbid anxiety disorders, and heart rate variability in physically healthy, unmedicated patients: implications for cardiovascular risk. *PLoS ONE* 7:e30777. doi: 10.1371/journal.pone.0030777

Kemp, A. H., Quintana, D. S., and Gray, M. A. (2011). Is heart rate variability reduced in depression without cardiovascular disease? *Biol. Psychiatry* 69, e3–e4. doi: 10.1016/j.biopsych.2010.07.030

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., and Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol. Psychiatry* 67, 1067–1074. doi: 10.1016/j.biopsych.2009.12.012

Kim, D. H., Lipsitz, L. A., Ferrucci, L., Varadhan, R., Guralnik, J. M., Carlson, M. C., et al. (2006). Association between reduced heart rate variability and cognitive impairment in older disabled women in the community: Women's Health and Aging Study I. *J. Am. Geriatr. Soc.* 54, 1751–1757. doi: 10.1111/j.1532-5415.2006.00940.x

Kleiger, R. E., Stein, P. K., and Bigger, J. T. (2005). Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 10, 88–101. doi: 10.1111/j.1542-474X.2005.10101.x

Koenig, J., Jarczok, M. N., Warth, M., Ellis, R. J., Bach, C., Hillecke, T. K., et al. (2014). Body mass index is related to autonomic nervous system activity as measured by heart rate variability–a replication using short term measurements. *J. Nutr. Health Aging* 18, 300–302. doi: 10.1007/s12603-014-0022-6

Krygier, J. R., Heathers, J. A. J., Shahrestani, S., Abbott, M., Gross, J. J., and Kemp, A. H. (2013). Mindfulness meditation, well-being, and heart rate variability: a preliminary investigation into the impact of intensive Vipassana meditation. *Int. J. Psychophysiol.* 89, 305–313. doi: 10.1016/j.ijpsycho.2013.06.017

Lemogne, C., Thomas, F., Consoli, S. M., Pannier, B., Jégo, B., and Danchin, N. (2011). Heart rate and completed suicide: evidence from the IPC cohort study. *Psychosom. Med.* 73, 731–736. doi: 10.1097/PSY.0b013e3182365dc7

Licht, C. M. M., de Geus, E. J. C., van Dyck, R., and Penninx, B. W. J. H. (2010). Longitudinal evidence for unfavorable effects of antidepressants on heart rate variability. *Biol. Psychiatry* 68, 861–868. doi: 10.1016/j.biopsych.2010.06.032

Licht, C. M. M., de Geus, E. J. C., Zitman, F. G., Hoogendijk, W. J. G., van Dyck, R., and Penninx, B. W. J. H. (2008). Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). *Arch. Gen. Psychiatry* 65, 1358–1367. doi: 10.1001/archpsyc.65.12.1358

Licht, C. M. M., Penninx, B. W., and de Geus, E. J. C. (2011). To include or not to include? A response to the meta-analysis of heart rate variability and depression. *Biol. Psychiatry* 69, e1; author reply e3–e4. doi: 10.1016/j.biopsych.2010.06.034

Lovibond, P. F., and Lovibond, S. H. (1995). The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behav. Res. Ther.* 33, 335–343. doi: 10.1016/0005-7967(94)00075-U

Malhi, G. S., Parker, G. B., and Greenwood, J. (2005). Structural and functional models of depression: from sub-types to substrates. *Acta Psychiatr. Scand.* 111, 94–105. doi: 10.1111/acp.2005.111.issue-2

Marshall, A., Altman, D. G., Holder, R. L., and Royston, P. (2009). Combining estimates of interest in prognostic modelling studies after multiple imputation: current practice and guidelines. *BMC Med. Res. Methodol.* 9:57. doi: 10.1186/1471-2288-9-57

Mayberg, H. S., Nemeroff, C., Krahl, S. E., Mcnamara, J., Frazer, A., Henry, T. R., et al. (2006). VNS therapy in treatment-resistant depression: clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology* 31, 1345–1355. doi: 10.1038/sj.npp.1301082

Pan, J., and Tompkins, W. J. (1985). A real-time QRS detection algorithm. *IEEE Trans. Biomed. Eng.* 32, 230–236. doi: 10.1109/TBME.1985.325532

Penttilä, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H. V., Tulppo, M. P., et al. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clin. Physiol.* 21, 365–376. doi: 10.1046/j.1365-2281.2001.00337.x

Picard, G., Tan, C. O., Zafonte, R., and Taylor, J. A. (2009). Incongruous changes in heart period and heart rate variability with vagotonic atropine: implications for rehabilitation medicine. *PM R* 1, 820–826. doi: 10.1016/j.pmrj.2009.07.017

Porges, S. W. (2011). *The Polyvagal Theory: Neurophysiological Foundations of Emotions, Attachment, Communication, and Self-regulation*, 1st Edn. New York: W. W. Norton & Company.

Quinn, C. R., Harris, A., Felmingham, K., Boyce, P., and Kemp, A. H. (2012a). The impact of depression heterogeneity on cognitive control in major depressive disorder. *Aust. N. Z. J. Psychiatry* 46, 1079–1088. doi: 10.1177/0004867412461383

Quinn, C., Harris, A., and Kemp, A. H. (2012b). The interdependence of subtype and severity: contributions of clinical and neuropsychological features to melancholia and non-melancholia in an outpatient sample. *J. Int. Neuropsychol. Soc.* 18, 361–369. doi: 10.1017/S1355617711001858

Quintana, D. S., Guastella, A. J., McGregor, I. S., Hickie, I. B., and Kemp, A. H. (2013a). Moderate alcohol intake is related to increased heart rate variability in young adults: implications for health and well-being. *Psychophysiology* 50, 1202–1208. doi: 10.1111/psyp.12134

Quintana, D. S., McGregor, I. S., Guastella, A. J., Malhi, G. S., and Kemp, A. H. (2013b). A meta-analysis on the impact of alcohol dependence on short-term resting-state heart rate variability: implications for cardiovascular risk. *Alcohol. Clin. Exp. Res.* 37(Suppl. 1), E23–E29. doi: 10.1111/j.1530-0277.2012.01913.x

Quintana, D. S., and Heathers, J. A. J. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Front. Psychol.* 5:805. doi: 10.3389/fpsyg.2014.00805

Rennie, K. L., Hemingway, H., Kumari, M., Brunner, E., Malik, M., and Marmot, M. (2003). Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants. *Am. J. Epidemiol.* 158, 135–143. doi: 10.1093/aje/kwg120

Reyes Del Paso, G. A., Langewitz, W., Mulder, L. J. M., Roon, A., and Duschek, S. (2013). The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* 50, 477–487. doi: 10.1111/psyp.12027

Saul, J. P. (1990). Beat-to-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *Physiology* 5, 32–37.

Saxena, A., Minton, D., Lee, D.-C., Sui, X., Fayad, R., Lavie, C. J., et al. (2013). Protective role of resting heart rate on all-cause and cardiovascular disease mortality. *Mayo Clin. Proc.* 88, 1420–1426. doi: 10.1016/j.mayocp.2013.09.011

Schafer, J. L. (1999). Multiple imputation: a primer. *Stat. Methods Med. Res.* 8, 3–15. doi: 10.1177/096228029900800102

Sheehan, D., Lecrubier, Y., Sheehan, K., Amorim, P., Janavs, J., Weiller, E., et al. (1998). The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* 59, 22. doi: 10.1016/S0924-9338(99)80239-9

Sjoberg, N., and Saint, D. A. (2011). A single 4 mg dose of nicotine decreases heart rate variability in healthy nonsmokers: implications for smoking cessation programs. *Nicotine Tob. Res.* 13, 369–372. doi: 10.1093/ntr/ntr004

Soares-Miranda, L., Sattelmair, J., Chaves, P., Duncan, G. E., Siscovick, D. S., Stein, P. K., et al. (2014). Physical activity and heart rate variability in older adults: the Cardiovascular Health Study. *Circulation* 129, 2100–2110. doi: 10.1161/CIRCULATIONAHA.113.005361

Stein, P. K., Barzilay, J. I., Domitrovich, P. P., Chaves, P. M., Gottdiener, J. S., Heckbert, S. R., et al. (2007). The relationship of heart rate and heart rate variability to non-diabetic fasting glucose levels and the metabolic syndrome: the Cardiovascular Health Study. *Diabet. Med.* 24, 855–863. doi: 10.1111/j.1464-5491.2007.02163.x

Tarvainen, M., and Niskanen, J. (2008). *Kubios HRV* (No. 13779), 2nd Edn, 1–53. Kuopio: Biosignal Analysis and Medical Imaging Group (BSAMIG), Department of Physics, University of Kuopio, Kuopio.

Thayer, J., and Fischer, J. E. (2013). Heart rate variability, overnight urinary norepinephrine, and plasma cholesterol in apparently healthy human adults. *Int. J. Cardiol.* 162, 240–244. doi: 10.1016/j.ijcard.2011.05.058

Thayer, J., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J., Yamamoto, S. S., and Brosschot, J. F. (2010). The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* 141, 122–131. doi: 10.1016/j.ijcard.2009.09.543

Thoemmes, F. (2012). Propensity score matching in SPSS. arXiv:1201.6385. Available at: http://arxiv.org/ftp/arxiv/papers/1201/1201.6385.pdf

van Ginkel, J. R., and Kroonenberg, P. M. (2014). Analysis of variance of multiply imputed data. *Multivariate Behav. Res.* 49, 78–91. doi: 10.1080/00273171.2013.855890

Williams, J. B. (1988). A structured interview guide for the Hamilton Depression Rating Scale. *Arch. Gen. Psychiatry* 45, 742–747. doi: 10.1007/s12160-009-9101-z

Conflict of Interest Statement: Patrick Hopkinson was an employee at Brain Resource Company when this study was conducted. Brain Resource (http://www.brainresource.com) and Johnson & Johnson Pharmaceutical Research and Development, RED Europe funded the research on which this study is based. However, funders played no role in analysis of data, interpretation of results, writing of the paper, or decision to submit for publication.

Received: 24 July 2014; accepted: 13 November 2014; published online: 27 November 2014.

*Citation: Kemp AH, Quintana DS, Quinn CR, Hopkinson P and Harris AWF (2014) Major depressive disorder with melancholia displays robust alterations in resting state heart rate and its variability: implications for future morbidity and mortality. Front. Psychol. **5**:1387. doi: 10.3389/fpsyg.2014.01387*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in
PSYCHOLOGY

REVIEW ARTICLE
published: 22 July 2014
doi: 10.3389/fpsyg.2014.00805

Considerations in the assessment of heart rate variability in biobehavioral research

Daniel S. Quintana[1,2] * and James A. J. Heathers[3]

[1] NORMENT, K.G. Jebsen Centre for Psychosis Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[2] Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway
[3] School of Psychology, University of Sydney, Sydney, NSW, Australia

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Mika Tarvainen, University of Eastern Finland, Finland
George E. Billman, The Ohio State University, USA

****Correspondence:***
Daniel S. Quintana, NORMENT, K.G. Jebsen Centre for Psychosis Research – TOP Study, Building 49, Oslo University Hospital, Ullevål, Kirkeveien 166, P. O. Box 4956 Nydalen, 0424 Oslo, Norway
e-mail: daniel.quintana@medisin.uio.no

Heart rate variability (HRV) refers to various methods of assessing the beat-to-beat variation in the heart over time, in order to draw inference on the outflow of the autonomic nervous system. Easy access to measuring HRV has led to a plethora of studies within emotion science and psychology assessing autonomic regulation, but significant caveats exist due to the complicated nature of HRV. Firstly, both breathing and blood pressure regulation have their own relationship to social, emotional, and cognitive experiments – if this is the case are we observing heart rate (HR) changes as a consequence of breathing changes? Secondly, experiments often have poor internal and external controls. In this review we highlight the interrelationships between HR and respiration, as well as presenting recommendations for researchers to use when collecting data for HRV assessment. Namely, we highlight the superior utility of within-subjects designs along with the importance of establishing an appropriate baseline and monitoring respiration.

Keywords: heart rate variability, autonomic nervous system, parasympathetic nervous system, psychophysiology, respiration, emotion

THE USE OF HRV IN EMOTION SCIENCE AND PSYCHOLOGY

The autonomic nervous system has been studied as a correlate of emotion for almost a century (Cannon, 1916). A central technique within this tradition of research is heart rate variability (HRV), which refers to a variety of methods for assessing the beat-to-beat change in the heart over time; these are used to approximate various aspects of autonomic outflow to the heart. Improvements in computing technology and miniaturization have made the electrocardiographic collection of inter-beat intervals (IBIs) accessible, and the analysis of the resulting beat-to-beat intervals trivial. One consequence of this access is a sustained interest in the application of HRV within the behavioral sciences, and in the psychology of emotion in particular. There are major biobehavioral theories that suggest that HRV can be used to investigate the central relationship between autonomic regulation and interpersonal interaction (Porges, 1995; Thayer and Lane, 2000). The neurovisceral integration model suggests that HRV is an index of the capacity for the central autonomic network (Benarroch, 1993) – which includes the brainstem, hypothalamus, and prefrontal cortex – to adjust to environmental demands (Thayer and Lane, 2000). Porges' polyvagal theory takes a phylogenetic approach (i.e., it observes evolutionary and developmental commonalities within the structure and function of the vertebrate autonomic nervous system), arguing that social engagement is centrally facilitated by outflow and functional organization of vagus nerve (Porges, 1995). Consistent with this theory, reduced HRV has been observed in psychiatric disorders characterized by poor social cognition and emotion regulation (Bär et al., 2007; Quintana et al., 2013b). Interestingly, psychiatric patients also demonstrate less HRV reactivity during different levels of mental loading in comparison to healthy controls (Valkonen-Korhonen et al., 2003), further highlighting the poor cardiorespiratory regulatory capacity of this population.

While it may be the case that HRV can be used as a neurobiological index of interpersonal interaction, significant caveats exist due to the complicated nature of HRV and consequently uncertainty regarding what information is actually provided by common HRV indices (Berntson et al., 1997; Malpas, 2002; Billman, 2011). Additionally, the relationship between HRV and vagal modulation is complex in itself with a large interindividual variation (Picard et al., 2009). The problem is further compounded by the co-modulation of various respiratory and circulatory factors, which occur via numerous mechanisms and over multiple time-scales. Moreover, both breathing and blood pressure regulation have their own directly mediated relationships to the tasks employed in social, emotional, and cognitive experiments – if this is the case, we often have a complicated question of interlocking causalities. For instance, are observed changes in heart period epiphenomena that can be more parsimoniously described by changes in breathing or blood pressure? If the direction of causality between experimental task and the coordinated response within cardiac, circulatory, and respiratory variables is poorly understood, simple relationships between task and output changes may be obscured. Finally, experiments are often poorly designed as uncontrolled variables within typical experimental environments may drastically influence HRV. Few papers ideally control for medication, food, and water consumption, bladder filling, time of day, and other extraneous factors (Tak et al., 2009; Heathers, 2014). The overall aim of this review is to highlight the interrelationships between the nature and extraneous control of HRV, with a particular emphasis on respiration, and discuss implications for research in emotion science and psychology. Firstly, a number of

important factors for the assessment of HRV in general and in emotion psychology in particular will be outlined. Secondly, solutions will be presented to reduce the potential impact of these factors.

CAVEATS AND CONSIDERATIONS

RESPIRATION IN HRV RESEARCH

Coupling between respiration and heart rate (HR) has a long research history, and was noted in classical animal studies predating the electrocardiogram, which noticed fluctuations with breathing of heart beat and blood pressure (Ludwig, 1847). Consequently, the typically functioning respiratory system is presently characterized by complex breath-to-breath variations in respiratory rate and depth (Bruce, 1996) coupled with both heart period and blood pressure oscillations in a network of continual co-modification. For instance, a decrease in respiratory frequency generally corresponds with a lengthening of the heart period (Bruce, 1996). The traditional experimental approach of assessing the impact of the manipulation of one of these variables on another has led to important advancements in the understanding of cardiorespiratory coupling. However, perturbing the cardiorespiratory system does not allow the observation of casual relationships during spontaneous activity. Procedures developed to examine the coupling between time series may facilitate the identification of directionality and strength of cardiorespiratory coupling during spontaneous activity but these traditionally have only provided a limited insight into causality (e.g., Granger causality; Granger, 1969). Indeed, cardiorespiratory interaction has been variously quantified as primarily respiration-to-heart rate (Rosenblum et al., 2002; Zhu et al., 2013) heart rate-to-respiration (Larsen et al., 1999; Tzeng et al., 2003) or neither (i.e., bidirectional; Porta et al., 2013). These differences are likely to strongly depend on the analytical technique employed, but the details of this are unclear.

The nature of cardiorespiratory coupling is of intense research interest, highlighted most centrally by a robust debate concerning the central (Eckberg, 2009) and baroreflex (Karemaker, 2009) mechanism contributions to respiratory sinus arrhythmia (RSA). There is also a common genetic influence on HRV and respiration (Kupper et al., 2005). To further complicate this already complex relationship, the degree of cardiorespiratory coupling depends on the respiratory rate. That is, as the respiratory rate increases, HR increases phase distance from respiration. For instance, a breathing rate of 5–6 breaths per minute corresponds with a phase angle increase of 90°, continuing to a phase angle of 180° with 10 breaths per minute (Angelone and Coulter, 1964). Indeed, a presumed tenet of RSA – that shorter R-R intervals should be coupled with the apogee of inspiration – only occurs at a slow respiratory rate of six breaths per minute (Vaschillo et al., 2004), around half the natural respiration rate. However, there is no relationship between cardiorespiratory coupling and baroreflex sensitivity or blood pressure variability (Tzeng et al., 2003).

Further, shared neural networks for respiratory and HR oscillations (Evans et al., 2009) suggest that the manipulation on breathing may also lead to unintended effects on HRV by removing some of the variance in HRV that may relevantly covary with experimental task. Intriguingly, the degree of coupling may be higher when HRV is increased and at lower breathing frequencies (Galletly and Larsen, 2001; Tzeng et al., 2003), suggesting that unhealthy populations or experiments that are designed to reduce HRV may be more prone to decoupling of cardiorespiratory oscillations. This observation is particularly relevant when comparing two populations that may display different breathing frequencies (e.g., anxious vs. non-anxious participants) or when an experimental manipulation modifies respiration. Notably, respiration is not a necessary condition to modify HR over time as variability is still observed (although significantly reduced) without mechanical respiratory input to the heart (Larsen et al., 1999). Conversely, individuals with no vagal input to the heart (e.g., heart transplant recipients) still demonstrate RSA (although to a much smaller degree) presumably due to mechanical effects on the sinoatrial node (Bernardi et al., 1989; Slovut et al., 1998). While respiration influences blood pressure via mechanical intrathoracic pressure changes, this is buffered by HRV (Toska and Eriksen, 1993; Elstad et al., 2001). The influence of respiration on blood pressure is likely to be caused by the mechanical influence on venous return, modulating cardiac output (Triedman and Saul, 1994) via changes in stroke volume, which in turn influences blood pressure (Elstad et al., 2001).

THE IMPACT OF RESPIRATION DURING SOCIAL-EMOTIONAL TASKS

Social-emotional tasks have been shown to reduce breathing variability (Vlemincx et al., 2011, 2012a), even for positively valenced emotions (Boiten, 1998), due to the "locked-in" attention often required during social-emotional tasks. Moreover, the mental stress that usually accompanies these tasks can also disorder general respiratory coordination (Vlemincx et al., 2012b). In addition to overall breathing variability, experimental stress induction can also influence the specific length of inspiration and expiration (Cohen et al., 1975). Thus, a social-emotional task that induces a change in respiratory time variables and/or depth may be indirectly influencing HRV. The rates of sighing also increase during these tasks (Vlemincx et al., 2011), with sighs shown to "reset" both respiratory variability and emotional states (Vlemincx et al., 2013). This is consistent with observations of increased sighing in a range of anxiety disorders (Abelson et al., 2001; Nardi et al., 2009), and increased sighing during experimentally induced stress (Vlemincx et al., 2012b). Finally, continual focused attention (e.g., during psychometrics tasks) has been shown in a number of studies (Mulder and Mulder, 1981; Aasman et al., 1987; Middleton et al., 1999) to reduce LF HRV, which creates further difficulties for interpretation.

There has been considerable debate on the necessity of controlling for respiration when assessing HRV. Denver et al. (2007) have argued against the need to control for respiration – at least for resting state recordings – given the important influence of breathing on HRV. To wit, by controlling for breathing in HRV recordings the researcher is removing an important influence on HRV (but see Grossman and Taylor, 2007). Denver et al. (2007) argue that if we assume that both respiration and heart beat oscillations are generated from the same central origin (e.g., Eckberg, 2009) then under resting state conditions controlling for respiration may not be necessary. Indeed, proponents for the control of respiration

assume (either explicitly or implicitly) that alterations in respiratory frequency bring provoke HRV changes (i.e., the direction of causality moves from respiration to HR) without considering that HR adjustments may provoke changes in respiratory drive (Tzeng et al., 2003).

One compromise solution is to measure a participant's natural breathing rate, and use the derived frequency for respiratory pacing (e.g., Elstad, 2012). While this approach has utility during resting state registration, this procedure may inadvertently influence HRV during emotional or cognitive tasks as the participant has to consciously follow the pacing cue, in addition to paying attention to the experimental task – dual attention, in a number of contexts, significantly increases task difficulty (Pashler, 1994). Zhang et al. (2010) argue that cardiorespiratory coupling during a cognitive task can be influenced either by activation of the motor cortex, which deceases cardiorespiratory coupling, or via increases in SNS activity from completing a cognitive task. However, here sympathetic outflow was indexed by normalized low frequency HRV – which is not straightforwardly related to SNS activity (e.g., Grassi and Esler, 1999; Moak et al., 2007; Goedhart et al., 2008; Billman, 2011, 2013a) – so this latter claim requires further empirical support using indices that more directly index cardiac sympathetic outflow.

Finally, slow respiratory rates (below 0.15 Hz) hinder the reliable estimation of RSA given the overlap with the LF component, which can be an issue for physically fit individuals, or with experimentally induced relaxation. While the RSA peak can be visually identified on a person-to-person basis, an objective algorithm based on a continuous wavelet transform has been developed to select variable HF bandwidth based on the power spectrum of the respiratory signal (Goren et al., 2006).

THE POORLY ADDRESSED NATURE OF HRV

While the collection of raw interbeat interval data is relatively straightforward process, several lines of evidence suggest that ancillary and interpretative factors surrounding HRV receive insufficient attention.

(1) Heart rate variability is affected by respiratory depth (Hirsch and Bishop, 1981) and frequency (Angelone and Coulter, 1964; Brown et al., 1993). Specifically, greater RSA magnitude occurs during higher tidal volumes and lower respiratory frequencies. In addition, basal respiratory frequency has a non-linear relationship with spectral power as breathing rate falls below approximately 0.15 Hz (as it occasionally does in athletes; Saboul et al., 2014). Thus, any task that increases respiratory tidal volume and/or reduces respiratory frequency (e.g., meditation; Krygier et al., 2013), or conversely decreases tidal volume and/or increases respiratory frequency (e.g., mental stress; Houtveen et al., 2002) is likely to indirectly modify HRV. More recently, it has also been shown that the inspiration:expiration (I:E) ratio also effects HRV (Strauss-Blasche et al., 2000). Specifically, HRV increases when short inspiration is followed by long expiration – which has implications for tasks that require speech production (Cysarz et al., 2004) and many forms of meditation, for instance. Even monitoring spontaneous breathing has been found to reduce respiratory variability (Cysarz and Büssing, 2005; Conrad et al., 2007). HR driven cardiorespiratory coupling also appears to increase when HRV is higher (Galletly and Larsen, 2001).

(2) While respiration has been most typically studied as the dominant physiological rhythm relevant to HRV, much less in known about chemosensory (Berthoud and Neuhuber, 2000; Niewinski et al., 2014) and circadian (Furlan et al., 1990; Guo and Stein, 2002; Bonnemeier et al., 2003) influences.

(3) Heart rate variability continues to be used to form an index of putative autonomic outflow by measuring a point on a simple continuum of parasympathetic/sympathetic activity. While this model is still popular, it is directly at odds with a great deal of available evidence; for instance, that neuropeptide Y directly mediates transmission between adrenergic and muscarinic neurons (Revington and McCloskey, 1990). This approach, generally focused around the use of the LF/HF ratio (the ratio of low frequency power to high frequency power) to represent "sympathovagal balance," has been criticized extensively for over two decades (e.g., Eckberg, 1997; Billman, 2013a). This obscures the interpretation of HRV from the approximately 65% of papers which still report metrics in this manner (Heathers, 2014). While it is clear that LF power does not represent sympathetic activity (Goldstein et al., 2011) it is important to note that there has also been robust debate surrounding the relationship between HF power and parasympathetic activity (for a review see Billman, 2011).

(4) Differences in the prevailing HR can influence HRV both mathematically, due to the inverse curvilinear relationship between HR and RR interval (Sacha and Pluta, 2008) and physiologically, via the augmenting or diminishing effect of the autonomic constituent of HRV (Billman, 2013b). Consequently, emotional interventions that reduce PNS activation could inflate reductions in HRV via HR increases that are independent of changes in cardiac autonomic nerve activity. Nevertheless, it is possible to mathematically correct for the influence of the prevailing HR on HRV (Sacha, 2013; Pradhapan et al., 2014), which may also improve the reproducibility of HRV (Sacha et al., 2013).

Notwithstanding the evidence, these important caveats do not discourage research in the social and psychological sciences, which equate HRV variously as an index of emotional regulation (Appelhans and Luecken, 2006), stress response (Berntson and Cacioppo, 2004), and interpersonal engagement (Butler et al., 2006). Moreover, over 32 studies have specifically investigated the effect of emotion on HRV in healthy participants (Kreibig, 2010).

THE NON-LINEAR NATURE OF HRV

Frequency analysis assumes the HR signal is stationary (Stratonovich, 1967) and that over time it can be modeled as the sum of cyclical processes, but this is demonstrably not the case. While removing slow or DC trends from short periods of HRV will create a quasi-stationary series (e.g., Tarvainen et al., 2002), HRV in general displays the characteristics of a non-linear signal, given the biological origin and the origin of HRV deriving from sum of processes that operate on a variety of time scales (Winfree, 2001; Piskorski and Guzik, 2007; Stein et al., 2008). The non-linear interaction of the PNS and SNS systems may also contribute to heart beat complexity observed in healthy participants (Levy, 1971). 1/*f*-like scaling of the heart beat signal, which

is characteristic of a heart beat series from a healthy individual (Ivanov et al., 1999; Goldberger et al., 2002), also points to a non-linear basis. A $1/f$ scaling of the heart beat signal ($\alpha = 1$) falls exactly between a completely random signal ($\alpha = 0.5$; i.e., white noise) and an entirely predictable signal ($\alpha = 1.5$). For instance, pathological heart rhythms tend to demonstrate Brownian noise (Peng et al., 1995). A complex interaction of linear and non-linear systems contribute to HRV (Voss et al., 2009), which suggests that measures of complexity may be a better measure of autonomic nervous system outflow (Kaplan et al., 1991). Indeed, non-linear measures of HRV have demonstrated improved prognostic information in heart failure patients with in comparison to linear HRV measures (Bigger et al., 1996; Huikuri et al., 2000). However, the utility of non-linear HRV measures have been questioned due to a lack of reproducibility (Tan et al., 2009).

Intriguingly, non-linear analysis indicates that some elderly patients with cardiovascular disease unexpectedly display increased HRV indices (Stein et al., 2005) due to erratic, non-respiratory sinus arrhythmia. These erratic rhythms have also been found to predict the onset of ventricular tachycardia (Mäkikallio et al., 1997) and mortality post-myocardial infarction (Stein et al., 2008). The source of this erratic non-respiratory sinus arrhythmia may be due to increased sympathetic activity (Tulppo et al., 1998), which is consistent with the higher concentrations of plasma noradrenaline observed in patients post-myocardial infarction (Christensen and Videbaek, 1974). Alternatively, erratic rhythms may be caused by poor coordination between the sinoatrial and atrioventricular nodes, which could reflect a pre-clinical manifestation of sick sinus syndrome (Stein et al., 2008).

A Poincaré plot is a visual, non-linear HRV index comprised of points that represent two consecutive heart periods, with any point above the identity line (a 45° slope that passes through the origin, which represents equal consecutive heart periods) representing a longer heart period, whereas points below the identity line represent a shortening of the heart period. A healthy participant typically displays a "comet" shaped plot (**Figure 1A**), with a wider dispersion of points as the beats lengthen. Even at different rates of breathing (ranging from 6 to 16 breaths/min) this shape persists in healthy participants (Guzik et al., 2007). On the other hand, patients with heart failure display atypical "torpedo," "fan," or "complex" (i.e., stepwise clusters of points) patterns (Woo et al., 1992). A torpedo shape (**Figure 1B**) is indicative of a lack of R-R interval increase when HR slows, whereas fan and complex patterns (**Figure 1C**) may represent general issues with cardiac autonomic regulation. Poincaré plots have been demonstrated to shown to display significant asymmetry in approximately 80% of individuals (Guzik et al., 2006; Piskorski and Guzik, 2007; Porta et al., 2008), with the plot "cloud" above the identity line appearing larger than the plot cloud below the line. Absent of long-term trends or very low frequency (VLF) power changes typically removed via detrending or high-pass filtering, HR acceleration will be matched with a roughly corresponding deceleration over time, and the Poincaré plots might be expected to be symmetrical. However, this commonly observed asymmetry in Poincaré plots suggests that HR accelerations operate in a different manner than decelerations, possibly due to baroreflex responses (Guzik et al., 2006). While the source of this asymmetry is unclear, it reinforces the fact that HRV is generated by complex non-linear dynamics. Together, this work emphasizes the importance of scrutinizing Poincaré plots for irregularities, particularly for populations characterized by low HRV (e.g., older participants), and urges caution with the central assumption that IBIs over time can be meaningfully devolved into the sum of sine waves as in traditional frequency-domain analysis.

EXTERNAL FACTORS THAT CAN INFLUENCE HRV

A number of external factors are usually controlled for in HRV research, including the intake of nicotine (Hayano et al., 1990; Sjoberg and Saint, 2011) and caffeine (Sondermeijer et al., 2002) preceding data collection. Cardioactive medication use, including some antidepressant classes (e.g., tricyclics; Kemp et al., 2010), some antipsychotic classes (e.g., clozapine; Cohen et al., 2001), benzodiazepines (Agelink et al., 2002), and antihypertensives (Schroeder et al., 2003) are also usually accounted for, although this may be somewhat difficult in practice when testing patient populations. Other factors that are usually accounted for include the time of day (Massin et al., 2000; van Eekelen et al., 2004), levels of habitual alcohol use (Quintana et al., 2013a,b),

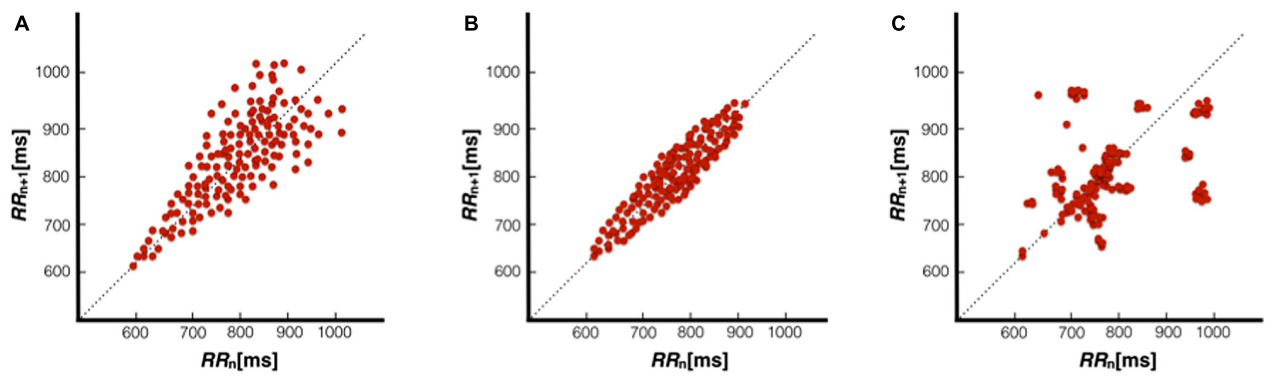


FIGURE 1 | Poincaré plots representative of a healthy individual with a comet pattern (A), heart failure patient with a torpedo pattern (B), and heart failure patient with a complex pattern (C). Figures adapted from Woo et al. (1992).

physical activity levels (Britton et al., 2007; Soares-Miranda et al., 2014), and age (O'Brien et al., 1986). Digestion of food and water are less commonly accounted for in HRV research, but both provoke a coordinated autonomic response. For instance, digesting food has been shown to reduce parasympathetic activity, even an hour after eating a 500 kcal meal (Lu et al., 1999). Even exposure to food-related cues elicits a similar response (Nederkoorn et al., 2000), suggesting a physiological response to the anticipation of a meal. Conversely, missing a meal (i.e., fasting) appears to have its own coordinated effects on HRV (Pivik et al., 2006), supporting the recommendation that participants consume a light meal approximately 2 h before the assessment of HRV (Tak et al., 2009). Water consumption has also been shown to increase HF-HRV in particular (Routledge et al., 2002), due to the vagal buffering response to the pressor effect provoked by hypo-osmotic fluids (Scott et al., 2001). Notably, this buffering response to the pressor effect is attenuated in older individuals (Jordan et al., 2000) and not observed in those with cardiac vagal denervation (Routledge et al., 2002). In addition, both bladder and gastric distension can also have an appreciable influence on HRV; these have been associated with increases in blood pressure and sympathetic outflow (Fagius and Karhuvaara, 1989; Rossi et al., 1998). However, papers only very rarely report that participants were asked to empty their bladder before experimental participation (Heathers, 2014).

POTENTIAL METHODOLOGICAL CONTROLS

WITHIN-SUBJECTS DESIGN OFFERS OPTIMAL EXPERIMENTAL CONTROL

In light of the complex interactions described above, a within-subjects design is the most appropriate method to explore the role of cardiorespiratory oscillations on behavior. Indeed, to appropriately detect a difference between groups, a sample size between 30 and 77, depending on the HRV metric used, is needed (Pinna et al., 2007). However, subgroups are commonly employed in these designs (e.g., gender, psychiatric comorbidities), which have been suggested to require 20 participants per cell (Simmons et al., 2011). Although some contexts make this difficult (i.e., comparison of psychiatric groups), within-subjects is the ideal design. The use of within-subjects design can eliminate any interindividual differences in coupling between HR, BP, and respiration. For instance, approximately 30% of individuals do not demonstrate any discernable synchrony between respiration and HR (Schäfer et al., 1998; Tzeng et al., 2003), with cardiorespiratory synchronization less likely to occur during higher breathing frequencies. While it is debatable if respiration should be controlled in HRV recordings, it is clear that sighs and long breaths have an effect on HRV as they generate non-sinus rhythm HR.

All of the caveats above can be minimized when individual comparisons are made between experimental points, that are as similar as possible. Most importantly, in the context of HRV, within-subjects designs better facilitate; (i) the removal of participants with atrial premature complexes and ventricular premature contractions, along with sighs, coughs, and gasps as such phenomena are easier to identify from multiple recordings if these are regular electrocardiographic errors or habitual behaviors; (ii) the elimination of individual differences in respiration rate, along with the avoidance of potential non-linear relationship of individual differences in respiration/HR relationship; (iii) the need for less participants (and consequently improved control over external variables due to repeat attendance under identical conditions); and (iv) a reduction in the impact of external factors such as medication, alcohol, nicotine, and recreational drug use.

DEFINING A "RESTING STATE" OR BASELINE

In an attempt to measure the effect of psychological task or group designation, much research assesses HRV during a resting state as a comparison to intervention. While informative, a more suitable method to interpret complex relationships between autonomic phenomena and psychological processes may be to perturb the cardiac autonomic system from complete rest. However, what constitutes a baseline needs to be carefully addressed depending on circumstances. A within-subjects experiment offers the most amount of control as a baseline is more likely to be similar.

Several caveats exist to the establishment of a baseline as an appropriate point of comparison. Firstly, the baseline HR needs to be able to support the respiratory signal without aliasing (Witte et al., 1988) – for instance, a normative breathing rate of 0.3 Hz can only be observed successfully in a HR faster than 0.6 Hz (i.e., 36 bpm). In a regular ECG, this criterion is often met. However, during supine recording, transient beats and intervals in healthy young people are frequently below 0.8 Hz (i.e., 48 bpm) – this may extend up to the entire IBI series in the case of physically fit individuals or any other participant displaying bradycardia. This corresponds with the fastest criterion for RSA in the HF-HRV band (i.e., 0.4 Hz). While this is an abnormal situation (see Sacha and Grzeszczak, 2002), it is a potential confound to the establishment of a baseline, especially if IBI series are filtered incorrectly (Grossman and Taylor, 2007). Secondly, physically fit participants may not have sinus rhythm appropriate for analysis in the first instance due to potential changes to the sinoatrial node – hearts of such individuals have often been assumed to be slower at rest due to higher vagal tone but the balance of evidence does not presently favor this explanation (Boyett et al., 2013). However, the resumption of "normal" sinus rhythm may be observed during exercise, orthostatic stress, etc. – if this is an experimental condition, then the transition from resting baseline is affected. Thirdly, tasks often compare passive eyes-open rest as a baseline to the performance of a psychomotor, attentional, or emotional task, for instance. It is possible that this conflates the difference between passive rest vs. the act of paying attention to task with the difference between passive rest vs. the specific task demands of the experiment in question. A popular alternative to complete rest is the Vanilla baseline (Jennings et al., 1992), which requires subjects to perform a trivial counting task requiring sustained attention but minimal cognitive load, as opposed to what the authors term "enforced relaxation." Other similar approaches have been attempted (e.g., Piferi et al., 2000). Finally, with individual recordings made over time, there is the complicated situation of the immediacy of baseline-to-experiment transition. HR is not stable over time, and can exhibit non-periodic phenomena or bifurcations, which may be in conflict with the assumption that an initial baseline well reflects a later experimental condition. Researchers must also consider the potential effect of decay between tasks if cardiorespiratory effects

are observed, what a normalization to baseline might look like, and of course the fact that secondary baselines may conflict with experimental instructions or manipulations. It is inherent from the above that an appropriate baseline is not a singular measurement with "correct" parameters under all circumstances, but rather the non-task situation that best controls for the presence of task comparison. In many situations, the comparison of a task to a "resting" state will therefore vary in appropriateness.

MONITORING RESPIRATION

As detailed above, basic changes in respiration can have a significant impact on HRV. Pneumotachography is the gold standard for the monitoring of tidal volume, however, the use of a closed face-mask required to do so is cumbersome and impractical for most research in emotion and psychological science (e.g., face-to-face interactions). In lieu of this, the use of a strain gage to index the expansion of the chest can give sufficient information – most importantly, a strain gage can identify gross deviations of typical cyclical respiration (e.g., sighs, coughs). Mirroring the importance of HR measures to reflect true sinus rhythm (as an ectopic beat does not represent ANS input to the SA node), "true" respiratory cycles must also be used to correctly draw inference on respiratory oscillations and coupling to HR. However, signals from strain gages do not necessarily have a linear relationship of circumference to signal (i.e., distension/signal output relationships may be different at different belt tensions) and that chest circumference is itself an indirect measure of the respiratory cycle (i.e., lung and chest wall volumes are not identical). In lieu of direct respiratory measures, established algorithms (Moody et al., 1985, 1986) that have been successively improved (e.g., Park et al., 2008; Langley et al., 2010) can also provide an appropriate surrogate measure of respiration from based on ECG signal morphology.

There is at present no satisfying solution for a totally non-invasive monitoring of tidal volume but in the meantime it seems prudent to monitor respiration at least to identify gross errors from normal cardiorespiratory analysis assumptions. For instance, healthy participants occasionally breathe at frequencies slower than 0.15 Hz (up to 35% of participants; Hoit and Lohmeier, 2000; Beda et al., 2007; Pinna et al., 2007) – this has also been observed in physically fit individuals (Saboul et al., 2014). Breathing below 0.15 Hz dramatically increases the observed power of RSA over that of typical breathing frequencies due to the involvement of the baroreflex. Consequently, this will dramatically affect measures of LF-HRV, HF-HRV, total spectral power and any ratio between spectral bands (e.g., LF/HF or LFnu). However, any such participant can be easily identified from the unitless cyclical information provided by a strain gage, and subsequently discarded from analysis. Alternatively, it may be possible to remove the immediate effect of slow breathing cycles using a continuous wavelet approach (or any spectral analysis method that handles discontinuity well, such as an averaged lomb-scargle periodogram) to identify affected areas. Naturally, areas affected by slower breathing can be compared within subjects to periods of regular sinus rhythm (if available) to determine the level of distortion present in either spectral band. At present, we are not aware of any work that proposes an acceptable amount of distortion.

CONCLUSION

Enthusiasm for HRV within emotion science is subsequent to it being seen as a source of accurate, cheap, and non-invasive insight into autonomic outflow. This position should be strongly tempered by the present considerations. Instead, it would be more reasonable to say that HRV presents an admixture of insight and significant layers of complication. The behavior of the heart over time is the end-state of multiple interlocking systems, which present their own individual challenges for researchers at a cellular, local, and systemic level.

It should be mentioned here that while this paper focuses solely on issues of traditional methodological control, there are other domains in which significant improvements in the experimental environment surrounding HRV might be gained. Most crucially, signal analytic requirements often receive surprisingly little attention, and decisions about type of spectral analysis, windowing, and data cleaning are crucial (e.g., Berntson and Stowell, 1998) but are often under-reported. Likewise, recent interest in data uploading and retention (e.g., Nosek et al., 2012) has received little systematic attention in cardiac psychophysiology so far, even though a) data retention is a American Psychological Association requirement (American psychological association [APA], 2001) and b) the ability to broadly access raw data is a potentially excellent control for the methodological and analytical issues outlined here, as well as a test bed for the development of future HRV metrics and meta-analysis.

The best case scenario for the continuing use of HRV is that the significant challenges and complications provided by interrelationships most crucially between respiration and blood pressure are acknowledged, and that experimental designs are improved by appropriately accounting for common external factors known to aggressively modify HRV. Careful consideration of these factors will help ensure researchers use more accurate and reproducible measures of autonomic outflow.

ACKNOWLEDGMENTS

The authors thank Maja Elstad, Tobias Kaufmann, and Douglas Angus for helpful comments on an earlier version of this manuscript. They also thank the reviewers of this manuscript for their constructive comments.

REFERENCES

Aasman, J., Mulder, G., and Mulder, L. J. (1987). Operator effort and the measurement of heart-rate variability. *Hum. Factors* 29, 161–170. doi: 10.1177/001872088702900204

Abelson, J. L., Weg, J. G., Nesse, R. M., and Curtis, G. C. (2001). Persistent respiratory irregularity in patients with panic disorder. *Biol. Psychiatry* 49, 588–595. doi: 10.1016/S0006-3223(00)01078-7

Agelink, M. W., Boz, C., Ullrich, H., and Andrich, J. (2002). Relationship between major depression and heart rate variability. Clinical consequences and implications for antidepressive treatment. *Psychiatry Res.* 113, 139–149. doi: 10.1016/S0165-1781(02)00225-1

American psychological association [APA]. (2001). *Publication Manual of the American Psychological Association.* Washington, DC: American Psychological Association.

Angelone, A., and Coulter, N. A. (1964). Respiratory sinus arrhythmia: a frequency dependent phenomenon. *J. Appl. Physiol.* 19, 479–482.

Appelhans, B. M., and Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Rev. Gen. Psychol.* 10, 229. doi: 10.1037/1089-2680.10.3.229

Bär, K. J., Boettger, M. K., Koschke, M., Schulz, S., Chokka, P., Yeragani, V. K., et al. (2007). Non-linear complexity measures of heart rate variability in acute schizophrenia. *Clin. Neurophysiol.* 118, 2009–2015. doi: 10.1016/j.clinph.2007.06.012

Beda, A., Jandre, F. C., Phillips, D. I., Giannella-Neto, A., and Simpson, D. M. (2007). Heart-rate and blood-pressure variability during psychophysiological tasks involving speech: influence of respiration. *Psychophysiology* 44, 767–778. doi: 10.1111/j.1469-8986.2007.00542.x

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin. Proc.* 68, 988–1001. doi: 10.1016/S0025-6196(12)62272-1

Bernardi, L., Keller, F., Sanders, M., Reddy, P., Griffith, B., Meno, F., et al. (1989). Respiratory sinus arrhythmia in the denervated human heart. *J. Appl. Physiol.* 67, 1447–1455.

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 34, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., and Cacioppo, J. T. (2004). "Heart rate variability: stress and psychiatric conditions," in *Dynamic Electrocardiography*, eds M. Malik and A. J. Camm (New York, NY: Futura), 57–64.

Berntson, G. G., and Stowell, J. R. (1998). ECG artifacts and heart period variability: don't miss a beat! *Psychophysiology* 35, 127–132. doi: 10.1111/1469-8986.3510127

Berthoud, H.-R., and Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Auton. Neurosci.* 85, 1–17. doi: 10.1016/S1566-0702(00)00215-0

Bigger, J. T., Steinman, R. C., Rolnitzky, L. M., Fleiss, J. L., Albrecht, P., and Cohen, R. J. (1996). Power law behavior of RR-interval variability in healthy middle-aged persons, patients with recent acute myocardial infarction, and patients with heart transplants. *Circulation* 93, 2142–2151. doi: 10.1161/01.CIR.93.12.2142

Billman, G. E. (2011). Heart rate variability – a historical perspective. *Front. Physiol.* 2:86. doi: 10.3389/fphys.2011.00086

Billman, G. E. (2013a). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* 4:26. doi: 10.3389/fphys.2013.00026

Billman, G. E. (2013b). The effect of heart rate on the heart rate variability response to autonomic interventions. *Front. Physiol.* 4:222. doi: 10.3389/fphys.2013.00222

Boiten, F. A. (1998). The effects of emotional behaviour on components of the respiratory cycle. *Biol. Psychol.* 49, 29–51. doi: 10.1016/S0301-0511(98)00025-8

Bonnemeier, H., Wiegand, U. K., Brandes, A., Kluge, N., Katus, H. A., Richardt, G., et al. (2003). Circadian profile of cardiac autonomic nervous modulation in healthy subjects. *J. Cardiovasc. Electrophysiol.* 14, 791–799. doi: 10.1046/j.1540-8167.2003.03078.x

Boyett, M. R., D'Souza, A., Zhang, H., Morris, G. M., Dobrzynski, H., and Monfredi, O. (2013). Viewpoint: is the resting bradycardia in athletes the result of remodeling of the sinoatrial node rather than high vagal tone? *J. Appl. Physiol.* 114, 1351–1355. doi: 10.1152/japplphysiol.01126.2012

Britton, A., Shipley, M., Malik, M., Hnatkova, K., Hemingway, H., and Marmot, M. (2007). Changes in heart rate and heart rate variability over time in middle-aged men and women in the general population (from the Whitehall II Cohort Study). *Am. J. Cardiol.* 100, 524–527. doi: 10.1016/j.amjcard.2007.03.056

Brown, T. E., Beightol, L. A., Koh, J., and Eckberg, D. L. (1993). Important influence of respiration on human RR interval power spectra is largely ignored. *J. Appl. Physiol.* 75, 2310–2317.

Bruce, E. N. (1996). Temporal variations in the pattern of breathing. *J. Appl. Physiol.* 80, 1079–1087.

Butler, E. A., Wilhelm, F. H., and Gross, J. J. (2006). Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. *Psychophysiology* 43, 612–622. doi: 10.1111/j.1469-8986.2006.00467.x

Cannon, W. B. (1916). *Bodily Changes in Pain, Hunger, Fear, and Rage: An Account of Recent Researches into the Function of Emotional Excitement.* New York: Appleton.

Christensen, N. J., and Videbaek, J. (1974). Plasma catecholamines and carbohydrate metabolism in patients with acute myocardial infarction. *J. Clin. Invest.* 54, 278–286. doi: 10.1172/JCI107763

Cohen, H., Loewenthal, U., Matar, M., and Kotler, M. (2001). Association of autonomic dysfunction and clozapine: heart rate variability and risk for sudden death in patients with schizophrenia on long-term psychotropic medication. *Br. J. Psychiatry* 179, 167–171. doi: 10.1192/bjp.179.2.167

Cohen, H. D., Goodenough, D. R., Witkin, H. A., Oltman, P., Gould, H., and Shulman, E. (1975). The effects of stress on components of the respiration cycle. *Psychophysiology* 12, 377–380. doi: 10.1111/j.1469-8986.1975.tb00005.x

Conrad, A., Müller, A., Doberenz, S., Kim, S., Meuret, A. E., Wollburg, E., et al. (2007). Psychophysiological effects of breathing instructions for stress management. *Appl. Psychophysiol. Biofeedback* 32, 89–98. doi: 10.1007/s10484-007-9034-x

Cysarz, D., and Büssing, A. (2005). Cardiorespiratory synchronization during Zen meditation. *Eur. J. Appl. Physiol.* 95, 88–95. doi: 10.1007/s00421-005-1379-3

Cysarz, D., Von Bonin, D., Lackner, H., Heusser, P., Moser, M., and Bettermann, H. (2004). Oscillations of heart rate and respiration synchronize during poetry recitation. *Am. J. Physiol. Heart Circ. Physiol.* 287, H579–H587. doi: 10.1152/ajpheart.01131.2003

Denver, J. W., Reed, S. F., and Porges, S. W. (2007). Methodological issues in the quantification of respiratory sinus arrhythmia. *Biol. Psychol.* 74, 286–294. doi: 10.1016/j.biopsycho.2005.09.005

Eckberg, D. L. (1997). Sympathovagal balance a critical appraisal. *Circulation* 96, 3224–3232. doi: 10.1161/01.CIR.96.9.3224

Eckberg, D. L. (2009). Point: counterpoint: respiratory sinus arrhythmia is due to a central mechanism vs. respiratory sinus arrhythmia is due to the baroreflex mechanism. *J. Appl. Physiol.* 106, 1740–1742. doi: 10.1152/japplphysiol.91107.2008

Elstad, M. (2012). Respiratory variations in pulmonary and systemic blood flow in healthy humans. *Acta Physiol.* 205, 341–348. doi: 10.1111/j.1748-1716.2012.02419.x

Elstad, M., Toska, K., Chon, K. H., Raeder, E. A., and Cohen, R. J. (2001). Respiratory sinus arrhythmia: opposite effects on systolic and mean arterial pressure in supine humans. *J. Physiol.* 536, 251–259. doi: 10.1111/j.1469-7793.2001.t01-1-00251.x

Evans, K. C., Dougherty, D. D., Schmid, A. M., Scannell, E., Mccallister, A., Benson, H., et al. (2009). Modulation of spontaneous breathing via limbic/paralimbic–bulbar circuitry: an event-related fMRI study. *Neuroimage* 47, 961–971. doi: 10.1016/j.neuroimage.2009.05.025

Fagius, J., and Karhuvaara, S. (1989). Sympathetic activity and blood pressure increases with bladder distension in humans. *Hypertension* 14, 511–517. doi: 10.1161/01.HYP.14.5.511

Furlan, R., Guzzetti, S., Crivellaro, W., Dassi, S., Tinelli, M., Baselli, G., et al. (1990). Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. *Circulation* 81, 537–547. doi: 10.1161/01.CIR.81.2.537

Galletly, D., and Larsen, P. (2001). Cardioventilatory coupling in heart rate variability: methods for qualitative and quantitative determination. *Br. J. Anaesth.* 87, 827–833. doi: 10.1093/bja/87.6.827

Goedhart, A. D., Willemsen, G., Houtveen, J. H., Boomsma, D. I., and De Geus, E. J. (2008). Comparing low frequency heart rate variability and preejection period: two sides of a different coin. *Psychophysiology* 45, 1086–1090. doi: 10.1111/j.1469-8986.2008.00710.x

Goldberger, A. L., Amaral, L. A., Hausdorff, J. M., Ivanov, P. C., Peng, C.-K., and Stanley, H. E. (2002). Fractal dynamics in physiology: alterations with disease and aging. *Proc. Natl. Acad. Sci. U.S.A.* 99, 2466–2472. doi: 10.1073/pnas.012579499

Goldstein, D. S., Bentho, O., Park, M. Y., and Sharabi, Y. (2011). LF power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp. Physiol.* 96, 1255–1261. doi: 10.1113/expphysiol.2010.056259

Goren, Y., Davrath, L. R., Pinhas, I., Toledo, E., and Akselrod, S. (2006). Individual time-dependent spectral boundaries for improved accuracy in time-frequency analysis of heart rate variability. *Biomed. Eng. IEEE Trans.* 53, 35–42. doi: 10.1109/TBME.2005.859784

Granger, C. W. (1969). Investigating causal relations by econometric models and cross-spectral methods. *Econometrica* 37, 424–438. doi: 10.2307/1912791

Grassi, G., and Esler, M. (1999). How to assess sympathetic activity in humans. *J. Hypertens.* 17, 719–734. doi: 10.1097/00004872-199917060-00001

Grossman, P., and Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol. Psychol.* 74, 263–285. doi: 10.1016/j.biopsycho.2005.11.014

Guo, Y.-F., and Stein, P. K. (2002). Circadian rhythm in the cardiovascular system: considerations in non-invasive electrophysiology. *Card. Electrophysiol. Rev.* 6, 267–272. doi: 10.1023/A:1016337210738

Guzik, P., Piskorski, J., Krauze, T., Schneider, R., Wesseling, K. H., Wykretowicz, A., et al. (2007). Correlations between the Poincaré plot and conventional heart rate

variability parameters assessed during paced breathing. *J. Physiol. Sci.* 57, 63–71. doi: 10.2170/physiolsci.RP005506

Guzik, P., Piskorski, J., Krauze, T., Wykretowicz, A., and Wysocki, H. (2006). Heart rate asymmetry by Poincaré plots of RR intervals. *Biomed. Tech.* 51, 272–275. doi: 10.1515/BMT.2006.054

Hayano, J., Yamada, M., Sakakibara, Y., Fujinami, T., Yokoyama, K., Watanabe, Y., et al. (1990). Short-and long-term effects of cigarette smoking on heart rate variability. *Am. J. Cardiol.* 65, 84–88. doi: 10.1016/0002-9149(90)90030-5

Heathers, J. A. (2014). Everything Hertz: methodological issues in short-term frequency-domain HRV. *Front. Physiol.* 5:177. doi: 10.3389/fphys.2014.00177

Hirsch, J. A., and Bishop, B. (1981). Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *Am. J. Physiol. Heart Circ. Physiol.* 241, H620–H629.

Hoit, J. D., and Lohmeier, H. L. (2000). Influence of continuous speaking on ventilation. *J. Speech Lang. Hear Res.* 43, 1240–1251. doi: 10.1044/jslhr.4305.1240

Houtveen, J. H., Rietveld, S., and Geus, E. J. (2002). Contribution of tonic vagal modulation of heart rate, central respiratory drive, respiratory depth, and respiratory frequency to respiratory sinus arrhythmia during mental stress and physical exercise. *Psychophysiology* 39, 427–436. doi: 10.1111/1469-8986.3940427

Huikuri, H. V., Mäkikallio, T. H., Peng, C.-K., Goldberger, A. L., Hintze, U., and Møller, M. (2000). Fractal correlation properties of RR interval dynamics and mortality in patients with depressed left ventricular function after an acute myocardial infarction. *Circulation* 101, 47–53. doi: 10.1161/01.CIR.101.1.47

Ivanov, P. C., Amaral, L. A. N., Goldberger, A. L., Havlin, S., Rosenblum, M. G., Struzik, Z. R., et al. (1999). Multifractality in human heartbeat dynamics. *Nature* 399, 461–465. doi: 10.1038/20924

Jennings, J. R., Kamarck, T., Stewart, C., Eddy, M., and Johnson, P. (1992). Alternate cardiovascular baseline assessment techniques: vanilla or resting baseline. *Psychophysiology* 29, 742–750. doi: 10.1111/j.1469-8986.1992.tb02052.x

Jordan, J., Shannon, J. R., Black, B. K., Ali, Y., Farley, M., Costa, F., et al. (2000). The pressor response to water drinking in humans a sympathetic reflex? *Circulation* 101, 504–509. doi: 10.1161/01.CIR.101.5.504

Kaplan, D., Furman, M., Pincus, S., Ryan, S., Lipsitz, L., and Goldberger, A. (1991). Aging and the complexity of cardiovascular dynamics. *Biophys. J.* 59, 945–949. doi: 10.1016/S0006-3495(91)82309-8

Karemaker, J. M. (2009). Counterpoint: respiratory sinus arrhythmia is due to the baroreflex mechanism. *J. Appl. Physiol.* 106, 1742–1743. doi: 10.1152/japplphysiol.91107.2008a

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., and Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol. Psychiatry* 67, 1067–1074. doi: 10.1016/j.biopsych.2009.12.012

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: a review. *Biol. Psychol.* 84, 394–421. doi: 10.1016/j.biopsycho.2010.03.010

Krygier, J. R., Heathers, J. A., Shahrestani, S., Abbott, M., Gross, J. J., and Kemp, A. H. (2013). Mindfulness meditation, well-being, and heart rate variability: a preliminary investigation into the impact of intensive Vipassana meditation. *Int. J. Psychophysiol.* 89, 305–313. doi: 10.1016/j.ijpsycho.2013.06.017

Kupper, N., Willemsen, G., Posthuma, D., De Boer, D., Boomsma, D. I., and De Geus, E. J. (2005). A genetic analysis of ambulatory cardiorespiratory coupling. *Psychophysiology* 42, 202–212. doi: 10.1111/j.1469-8986.2005.00276.x

Langley, P., Bowers, E. J., and Murray, A. (2010). Principal component analysis as a tool for analyzing beat-to-beat changes in ECG features: application to ECG-derived respiration. *Biomed. Eng. IEEE Trans.* 57, 821–829. doi: 10.1109/TBME.2009.2018297

Larsen, P., Trent, E., and Galletly, D. (1999). Cardioventilatory coupling: effects of IPPV. *Br. J. Anaesth.* 82, 546–550. doi: 10.1093/bja/82.4.546

Levy, M. N. (1971). Sympathetic-Parasympathetic Interactions in the Heart. *Circ. Res.* 29, 437–445. doi: 10.1161/01.RES.29.5.437

Lu, C.-L., Zou, X., Orr, W. C., and Chen, J. (1999). Postprandial changes of sympathovagal balance measured by heart rate variability. *Dig. Dis. Sci.* 44, 857–861. doi: 10.1023/A:1026698800742

Ludwig, C. (1847). Beiträge zur kenntniss des einflusses der respirationsbewegung auf den blutlauf im aortensystem. *Arch. Anat. Physiol.* 13, 242–302.

Mäkikallio, T. H., Seppänen, T., Airaksinen, K., Koistinen, J., Tulppo, M. P., Peng, C.-K., et al. (1997). Dynamic analysis of heart rate may predict subsequent ventricular tachycardia after myocardial infarction. *Am. J. Cardiol.* 80, 779–783. doi: 10.1016/S0002-9149(97)00516-X

Malpas, S. C. (2002). Neural influences on cardiovascular variability: possibilities and pitfalls. *Am. J. Physiol. Heart Circ. Physiol.* 282, H6–H20.

Massin, M. M., Maeyns, K., Withofs, N., Ravet, F., and Gérard, P. (2000). Circadian rhythm of heart rate and heart rate variability. *Arch. Dis. Child.* 83, 179–182. doi: 10.1136/adc.83.2.179

Middleton, H., Sharma, A., Agouzoul, D., Sahakian, B., and Robbins, T. (1999). Contrasts between the cardiovascular concomitants of tests of planning and attention. *Psychophysiology* 36, 610–618. doi: 10.1111/1469-8986.3650610

Moak, J. P., Goldstein, D. S., Eldadah, B. A., Saleem, A., Holmes, C., Pechnik, S., et al. (2007). Supine low-frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Heart Rhythm* 4, 1523–1529. doi: 10.1016/j.hrthm.2007.07.019

Moody, G. B., Mark, R. G., Bump, M. A., Weinstein, J. S., Berman, A. D., Mietus, J. E., et al. (1986). Clinical validation of the ECG-derived respiration (EDR) technique. *Comput. Cardiol.* 13, 507–510.

Moody, G. B., Mark, R. G., Zoccola, A., and Mantero, S. (1985). Derivation of respiratory signals from multi-lead ECGs. *Comput. Cardiol.* 12, 113–116.

Mulder, G., and Mulder, L. J. M. (1981). "Task-related cardiovascular stress," in *Attention and Performance IX*, eds J. Long and A. D. Baddeley (Hillsdale: Lawrence Erlbaum Associates), 591–606.

Nardi, A. E., Freire, R. C., and Zin, W. A. (2009). Panic disorder and control of breathing. *Respir. Physiol. Neurobiol.* 167, 133–143. doi: 10.1016/j.resp.2008.07.011

Nederkoorn, C., Smulders, F., and Jansen, A. (2000). Cephalic phase responses, craving and food intake in normal subjects. *Appetite* 35, 45–55. doi: 10.1006/appe.2000.0328

Niewinski, P., Tubek, S., Banasiak, W., Paton, J. F., and Ponikowski, P. (2014). Consequences of peripheral chemoreflex inhibition with low-dose dopamine in humans. *J. Physiol.* 592(Pt 6), 1295–1308. doi: 10.1113/jphysiol.2012.266858

Nosek, B. A., Spies, J. R., and Motyl, M. (2012). Scientific utopia II. Restructuring incentives and practices to promote truth over publishability. *Perspect. Psychol. Sci.* 7, 615–631. doi: 10.1177/1745691612459058

O'Brien, I., O'Hare, P., and Corrall, R. (1986). Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. *Br. Heart J.* 55, 348–354. doi: 10.1136/hrt.55.4.348

Park, S. B., Noh, Y. S., Park, S. J., and Yoon, H. R. (2008). An improved algorithm for respiration signal extraction from electrocardiogram measured by conductive textile electrodes using instantaneous frequency estimation. *Med. Biol. Eng. Comput.* 46, 147–158. doi: 10.1007/s11517-007-0302-y

Pashler, H. (1994). Dual-task interference in simple tasks: data and theory. *Psychol. Bull.* 116, 220–244. doi: 10.1037/0033-2909.116.2.220

Peng, C.-K., Havlin, S., Hausdorff, J., Mietus, J., Stanley, H., and Goldberger, A. (1995). Fractal mechanisms and heart rate dynamics: long-range correlations and their breakdown with disease. *J. Electrocardiol.* 28, 59–65. doi: 10.1016/S0022-0736(95)80017-4

Picard, G., Tan, C. O., Zafonte, R., and Taylor, J. A. (2009). Incongruous changes in heart period and heart rate variability with vagotonic atropine: implications for rehabilitation medicine. *PM R* 1, 820–826. doi: 10.1016/j.pmrj.2009.07.017

Piferi, R. L., Kline, K. A., Younger, J., and Lawler, K. A. (2000). An alternative approach for achieving cardiovascular baseline: viewing an aquatic video. *Int. J. Psychophysiol.* 37, 207–217. doi: 10.1016/S0167-8760(00)00102-1

Pinna, G., Maestri, R., Torunski, A., Danilowicz-Szymanowicz, L., Szwoch, M., La Rovere, M., et al. (2007). Heart rate variability measures: a fresh look at reliability. *Clin. Sci.* 113, 131–140. doi: 10.1042/CS20070055

Piskorski, J., and Guzik, P. (2007). Geometry of the Poincaré plot of RR intervals and its asymmetry in healthy adults. *Physiol. Meas.* 28, 287–300. doi: 10.1088/0967-3334/28/3/005

Pivik, R., Dykman, R., Tennal, K., and Gu, Y. (2006). Skipping breakfast: gender effects on resting heart rate measures in preadolescents. *Physiol. Behav.* 89, 270–280. doi: 10.1016/j.physbeh.2006.06.001

Porges, S. W. (1995). Orienting in a defensive world: mammalian modifications of our evolutionary heritage. A polyvagal theory. *Psychophysiology* 32, 301–318. doi: 10.1111/j.1469-8986.1995.tb01213.x

Porta, A., Casali, K. R., Casali, A. G., Gnecchi-Ruscone, T., Tobaldini, E., Montano, N., et al. (2008). Temporal asymmetries of short-term heart period variability are linked to autonomic regulation. *Am. J. Physiol. Regul. Int. Comp. Physiol.* 295, R550–R557. doi: 10.1152/ajpregu.00129.2008

Porta, A., Castiglioni, P., Di Rienzo, M., Bassani, T., Bari, V., Faes, L., et al. (2013). Cardiovascular control and time domain Granger causality: insights from

selective autonomic blockade. *Philos. Trans. Royal Soc. A Math. Phys. Eng. Sci.* 371, 20120161. doi: 10.1098/rsta.2012.0161

Pradhapan, P., Tarvainen, M. P., Nieminen, T., Lehtinen, R., Nikus, K., Lehtimäki, T., et al. (2014). Effect of heart rate correction on pre- and post-exercise heart rate variability to predict risk of mortality—an experimental study on the FINCAVAS cohort. *Front. Physiol.* 5:208. doi: 10.3389/fphys.2014.00208

Quintana, D. S., Guastella, A. J., Mcgregor, I. S., Hickie, I. B., and Kemp, A. H. (2013a). Moderate alcohol intake is related to increased heart rate variability in young adults: implications for health and well-being. *Psychophysiology* 50, 1202–1208. doi: 10.1111/psyp.12134

Quintana, D. S., Mcgregor, I. S., Guastella, A. J., Malhi, G. S., and Kemp, A. H. (2013b). A Meta-analysis on the impact of alcohol dependence on short-term resting-state heart rate variability: implications for cardiovascular risk. *Alcohol. Clin. Exp. Res.* 37, E23–E29. doi: 10.1111/j.1530-0277.2012.01913.x

Revington, M., and McCloskey, D. (1990). Sympathetic-parasympathetic interactions at the heart, possibly involving neuropeptide Y, in anaesthetized dogs. *J. Physiol.* 428, 359–370.

Rosenblum, M. G., Cimponeriu, L., Bezerianos, A., Patzak, A., and Mrowka, R. (2002). Identification of coupling direction: application to cardiorespiratory interaction. *Phys. Rev. E* 65, 041909. doi: 10.1103/PhysRevE.65.041909

Rossi, P., Andriesse, G., Oey, P., Wieneke, G., Roelofs, J., and Akkermans, L. (1998). Stomach distension increases efferent muscle sympathetic nerve activity and blood pressure in healthy humans. *J. Neurol. Sci.* 161, 148–155. doi: 10.1016/S0022-510X(98)00276-7

Routledge, H. C., Chowdhary, S., Coote, J. H., and Townend, J. N. (2002). Cardiac vagal response to water ingestion in normal human subjects. *Clin. Sci.* 103, 157–162. doi: 10.1042/CS20010317

Saboul, D., Pialoux, V., and Hautier, C. (2014). The breathing effect of the LF/HF ratio in the heart rate variability measurements of athletes. *Eur. J. Sport Sci.* 14, S282–S288. doi: 10.1080/17461391.2012.691116

Sacha, J. (2013). Why should one normalize heart rate variability with respect to average heart rate. *Front. Physiol.* 4:306. doi: 10.3389/fphys.2013.00306

Sacha, J., and Grzeszczak, W. (2002). Remnants of respiratory peaks in heart rate variability spectrum of an adult man with tachypnea and bradycardia. *Folia Cardiol.* 9, 67–74.

Sacha, J., and Pluta, W. (2008). Alterations of an average heart rate change heart rate variability due to mathematical reasons. *Int. J. Cardiol.* 128, 444–447. doi: 10.1016/j.ijcard.2007.06.047

Sacha, J., Sobon, J., Sacha, K., and Barabach, S. (2013). Heart rate impact on the reproducibility of heart rate variability analysis. *Int. J. Cardiol.* 168, 4257–4259. doi: 10.1016/j.ijcard.2013.04.160

Schäfer, C., Rosenblum, M. G., Kurths, J., and Abel, H.-H. (1998). Heartbeat synchronized with ventilation. *Nature* 392, 239–240. doi: 10.1038/32567

Schroeder, E. B., Liao, D., Chambless, L. E., Prineas, R. J., Evans, G. W., and Heiss, G. (2003). Hypertension, blood pressure, and heart rate variability the Atherosclerosis Risk In Communities (ARIC) study. *Hypertension* 42, 1106–1111. doi: 10.1161/01.HYP.0000100444.71069.73

Scott, E. M., Greenwood, J. P., Gilbey, S. G., Stoker, J. B., and Mary, D. (2001). Water ingestion increases sympathetic vasoconstrictor discharge in normal human subjects. *Clin. Sci.* 100, 335–342. doi: 10.1042/CS20000177

Simmons, J. P., Nelson, L. D., and Simonsohn, U. (2011). False-positive psychology undisclosed flexibility in data collection and analysis allows presenting anything as significant. *Psychol. Sci.* 22, 1359–1366. doi: 10.1177/0956797611417632

Sjoberg, N., and Saint, D. A. (2011). A single 4 mg dose of nicotine decreases heart rate variability in healthy nonsmokers: implications for smoking cessation programs. *Nicotine Tob. Res.* 13, 369–372. doi: 10.1093/ntr/ntr004

Slovut, D. P., Wenstrom, J. C., Moeckel, R. B., Wilson, R. F., Osborn, J. W., and Abrams, J. H. (1998). Respiratory sinus dysrhythmia persists in transplanted human hearts following autonomic blockade. *Clin. Exp. Pharmacol. Physiol.* 25, 322–330. doi: 10.1111/j.1440-1681.1998.tb02358.x

Soares-Miranda, L., Sattelmair, J., Chaves, P., Duncan, G., Siscovick, D. S., Stein, P. K., et al. (2014). Physical activity and heart rate variability in older adults: the cardiovascular health study. *Circulation* 129, 2100–2110. doi: 10.1161/CIRCULATIONAHA.113.005361

Sondermeijer, H. P., Van Marle, A. G., Kamen, P., and Krum, H. (2002). Acute effects of caffeine on heart rate variability. *Am. J. Cardiol.* 90, 906–907. doi: 10.1016/S0002-9149(02)02725-X

Stein, P. K., Domitrovich, P. P., Hui, N., Rautaharju, P., and Gottdiener, J. (2005). Sometimes higher heart rate variability is not better heart rate variability: results of graphical and nonlinear analyses. *J. Cardiovasc. Electrophysiol.* 16, 954–959. doi: 10.1111/j.1540-8167.2005.40788.x

Stein, P. K., Le, Q., and Domitrovich, P. P. (2008). Development of more erratic heart rate patterns is associated with mortality post–myocardial infarction. *J. Electrocardiol.* 41, 110–115. doi: 10.1016/j.jelectrocard.2007.11.005

Stratonovich, R. L. (1967). *Topics in the Theory of Random Noise.* New York: Gordon and Breach.

Strauss-Blasche, G., Moser, M., Voica, M., Mcleod, D., Klammer, N., and Marktl, W. (2000). Relative timing of inspiration and expiration affects respiratory sinus arrhythmia. *Clin. Exp. Pharmacol. Physiol.* 27, 601–606. doi: 10.1046/j.1440-1681.2000.03306.x

Tak, L. M., Riese, H., De Bock, G. H., Manoharan, A., Kok, I. C., and Rosmalen, J. G. (2009). As good as it gets? A meta-analysis and systematic review of methodological quality of heart rate variability studies in functional somatic disorders. *Biol. Psychol.* 82, 101–110. doi: 10.1016/j.biopsycho.2009.05.002

Tan, C. O., Cohen, M. A., Eckberg, D. L., and Taylor, J. A. (2009). Fractal properties of human heart period variability: physiological and methodological implications. *J. Physiol.* 587, 3929–3941. doi: 10.1113/jphysiol.2009.169219

Tarvainen, M. P., Ranta-aho, P. O., and Karjalainen, P. A. (2002). An advanced detrending method with application to HRV analysis. *IEEE Trans. Biomed. Eng.* 49, 172–175. doi: 10.1109/10.979357

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Toska, K., and Eriksen, M. (1993). Respiration-synchronous fluctuations in stroke volume, heart rate and arterial pressure in humans. *J. Physiol.* 472, 501–512.

Triedman, J. K., and Saul, J. P. (1994). Blood pressure modulation by central venous pressure and respiration. Buffering effects of the heart rate reflexes. *Circulation* 89, 169–179. doi: 10.1161/01.CIR.89.1.169

Tulppo, M. P., Mäkikallio, T. H., Seppänen, T., Airaksinen, J. K. E., and Huikuri, H. V. (1998). Heart rate dynamics during accentuated sympathovagal interaction. *Am. J. Physiol. Heart Circ. Physiol.* 274, H810–H816.

Tzeng, Y. C., Larsen, P. D., and Galletly, D. C. (2003). Cardioventilatory coupling in resting human subjects. *Exp. Physiol.* 88, 775–782. doi: 10.1113/eph8802606

Valkonen-Korhonen, M., Tarvainen, M. P., Ranta-Aho, P., Karjalainen, P. A., Partanen, J., Karhu, J., et al. (2003). Heart rate variability in acute psychosis. *Psychophysiology* 40, 716–726. doi: 10.1111/1469-8986.00072

van Eekelen, A. P., Houtveen, J. H., and Kerkhof, G. A. (2004). Circadian variation in cardiac autonomic activity: reactivity measurements to different types of stressors. *Chronobiol. Int.* 21, 107–129. doi: 10.1081/CBI-120027983

Vaschillo, E., Vaschillo, B., and Lehrer, P. (2004). Heartbeat synchronizes with respiratory rhythm only under specific circumstances. *Chest J.* 126, 1385–1387. doi: 10.1378/chest.126.4.1385-a

Vlemincx, E., Abelson, J. L., Lehrer, P. M., Davenport, P. W., Van Diest, I., and Van Den Bergh, O. (2013). Respiratory variability and sighing: a psychophysiological reset model. *Biol. Psychol.* 93, 24–32. doi: 10.1016/j.biopsycho.2012.12.001

Vlemincx, E., Diest, I., and Bergh, O. (2012a). Imposing respiratory variability patterns. *Appl. Psychophysiol. Biofeedback* 37, 153–160. doi: 10.1007/s10484-012-9187-0

Vlemincx, E., Van Diest, I., and Van Den Bergh, O. (2012b). A sigh following sustained attention and mental stress: effects on respiratory variability. *Physiol. Behav.* 107, 1–6. doi: 10.1016/j.physbeh.2012.05.013

Vlemincx, E., Taelman, J., De Peuter, S., Van Diest, I., and Van Den Bergh, O. (2011). Sigh rate and respiratory variability during mental load and sustained attention. *Psychophysiology* 48, 117–120. doi: 10.1111/j.1469-8986.2010.01043.x

Voss, A., Schulz, S., Schroeder, R., Baumert, M., and Caminal, P. (2009). Methods derived from nonlinear dynamics for analysing heart rate variability. *Philos. Trans. Royal Soc. A Math. Phys. Eng. Sci.* 367, 277–296. doi: 10.1098/rsta.2008.0232

Winfree, A. T. (2001). *The Geometry of Biological Time.* New York: Springer. doi: 10.1007/978-1-4757-3484-3

Witte, H., Zwiener, U., Rother, M., and Glaser, S. (1988). Evidence of a previously undescribed form of respiratory sinus arrhythmia (RSA)—the physiological manifestation of "cardiac aliasing". *Pflügers Arch.* 412, 442–444. doi: 10.1007/BF01907565

Woo, M. A., Stevenson, W. G., Moser, D. K., Trelease, R. B., and Harper, R. M. (1992). Patterns of beat-to-beat heart rate variability in advanced

heart failure. *Am. Heart J.* 123, 704–710. doi: 10.1016/0002-8703(92)90510-3

Zhang, J., Yu, X., and Xie, D. (2010). Effects of mental tasks on the cardiorespiratory synchronization. *Respir. Physiol. Neurobiol.* 170, 91–95. doi: 10.1016/j.resp.2009.11.003

Zhu, Y., Hsieh, Y.-H., Dhingra, R. R., Dick, T. E., Jacono, F. J., and Galán, R. F. (2013). Quantifying interactions between real oscillators with information theory and phase models: application to cardiorespiratory coupling. *Phys. Rev. E* 87, 022709. doi: 10.1103/PhysRevE.87.022709

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 06 June 2014; accepted: 07 July 2014; published online: 22 July 2014.

Citation: Quintana DS and Heathers JAJ (2014) Considerations in the assessment of heart rate variability in biobehavioral research. Front. Psychol. ***5****:805. doi: 10.3389/fpsyg.2014.00805*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in Psychology

ORIGINAL RESEARCH
published: 25 March 2015
doi: 10.3389/fpsyg.2015.00300

Neuroanatomical substrates for the volitional regulation of heart rate

*Catherine L. Jones[1,2], Ludovico Minati[1,3], Yoko Nagai[1,2], Nick Medford[1,2,4,5], Neil A. Harrison[1,2,4,5], Marcus Gray[6], Jamie Ward[2,4,7] and Hugo D. Critchley[1,2,4,5]**

[1] Clinical Imaging Sciences Centre, Brighton and Sussex Medical School, University of Sussex, Brighton, UK, [2] Department of Psychiatry and Sackler Centre for Consciousness Science, Clinical Imaging Sciences Centre, University of Sussex, Brighton, UK, [3] IRCCS Istituto Neurologico Carlo Besta, Milano, Italy, [4] Sackler Centre for Consciousness Science, University of Sussex, Brighton, UK, [5] Sussex Partnership NHS Foundation Trust, Worthing, UK, [6] Gehrmann Laboratory, University of Queensland, Brisbane, QLD, Australia, [7] School of Psychology, University of Sussex, Brighton, UK

The control of physiological arousal can assist in the regulation of emotional state. A subset cortical and subcortical brain regions are implicated in autonomic control of bodily arousal during emotional behaviors. Here, we combined human functional neuroimaging with autonomic monitoring to identify neural mechanisms that support the volitional regulation of heart rate, a process that may be assisted by visual feedback. During functional magnetic resonance imaging (fMRI), 15 healthy adults performed an experimental task in which they were prompted voluntarily to increase or decrease cardiovascular arousal (heart rate) during true, false, or absent visual feedback. Participants achieved appropriate changes in heart rate, without significant modulation of respiratory rate, and were overall not influenced by the presence of visual feedback. Increased activity in right amygdala, striatum and brainstem occurred when participants attempted to increase heart rate. In contrast, activation of ventrolateral prefrontal and parietal cortices occurred when attempting to decrease heart rate. Biofeedback enhanced activity within occipito-temporal cortices, but there was no significant interaction with task conditions. Activity in regions including pregenual anterior cingulate and ventral striatum reflected the magnitude of successful task performance, which was negatively related to subclinical anxiety symptoms. Measured changes in respiration correlated with posterior insula activation and heart rate, at a more lenient threshold, change correlated with insula, caudate, and midbrain activity. Our findings highlight a set of brain regions, notably ventrolateral prefrontal cortex, supporting volitional control of cardiovascular arousal. These data are relevant to understanding neural substrates supporting interaction between intentional and interoceptive states related to anxiety, with implications for biofeedback interventions, e.g., real-time fMRI, that target emotional regulation.

Keywords: autonomic, biofeedback, brain imaging, emotion, heart rate, interoception

OPEN ACCESS

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Martin Walter, Otto-von-Guericke-Universität Magdeburg, Germany
Andrea Caria, Eberhard Karls Universität Tübingen, Germany
Agustin Ibanez, Institute of Cognitive Neurology, Argentina

****Correspondence:***
Hugo D. Critchley, Clinical Imaging Sciences Centre, Brighton and Sussex Medical School, University of Sussex, Brighton BN1 9RR, UK
h.critchley@bsms.ac.uk

Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *11 November 2014*
Accepted: *02 March 2015*
Published: *25 March 2015*

Citation:
Jones CL, Minati L, Nagai Y, Medford N, Harrison NA, Gray M, Ward J and Critchley HD (2015) Neuroanatomical substrates for the volitional regulation of heart rate. Front. Psychol. 6:300. doi: 10.3389/fpsyg.2015.00300

Introduction

States of physiological bodily arousal, including increased heart rate, are integral to the expression of negative emotions, including anxiety, and feed back to intensify affective feelings. Interventions

that specifically target physiological arousal can diminish anxiety symptoms and emotional reactivity (Bonn et al., 1972). Physiological relaxation techniques, with or without biofeedback, contribute to strategies for anxiety management, often alongside cognitive behavioral therapy (Borkovec and Costello, 1993). Further, the intentional regulation of emotional states engages brain regions implicated in the control of peripheral as well as central arousal (Buhle et al., 2014). However, physiological arousal itself also accompanies non-emotional behavioral states, notably physical activity, which do not typically evoke negative feelings. One explanation for this discrepancy lies in the predictability and sense of control of internal physiological state mediated by the autonomic nervous system (Paulus and Stein, 2006; Seth, 2013).

Autonomic responses are integrated with emotional and motivational behaviors. Correspondingly, brain regions controlling behavior also directly or indirectly influence internal bodily arousal states via the autonomic nervous system. These bodily changes are themselves linked to activation within discrete brain regions. For example, stimulation of the human insula can evoke visceromotor changes (Penfield and Faulk, 1955; Oppenheimer et al., 1992) and insula damage may result in autonomic dysregulation (Oppenheimer et al., 1992; Meyer et al., 2004; Jones et al., 2010). Neuroimaging evidence also implicates regions including insula, anterior cingulate, and amygdala in interoception (sensing and representing the physiological state of the body) and accompanying feelings states (Damasio, 1994; Craig, 2002, 2009; Critchley et al., 2004; Harrison et al., 2010).

These same set of brain regions contribute to brain networks that are also implicated in executive, cognitive, and social functioning (Seeley et al., 2007; Sridharan et al., 2008; Limongi et al., 2014). Connectivity within such networks appear dynamically related to changes in peripheral cardiovascular state: thus, during the resting state, increases in heart rate variability fluctuate with increases in connectivity from dorsal anterior cingulate and amygdala to other cortical (cingulate insula and dorsolateral prefrontal cortex) and subcortical (basal ganglia and midbrain) centers (Chang et al., 2013). Cardiorespiratory effects similarly, contribute to connectivity strength within the 'default mode' network (encompassing medial prefrontal/rostral cingulate and medial parietal lobe). Removal of variance from physiological bodily responses diminishes experimental sensitivity to task-related changes in brain activity (van Buuren et al., 2009). Nevertheless, these passive relationships raise important questions regarding the functional impact of such heart-brain interactions.

The neural mechanisms supporting this link between peripheral arousal and emotional feelings have attracted therapeutic attention. Biofeedback of brain activity (neurofeedback) has been explored in this context: here, the immediate explicit (visual) presentation of changes in neural activation or connectivity can be used as a 'training signal' that enables a participant to learn to wilfully modulate neural responses to affect associated psychophysiological processes. For example, interventions that target insular cortex (or connected brain regions) have been explored in the management of affective symptoms and chronic pain disorders. Anterior insula in particular, has been the target of neurofeedback studies using real-time functional magnetic resonance imaging (fMRI; e.g., Caria et al., 2007; Frank et al., 2012). Autonomic biofeedback tasks (using peripheral response) can also be used to extend knowledge about neural substrates supporting the functional integration of cognition and internal bodily states of arousal: the proposed role of anterior insula as the substrate for (emotional) feeling states arising from internal visceral states (Craig, 2002, 2009; Critchley and Harrison, 2013), predicts that this region is likely to be involved in the volitional/intentional regulation of physiological state. Similar arguments apply also to closely connected regions such as anterior cingulate cortex, which is implicated in both emotional autonomic arousal and emotion awareness (Lane et al., 1998). In fact, anterior cingulate cortex is observed to be activated during performance of electrodermal biofeedback tasks (Critchley et al., 2001, 2002a; Nagai et al., 2004a).

In the present paper, we focused on the control of heart rate. At rest, heart rate modulation is achieved through changing the balance between both sympathetic and parasympathetic drive, hence it is closely related to baroreflex mechanisms that underlie heart rate variability. We chose to focus on identifying regional brain centers contributing to the active/intentional regulation of heart rate (arguably, a more intuitively accessible physiological response than heart rate variability). We tested the notion that both sensing internal bodily states and regulating these states activate cortical regions such as insula, where ascending interoceptive representation appear to be integrated with conscious perception (Critchley et al., 2002b; Gianaros et al., 2012; Gray et al., 2012). We investigated the ability to wilfully modify heart rate, in the presence of biofeedback (visual feedback of their actual heart rate), no feedback, or false feedback. A key prediction was that the insula, alongside anterior cingulate cortex, and dorsal brainstem, would be engaged during biofeedback regulation of heart rate. Thus, we predicted that the presence and veracity of the feedback would modulate both behavior (successful performance of the task) and associated neural activity within brain regions supporting regulation and representation of autonomic bodily responses. Ultimately, we were motivated by a perceived relevance to emotional regulation and anxiety (e.g., Clark, 1986; Beck and Clark, 1997; Paulus and Stein, 2006; Dunn et al., 2010; Domschke et al., 2011). Hence participants also completed an anxiety inventory to test the prediction that effective autonomic regulation (i.e., successfully increasing and decreasing heart rate) would be related to *reduced* levels of anxiety. To our knowledge, this is the first study to investigate the effect of feedback on the modulation of heart rate while using fMRI to map the neural representations. A further novel aspect is the exploration of the relationship between anxiety symptoms and the capacity for volitional autonomic regulation.

Materials and Methods

Participants

Fifteen right-handed, healthy participants (Five male), mean age 25 $\pm$ 10 years, were enrolled. All participants were screened

to exclude neurological and psychiatric disorders. The study was approved by the Brighton and Sussex Medical School Research Governance and Ethics Committee. Each participant was fully informed and gave written consent to take part in this neuroimaging study entitled 'Biofeedback control of heart rate.'

Experimental Design

Participants knowingly engaged with an experimental task involving intentional modulation of heart rate. The experiment involved six task conditions within a 2 × 3 factorial design. One factor was the objective, i.e., direction of intended heart rate change, where participants were required to try to increase or decrease their heart rate as an index of cardiovascular arousal ['arousal'/increase and 'relaxation'/decrease. The use of terms arousal and relaxation to refer to these physiological/autonomic changes, associated with increased sympathetic and decreased parasympathetic effects, is well established within the literature (e.g., from our own laboratory Critchley et al., 2000a,b, 2001, 2002a)]. The second factor, presence and veracity of visual feedback had three levels (true feedback – which accurately reflected heart rate; false feedback – randomly fluctuating information and absent feedback – no feedback given). Each condition was performed twice by each participant and presented in pseudo-random sequences that avoided immediate repetition of the same task condition.

Heart rate and breathing rate were monitored continuously throughout the tasks. The biofeedback signal of physiological relaxation and arousal was represented by a visible thermometer with a blue bar that reflected heart rate (**Figure 1**). The starting point (near top or bottom) and approximate sensitivity was tailored for each participant by task condition. In the relaxation conditions, participants were instructed by a cue to try to make the bar go down by physiologically relaxing, conversely in the arousal task participants were instructed to make the bar rise by becoming more physiologically aroused. Thus the nebulous terms relaxation and arousal were operationalized, as in previous studies, to refer to heart rate decreases and increases, respectively. In the false feedback condition, the bar fluctuated following a smooth random walk. In the no-feedback condition there was no bar, with only the outline of a thermometer displayed. All participants knew that the purpose of the study was to control their own heart rate. Participants were informed that the thermometer reflected their heart rate, where a rise in heart rate was displayed as a rise in the level depicted on the thermometer which signaled an increase in physiological arousal. Similarly, participants were told that a drop in heart rate was displayed as a lowering of the level depicted on the thermometer which in turn signaled a reduction in physiological arousal, which we termed 'relaxation.' They were advised (when possible) to use the feedback and were also told that, during scanning, the feedback would be altered or removed for some trials, which might make it harder for them to achieve the required increase or decrease in arousal/heart rate. Practice trials were performed before scanning, in which participants were given only true heart rate feedback and were instructed to make to bar go down by relaxing and to make the bar rise by increasing the level of arousal, operationalized to mean heart rate, and reinforced by this practice session. These instructions were carried over to their performance within the scanner. Here again, the instruction to volitionally increase heart rate was displayed by the visual cue 'AROUSAL' and the instruction to volitionally decrease heart rate was displayed by the visual cue 'RELAXATION' at the start of each 90 s block (replacing the text shown in the upper part of **Figure 1**). The false feedback conditions were not explicitly distinguished from the true feedback conditions.

Participants were naïve to the biofeedback exercise before the day of the experiment (i.e., in contrast to previous biofeedback tasks they were not over-trained). In the instructions, Participants were explicitly instructed not to close their eyes (a natural behavior when trying to relax) and told to try and maintain a constant breathing rate. Task conditions alternated between relaxation and arousal conditions with feedback blocks presented in a pseudo-random manner. Each block lasted for 90 s (**Figure 1**).

Physiological Monitoring

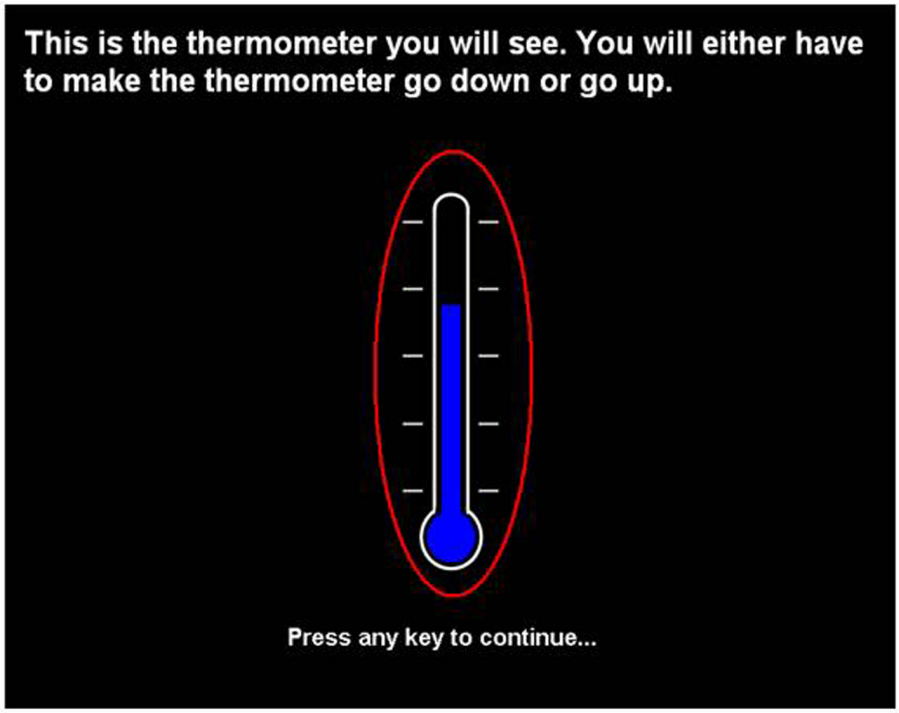
Each participant was monitored during fMRI using pulse oximetry (Nonin 8600FO, Nonin Inc., Plymouth, MN, USA) with the sensor taped to the middle finger of the left hand. The fibreoptic cable was passed through the guide tube from the Faraday cage of the MRI scanner room to the control room where the analog outputs of the apparatus were fed via an A/D converter (CED1401) to a computer running Spike 2 Software (Cambridge Electronic Design, Cambridge, UK) and to a stimulus-control computer running Matlab (MathWorks, Nantick, MA, USA). The biofeedback components of the tasks (**Figure 1**) were run on this computer using Matlab scripts developed in-house: the signal was low-pass filtered at 1 Hz and processed with a peak-picking algorithm yielding beat-by-beat heart rate measurements. The resulting signal was epoched in the [−0.5,5] s peristimulus range and averaged across trials. Respiratory motor function was recorded within the MRI environment via respiratory bands, a technique referred to as remote pressure sensor respiratory plethysmography (Caldiroli and Minati, 2007). Again the signal was low-pass filtered at 1 Hz and processed with a peak-picking algorithm yielding breathing rate measurements (**Figure 2**).

Behavioral Analysis

A 3 × 2 repeated measures ANOVA [feedback (True, None, False) × objective (Relaxation, Arousal)] was performed with heart rate as the dependent variable. A second ANOVA was conducted using breathing rate as a dependent variable. Each participant was debriefed to establish what particular strategies may have been used when performing the task. After debriefing participants also completed the Beck Anxiety Inventory (BAI, Beck et al., 1988). While none of the participants were above the clinical threshold for anxiety, scores were compared against performance on the biofeedback tasks.

Neuroimaging Data Acquisition and Analysis

Each participant underwent neuroimaging on a Siemens Avanto 1.5 Tesla magnetic resonance imaging scanner. The participant was placed in the scanner with their head gently, yet firmly restrained within the head coil using vacuum cushions. During



Objective	True Feedback (heart rate biofeedback)	No Feedback	False Feedback (random)
Relaxation	2 scan blocks	2 scan blocks	2 scan blocks
Arousal	2 scan blocks	2 scan blocks	2 scan blocks

FIGURE 1 | Visual display of feedback 'thermometer.' There were six task conditions within the experiment which embodied a 2 × 3 factorial design. The factors were (1) the direction of intended heart rate change (increase = 'arousal'/and decrease = 'relaxation') and (2) the presence and veracity of visual feedback (true feedback accurately reflecting heart rate; false feedback, i.e., random fluctuation and absent feedback). Each condition was performed twice by the participant and presented in pseudorandom sequences that avoided immediate repetition of the same task condition. Participants were instructed to use arousal to make the thermometer level rise in the increased heart rate conditions and relaxation in the lower in the heart rate conditions. Below are tabulated the biofeedback task conditions undertaken during the course of the experiment.

performance of the biofeedback tasks, T2*-weighted echo planar data were acquired with near complete brain coverage (bicommissural orientation for 21 slices, 5 mm thickness, no gap, TR = 2000 ms, TE = 50 ms, in-slice resolution 2 × 2 mm, matrix 80 × 128). A T1-weighted whole brain, high resolution structural scan was obtained at the end of the scanning study (magnetization-prepared rapid gradient-echo sequence, 0.9 mm isotropic voxels; TR = 1160 ms, TE = 4.44 ms, FoV 230 mm × 230 mm, matrix size 256 × 256, 50 slices) and used to co-register the functional dataset and screen for potential anatomical abnormalities.

Neuroimaging time-series datasets were analyzed as a block design using statistical parametric mapping (SPM8; Wellcome Trust Centre for Neuroimaging, UCL, UK) implemented in Matlab. Functional scans were realigned to correct for participant movements, slice-timing corrected, and co-registered with individual anatomy. Subsequently, all scans were transformed into MNI space. The scans were smoothed using an 8 mm full width at half maximum Gaussian filter. Two separate analyses were carried out: the first analysis tested for relationships between regional (blood oxygenation-level dependent, BOLD) activity and the different task conditions. The second analysis tested for regional activity sensitive to physiological fluctuation (heart rate and breathing rate) over the whole experiment.

In the first analysis, a design matrix modeled the six condition regressors for each participant, i.e., true_relaxation, true_arousal, none_relaxation, none_arousal, false_relaxation, false_arousal. There was no significant effect of the task conditions on head movement. Nevertheless, to improve sensitivity to neuraly mediated signal changes during the experiment, six movement regressors from the initial functional realignment were included in the

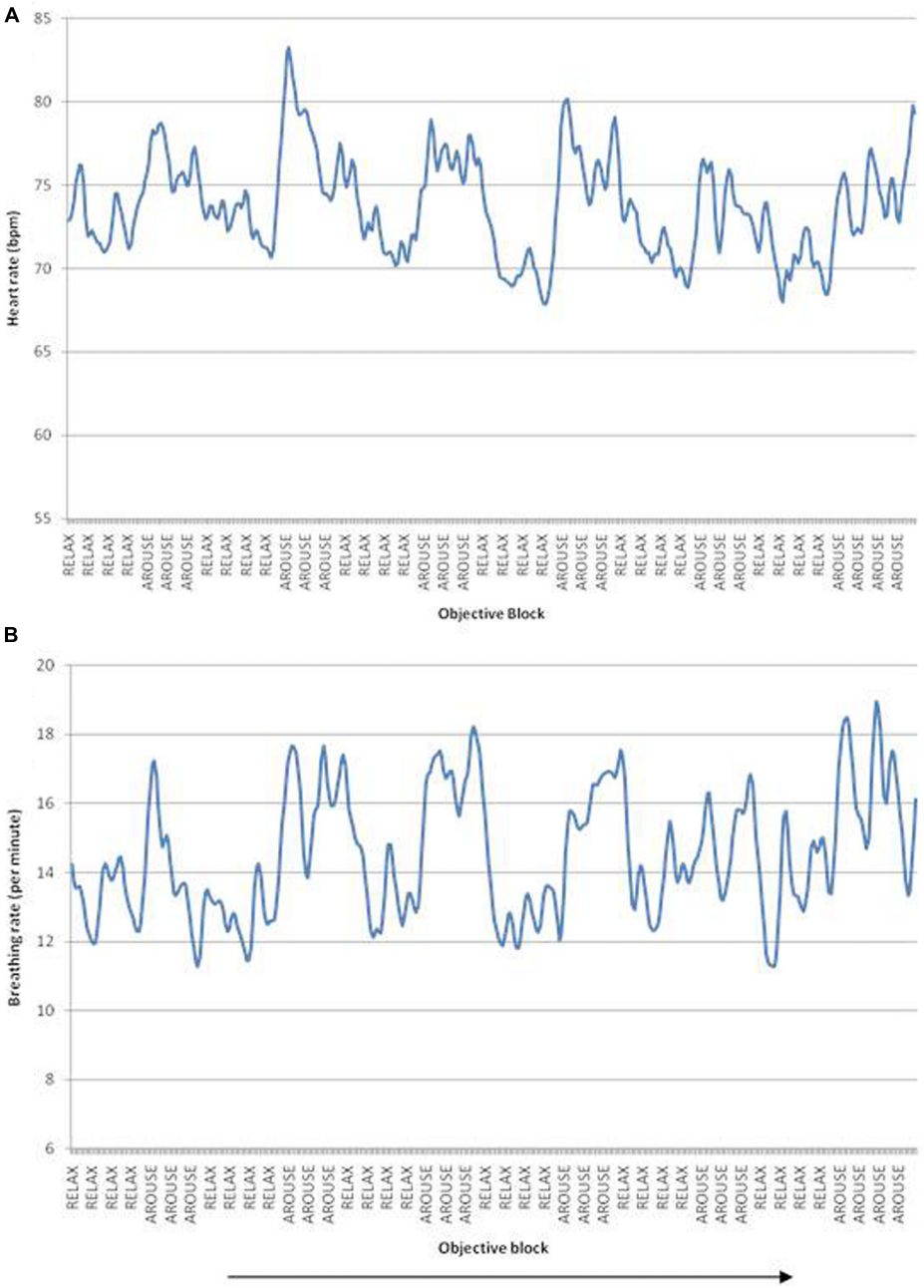


FIGURE 2 | (A) Heart rate fluctuations over the scanning duration, peaks correspond to increased heart rate/arousal blocks, troughs correspond to decreased heart rate/relaxation blocks. **(B)** Breathing rate fluctuations over time. Arrow indicates passage of time. Data illustrated from a representative single participant.

design matrix as nuisance variables. The statistical maps were then entered into a second-level (i.e., group) random effects analysis, where a 2-way factorial analysis was employed to determine the presence of main effects and interactions between feedback and objective on regional activity at the population level. In the second analysis, an individual design matrix was created for each participant that included heart rate and breathing rate as regressors of interest. Again, movement parameters were included as nuisance regressors. These statistical maps were entered into second-level (i.e., group) analysis and one-sample *t*-tests were used to evaluate the significance of the effect of heart rate and breathing rate on regional neural (haemodynamic) response. In the neuroimaging results, activations which survive family-wise error (FWE) correction ($p < 0.05$) at the cluster level are reported, unless otherwise stated. Descriptions of anatomical location were determined using the anatomical toolbox for SPM (Eickhoff et al., 2005) and in addition the atlas of Duvernoy (1991).

Results

Behavioral Results

Heart rate changed in accordance with the task instructions: across participants, heart rate averaged 76 bpm for the intended arousal condition compared to 72 bpm for the intended relaxation blocks. Thus task objective had a significant effect on heart rate, $F(2,14) = 19.2$, $p < 0.01$, $\eta^2 = 0.58$, with (see **Figures 2** and **3A**). Surprisingly, however, there was no suprathreshold main effect of feedback type on heart rate across participants and no overall interaction between objective and feedback on heart rate. This suggests that as a group, participants were able to increase or decrease their heart rate according to the objective, but the presence of feedback did not significantly impact performance. There was a trend for heart rate to increase more in the accurate biofeedback condition during the intended arousal conditions (**Figures 2A** and **3A**).

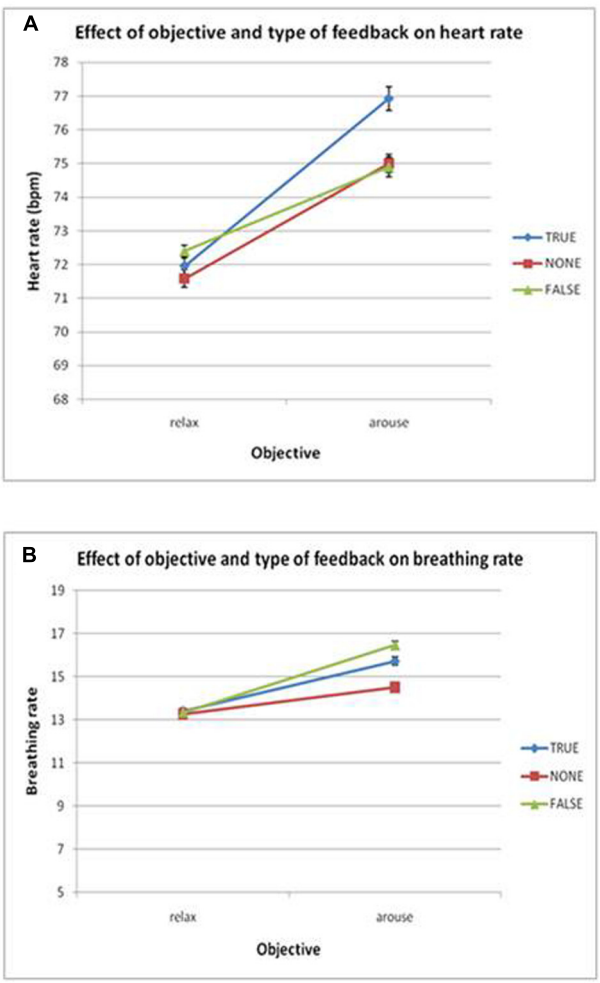


FIGURE 3 | (A) Plots the group effects of objective and feedback on heart rate. There was a significant main effect of objective ($p < 0.05$) but no interaction of objective and feedback. **(B)** Breathing rate, no significant main effects or interactions were found. Error bars show SEM.

Using breathing rate as the dependent variable, we observed no significant main effects or interactions of objective and/or feedback on breathing rate, indicating that overall participants were able to modulate heart rate without significantly changing their breathing rate (see **Figures 2B** and **3B**).

Participants' ability to volitionally regulate their heart rate, measured by percentage heart rate change in the intended direction (prompted increase or decrease) was negatively correlated with anxiety scores on the BAI, $r = -0.58$, $p < 0.05$. This suggests that participants who were less able to regulate their heart rate during this experiment experienced more anxiety symptoms (**Figure 4**).

Neuroimaging Results

Main Effect of Objective (Cardiovascular Relaxation/Arousal) on Brain Activity

The main effect on brain activity of intending to decrease heart rate was assessed by comparing relaxation and arousal tasks. Clusters of increased activity were observed in the right ventrolateral prefrontal cortex) and in the right inferior parietal lobule (see **Figure 5A**). Conversely, the main effect of intending to increase heart rate was assessed by conducting the reverse contrast, comparing arousal against relaxation conditions: this revealed greater activity within the left caudate, left midbrain, left posterior central gyrus, left cerebellar vermis, and a cluster encompassing regions of right amygdala and anterior insula (**Table 1**).

Main Effect of Feedback Type on Brain Activity

We first tested for a main effect of receiving veridical biofeedback across relaxation and arousal conditions (True > None + False). The presence of true biofeedback was associated with enhanced activation within the right occipito-temporal gyrus (Brodmann area 37). To ascertain whether this activation reflected biofeedback *per se*, or if it was driven by a visual representation of the

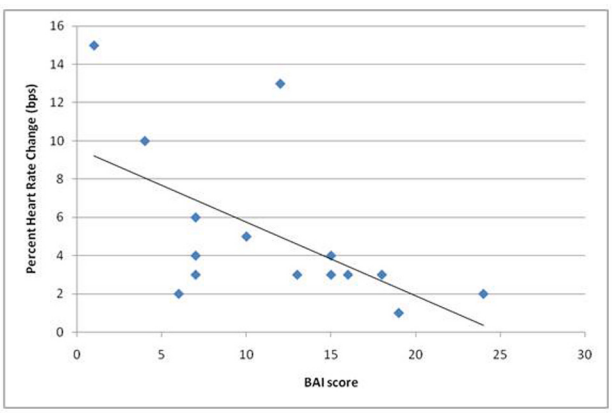


FIGURE 4 | Shows a significant negative correlation ($r = -0.58$) in performance on the task (given as average percent heart rate change across objective conditions when in the correct direction) and scores on the Beck Anxiety Inventory (BAI) scale (completed on the day of scanning).

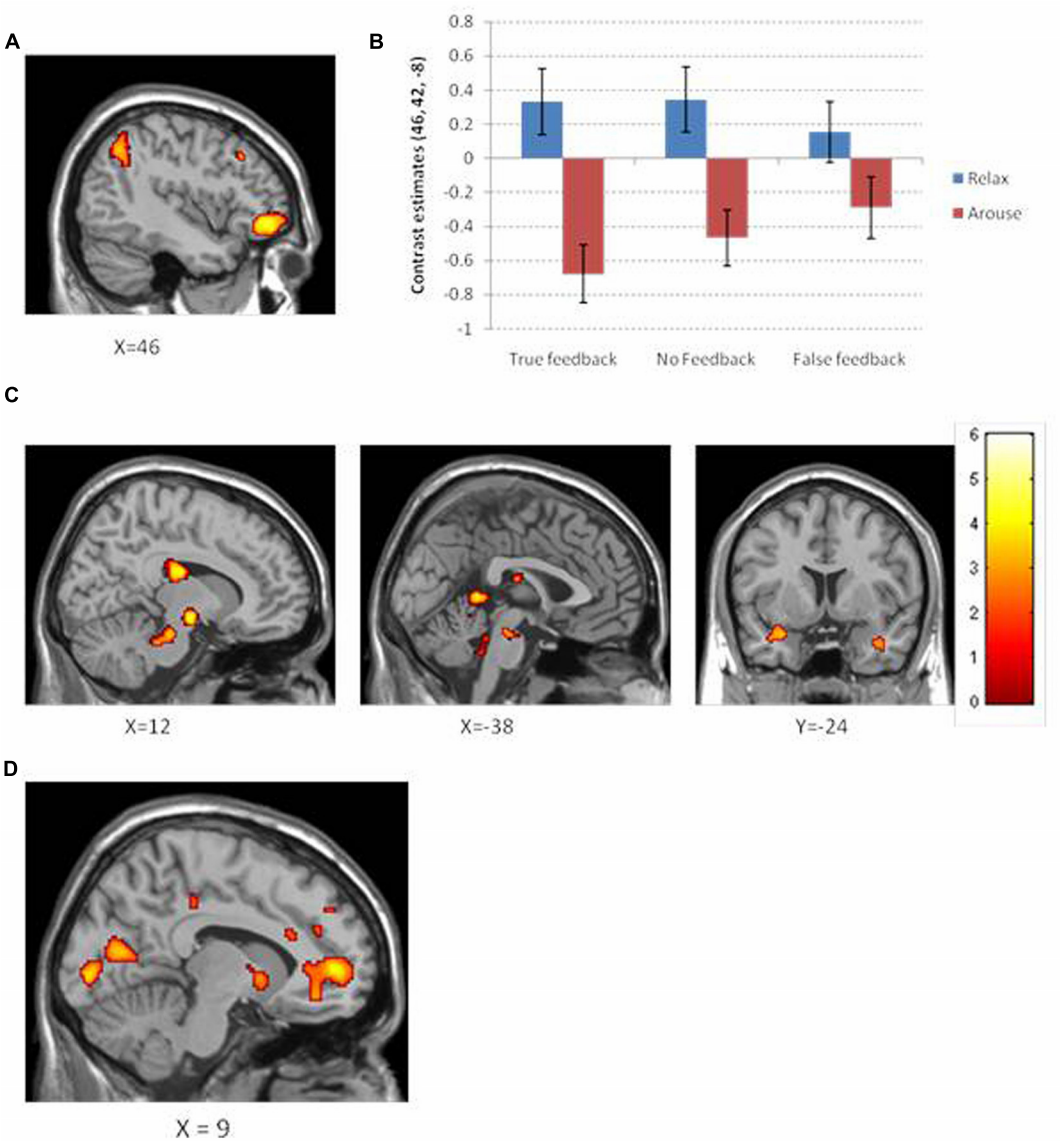


FIGURE 5 | (A) Neural activation for the contrast relaxation >arousal observed in the right ventrolateral prefrontal cortex and right intraparietal lobule. **(B)** A plot of the contrast estimates at peak ventrolateral prefrontal voxel for objective and feedback type. Interestingly, the difference in relaxation and arousal for each feedback condition appears to mirror that observed in the behavioral data. **(C)** Neural activation for the contrast arousal >relaxation across different brain slices shows increased activation in the caudate, midbrain, and the insula/amygdaloid complex. Color bar corresponds to color maps on brain images which reflect the t statistic. **(D)** Neural activation which significantly correlates with ability to perform the task objective as measured by average percent change in heart rate. Increased activation shown for regions close to the midline within pregenual anterior cingulate, ventral striatum, and primary visual cortex.

moving thermometer, we performed separate contrasts of interest (**Table 2**). Significant activity was observed in the same region of the occipital-temporal gyrus for both true feedback and false feedback when they were compared to no feedback. However, this region was not activated when comparing true feedback to false feedback and vice versa. This suggests that this activation was primarily concerned with visual aspects of the feedback i.e., the moving bar. Blocks in which no feedback was received were associated with activity in left posterior cingulate and left anterior cingulate ($p < 0.05$ corrected) but interestingly only in the intended arousal condition (the same effect was not seen for relaxation).

Modulation of Brain Activity Related to Objective (Cardiovascular Relaxation/Arousal) By Feedback Type

By examining the interaction between feedback and objective, we attempted to identify regions where heart rate relaxation/arousal related activity was modulated by the type of feedback. There were no clusters of activity reflecting this interaction at a FWE

TABLE 1 | Regional activity associated with main effect of task objective: decreasing heart rate (relaxation) versus increasing heart rate (arousal).

Region	Side	Coordinates of peak activity (MNI)	Voxels	Peak *T* score
(A) Regional brain activity associated with intended heart rate decrease				
Ventrolateral PFC	Right	46 42 –8	323	5.50
Inferior parietal lobule	Right	50–56 48	355	5.15
(B) Regional activity associated with intended heart rate increase				
Caudate	Left	–16 –28 26	366	4.97
Midbrain	Left	–10 –10 –12	99	4.96
Cerebellar vermis		0 46 2	189	4.91
Amygdala, /insula	Right	32 10 –22	172	4.42

TABLE 2 | Regional activity associated with main effects of feedback type (True/None/False).

Region	Side	Coordinates of peak activity (MNI)	Voxels	T score
(A) Regional brain activity associated with biofeedback (true feedback)				
True > None				
Occipito-temporal gyrus	Right	50 –66 –2	544	5.81
True > False				
NSA				
(B) Regional activity associated with no feedback				
None > True				
Posterior insula	Right	38 –18 18	40	3.51*
None > False				
NSA				
(C) Regional activity associated with false feedback				
False > None				
Occipito- temporal gyrus	Right	46 –66 2	740	5.87
False > True				
NSA				

*NSA, no suprathreshold activity. Clusters reported at p < 0.05 corrected. *SVC, small volume corrected.*

correction (or at a more permissive uncorrected threshold of $p < 0.001$). To obtain an impression of how feedback type may have differentially activated brain regions involved in relaxation and arousal, individual *t*-tests were performed to assess the effects of intended relaxation and intended arousal under each feedback condition (**Table 3**; **Figure 5**). In these tests, of note are the observations first; that the absence of feedback during the intended arousal conditions evoked greater engagement of right insula/amygdaloid complex, and second; that the presentation of false or no feedback during the intended relaxation condition enhanced activation within regions of subgenual cingulate cortex.

Brain Activity Related to the Magnitude of Successful Task Performance

We performed a group-level analysis to test for regional activity correlating with successful task performance, defined as the average percent change in heart rate across objective conditions. Regional activity covarying with performance was observed within pregenual anterior cingulate, angular gyrus,

TABLE 3 | *Post hoc* tests showing regions of activation associated with feedback × objective.

Region	Side	Coordinates of peak activity (MNI)	Voxels	T score
(1) True Feedback				
(A) Heart Rate Increase > Decrease (relaxation > arousal)				
Superior temporal gyrus	Right	62 –46 –4	136	4.93
Intraparietal sulcus*	Right	32 –66 48	318	4.23
vlPFC	Right	46 42 –8	117	3.91
(B) Increase > Decrease				
Thalamus	Right	2 –16 16	74	4.13
(2) No Feedback				
(A) Decrease > Increase				
Orbitofrontal gyrus/anterior insula*	Right	22 28 –8	37	4.68
(B) Increase > Decrease				
Insula/amygdala*	Right	32 10 –24	72	4.39
(3) False Feedback				
(A) Decrease > Increase				
Subgenual anterior cingulate	Left	–12 36 –2	170	4.71
(B) Increase > Decrease				
NSA	NSA	NSA	NSA	NSA

**Nominal description of region refers to anatomical extent of cluster which may encompass more than one region, the coordinates of the peak voxel represent the location maxima of the cluster. NSA, no suprathreshold activity.*

middle temporal gyrus, temporal pole, ventral striatum, and primary visual cortex (calcarine cortices; $p < 0.05$ corrected; **Figure 5D**).

Brain Activity Mapping Heart Rate and Breathing Rate Across Experimental Conditions

We further tested for regional brain activity related to changes in heart rate (the primary task objective) and also breathing rate during each scan. These physiological variables formed the two single regressors within the same analytic design matrix. Breathing rate over the course of the experiment was associated with significant changes within the right insula. This is unlikely to reflect a consequence of the instruction to try to maintain a constant breathing rate across increased and decreased heart rate blocks, since task conditions were implicitly controlled for by inclusion of heart rate within the same regression analysis. At an uncorrected threshold only ($p < 0.001$), heart rate changes correlated with activity within periaqueductal gray matter, right caudate nucleus, and right insula cortex.

Discussion

The central regulation of internal bodily states is crucial to adaptive behavior, and controlled proximately through autonomic nervous system and viscerosensory afferents. Most psychological models for understanding the interaction between mind and body underplay organ specificity and patterning across peripheral responses (Harrison et al., 2010). Nevertheless, emotional and motivational feelings are linked to the prediction and signaling of physiological 'interoceptive' state (Seth, 2013; Seth and

Critchley, 2013). Thus, by studying brain mechanisms controlling autonomic reactivity, specifically those underlying the generation and feedback representation of changes in internal state, a more comprehensive integrated neurobiological account of affective behavior can be achieved.

The present study illustrated neural mechanisms associated with the volitional modulation of heart rate. Individual task performance varied across participants; even so, the aim of eliciting increases and decreases in heart rate for intended arousal and intended relaxation, respectively, was achieved by all but one of the participants. Most strikingly, the activity within ventrolateral prefrontal cortex (Brodmann areas 44, 45, and 47; with peak activation in the lateral orbitofrontal/inferior frontal gyrus or Brodmann area 47) was enhanced during 'active relaxation' conditions intended to decrease heart rate. This broad region is implicated in the cognitive appraisal of emotional events and corresponding behavioral control (to meet task demands; Lee and Siegle, 2012). Furthermore, ventrolateral prefrontal cortex receives motivational and emotional information from the orbitofrontal cortex and subcortical areas (midbrain, hypothalamus, and striatum) and, in non-human primates, supports the computation of the behavioral significance of external events for goal directed behavior (Sakagami and Pan, 2007). Ventrolateral prefrontal cortex is likely to influence autonomic function indirectly through influences on a network incorporating visceral cingulate and insular cortices alongside amygdala and dorsal brainstem. Previous neuroimaging studies of emotion regulation also implicate this ventrolateral prefrontal region in the volitional control of physiological arousal (Beauregard et al., 2001; Critchley et al., 2002a), and during the voluntary suppression of negative affect during cognitive reappraisal (Phan et al., 2005). This prefrontal region is also involved in evaluating and gating the influence of contextual emotional information in decision-making (Beer et al., 2006); for example, it is engaged when decisions are made in states of high, but not low, urgency, suggesting that it may suppress anxiety and emotional arousal associated with risky decision-making (Jones et al., 2011). The present study extends these data by indicating that ventrolateral prefrontal cortex has a steering role in the intentional regulation of bodily arousal.

Activation within right inferior parietal lobule was also enhanced during intentional relaxation/heart rate reduction. This region is implicated in directed attention toward external stimuli (Fink et al., 1996), and earlier data suggest a shared neural substrate for selective attention and autonomic arousal (Critchley et al., 2000b). Interestingly in the present study, this region was engaged during relaxation conditions particularly when receiving veridical biofeedback, consistent with its potential role as a substrate for body-centered integration of external feedback signals with internal arousal state. Conversely, the intention to increase heart rate through enhancing one's state of arousal activated regions within the amygdala, midbrain, and caudate. Amygdala activation is linked to the generation of transient sympathetic (electrodermal) response (Phelps et al., 2001; Williams et al., 2001) and suppression of the baroreflex, allowing blood pressure and heart rate to rise together (Gianaros et al., 2012). The amygdala contributes to a network of regions including anterior cingulate cortex, insula and periaqueductal grey matter (PAG), which mediates cardiovascular reactions to psychological stressors (Gianaros et al., 2004, 2012; Gray et al., 2009; Wager et al., 2009). Additionally, both caudate and midbrain are implicated in autonomic nervous system regulation and dysregulation (e.g., Beacher et al., 2009; Gray et al., 2009). The present study adds to this literature by highlighting the capacity for individuals to engage volitionally this set of subcortical brain regions. While intentional behavioral responses are typically thought to originate from processes within prefrontal cortex, the present study suggests that intentional changes in autonomic arousal state may also be engendered through more direct recruitment of a select network of subcortical structures linked to motivational behavior.

Across task conditions, we showed an interesting relationship between neural activity and the participants' success at performing the instructed directional change in heart rate. This success was quantified as the magnitude of increase in heart rate during the intended increase/arousal conditions and the magnitude of decrease in heart rate during the intended decrease/relaxation conditions. Successful performance was associated with activation across regions including pregenual anterior cingulate, ventral striatum, and early visual cortex (illustrated **Figure 5D**) and lateral parietal and temporal cortices and temporal pole. Interestingly, earlier neuroimaging studies of biofeedback, with sympathetic electrodermal signals, implicate similar a pregenual cingulate response to task success alongside amygdala/rostral temporal lobe that also predicting the rate of (successful) physiological relaxation (Critchley et al., 2001). Our present findings includes the ventral striatum in these processes, suggesting the presence of a reward (prediction error) signal that bridges the cognitive intention to perform the task linked to the monitoring of physiological change. The fact that these findings occurred independently of perturbation in visual feedback also suggests that anterior cingulate, ventral striatal, and temporal regions are coupled to internalized interoceptive information.

We observed no formal interaction between visual feedback and task objective. It was not predicted that the experimental feedback manipulations would have little impact on task performance or associated brain activity. The main feedback-related observations were of visual cortex activation by true and false visual feedback, and of enhanced activity within posterior insula in the absence of feedback. The latter observation is in keeping with the notion of greater attention-driven engagement of interoceptive process mediated by insula cortex in the absence of veridical feedback, although previous studies also present this argument for increases in insula activity evoked by false feedback (Critchley et al., 2002a). Overall, the volitional control of heart rate seemed to be a concept that all participants could grasp and attain from minimal practice with veridical feedback before the scanning session. However, in the scanner, the feedback appeared to provide little additional value for the participants to achieve intended changes in physiological state. Interestingly, this seems to suggest that heart signals (in contrast to other internal autonomic responses such as electrodermal activity) are more readily accessible for volitional control, at least for those who score lower on anxiety measures. Retrospectively, it is an omission that we did not explore this further by measuring individual differences

in interoceptive ability using heart rate detection tasks (Garfinkel et al., 2015). There was some indication from the planned simple contrasts (presented in **Table 3**) that the quality of visual feedback modulated the neuro cognitive strategies employed to reach the target volitional state of increased or decreased heart rate. This is consistent with the observation that, with extensive training, people can become more adept at using biofeedback as a means to regulate efficiently their arousal state (Nagai et al., 2004b). Ultimately, the biofeedback manipulation only partially addressed a secondary question of this study regarding mechanisms for volitional manipulation of heart rate and their neural correlates.

One hypothesis was that 'viscerosensory' insular cortex, including anterior insula, would contribute to the neural circuitry supporting the volitional regulation of heart rate, by virtue of its evident role in the integration of cognitive, exteroceptive, and interoceptive information, and its relationship to forebrain visceromotor regions including anterior cingulate and amygdala: human insula is implicated in autonomic control, interoceptive representations, and emotional feelings (Penfield and Faulk, 1955; Oppenheimer et al., 1992; Craig, 2002; Critchley et al., 2004; Meyer et al., 2004; Harrison et al., 2010; Jones et al., 2010). Yet it is also clear that the insula does not act in isolation, neither in its contribution to autonomic regulation, nor as a substrate for feelings states and interoception. Anterior insula, along with anterior cingulate and amygdala, is implicated in 'translating' interoceptive bodily signals into feeling states (Craig, 2002, 2009; Critchley et al., 2004; Gray et al., 2007; Singer et al., 2009). It is also implicated as one cortical hub within a network for salience and self- representation (Seeley et al., 2007; Sridharan et al., 2008) and connects with frontotemporal hubs that contribute to contextual social as well as emotional behavior (Ibanez and Manes, 2012; Limongi et al., 2014). In these scenarios, anterior insula is prosed to serve as a comparator within the more distributed predictive coding of emotion, putatively receiving efference copies of descending signals from anterior cingulate and prefrontal cortices (Critchley, 2005; Paulus and Stein, 2006; Singer et al., 2009; Critchley and Seth, 2012; Seth, 2013). It is therefore noteworthy that in the present study, we did not observe marked engagement of anterior insular cortex across the different task conditions. Increased anterior insula engagement when decreasing heart rate in the absence of a feedback signal is present, but its interpretation tempered by the absence of an overarching interaction. The most parsimonious account is that we showed little behavioral or neural evidence for the integration of exteroceptive feedback information with interocetive processes toward successful task performance. Task-related increases in heart rate/arousal preferentially engaged ventrolateral prefrontal cortex, rather than anterior insula. While activity related to successful task performance evoked change within insula, including a region of anterior insula, even this effect was attenuated relative to the activity observed the pregenual cingulate, ventral striatum and even primary visual cortex. In earlier biofeedback studies of electrodermal activity, anterior insula engagement is typically associated with interference caused by perturbed feedback (e.g., Critchley et al., 2002a) and there was weak evidence showing a similar effect in the present study.

We anticipated that scan-by-scan fluctuation in measured physiological indices, i.e., heart rate and respiration, would be reflected in changes in neural activity within posterior insula (implicated as primary interoceptive cortex). We did observe activity within right posterior insula related to increased respiration and a weaker positive correlation with heart rate change. However, there was a stronger correlation between heart rate and activity within both right caudate nucleus and midbrain (PAG). The integrity of the caudate is linked to autonomic response tendencies (Beacher et al., 2009). Caudate activity, alongside insula and dorsal cingulate, predicts heart rate changes to emotional stimuli (Critchley et al., 2005) and at a network level, the connectivity between caudate, cingulate, insula, and midbrain is coupled to resting state fluctuations in heart rate variability (Chang et al., 2013). Together these data suggest a proximate network of brain regions supports the representation of heart rate signals, which are selectively engaged in their volitional control. These inferences merit further investigation. Different methodological approaches may shed more light: for example, we focused on heart rate change (measured with pulse oximetry) as an intuitively accessible interoceptive response: however, potentially more accurate methods for mapping the central control of heart rate involve measuring heart rate variability, reflected in changing intervals between successive heartbeats (on electrocardiography: R–R intervals). Heart rate variability reflects homeostatic regulation through sympathetic and parasympathetic axes of the autonomic nervous system, where high frequency components index vagus-mediated coupling of cardiac control with respiration (Napadow et al., 2008). Somewhat impressively, participants were able to induce significant changes in their heart rate while maintaining a consistent breathing rate, suggesting cognitive mechanisms are sufficient to fulfill the task objectives, bypassing the need to evoke cardiorespiratory reflexes through explicitly modulating the rate or depth of breathing. Typically, humans regulate their breathing in order to become more relaxed, e.g., in yoga or meditation. In emotional situations involving high physiological arousal, breathing increases to provide muscles with more oxygen as part of the fight or flight response. Such effects did not account for task-associated changes in heart rate in the present study. Nevertheless, while participants were asked to maintain a constant breathing rate, their breathing fluctuated across the course of the experiment and was tracked by activity within right insular cortex. This finding is consistent with evidence from studies in humans and other animals that map reciprocal respiratory projections between insula and vagus nerve (Radna and MacLean, 1981) and which show strong inhibitory effects of insula stimulation on respiration rate (Kaada and Jasper, 1952; Hoffman and Rasmussen, 1953).

Interestingly, there was a significant negative correlation between the ability of participants to modulate their heart rate intentionally and anxiety scores. Those who were rated as more anxious were less able to meet the task objective (i.e., increase or decrease their heart rate). Our findings suggest that greater anxiety is associated with impaired capacity for physiological control and, by implication, a relatively reduced ability to contain emotional arousal responses as effectively as their less-anxious individuals. This observation in a subclinical group qualifies

evidence showing that individuals with clinical anxiety show heightened sensitivity to interoceptive cues (Domschke et al., 2011) and associating autonomic dysregulation with anxiety disorders (Wilhelm et al., 2001). Self-regulation of autonomic arousal may be applied as every day countermeasures or in therapeutic interventions to enhance the regulation of emotions states, notably anxiety. The contribution of bodily arousal states is well-recognized, highlighting a link between interoceptive processes to anxiety symptoms (Clark, 1986). However, the simplified hypothesis that individual differences in (heightened) interoceptive sensitivity, quantified for example by assessing an individual's accuracy in detecting own heartbeats at rest, predisposes to anxiety has limited validity in both normative and clinical populations. Overrepresentation of individuals with enhanced interoceptive processing (heartbeat detection) is observed in populations with anxiety (Pollatos et al., 2007; Dunn et al., 2010; Terasawa et al., 2013), although the finding is not always demonstrated (Asmundson et al., 1993; Craske et al., 2001). This inconsistency reflects a complexity addressed by the theoretical proposal, backed by data, of dissociable cognitive dimensions to interoception wherein awareness and subjective perception/interpretation of bodily response may diverge significantly from objective measures of interoceptive sensitivity accuracy (Garfinkel and Critchley, 2013; Garfinkel et al., 2015). Discrepancy between these subjective (prediction/interpretation) and objective (interoceptive accuracy) dimensions is proposed to give rise to emotional symptoms through 'interoceptive prediction error' signaling an impaired sense of control of internal physiological state (Paulus and Stein, 2006; Seth, 2013). Our finding, in a subclinical group, that better volitional control of heart rate predicts lower levels of state anxiety is consistent with this concept. Equally, the counter-argument that anxiety impairs non-specifically performance of volitional control tasks must acknowledge the bidirectional psychophysiological dynamics of symptom expression.

Human anxiety consists of a complex pattern of cognitive, affective, physiological, and behavioral changes in response to threat, loss, or perceived negative outcome (Beck and Clark, 1997). The finding that individuals with greater anxiety are significantly less able to volitionally modulate their heart rate without prior training has clinical implications for treatment approaches. Although, the presence of biofeedback did not significantly improve participant's ability to regulate their heart rate on this one occasion, heart rate feedback retains potential therapeutic utility for anxiety patients. Visual heart rate feedback is reported to facilitate exposure treatment of animal phobic patients (Nunes and Marks, 1975) and auditory heart rate feedback enhances claustrophobia treatment (Telch et al., 2000). Conversely, increased anxiety can be induced by false heart rate feedback in patients with panic disorder (Ehlers et al., 1988). Thus, heart rate based biofeedback paradigms have the potential to enhance 'interoceptive exposure' in the management of anxiety disorders. There is evidence to support the notion that autonomic biofeedback training may also diminish symptoms in other patient groups with stress-sensitive neuropsychiatric and medical disorders, including epilepsy (Nagai et al., 2004a; Micoulaud-Franchi et al., 2014), tic disorder (Nagai et al., 2009) and cardiovascular disease (Moravec and McKee, 2011). There is therefore broader utility of biofeedback approaches in managing dissociative neuropsychiatric symptoms (Sedeño et al., 2014).

There are limitations to this study: training participants in performing biofeedback prior to scanning and ensuing all could carry out biofeedback to a reasonable standard may have reduced participant variability in task performance and increased the chance of observing feedback-specific influences. Also, the instructions for participants to increase or decrease their level of arousal may have biased them toward engaging mechanisms that go beyond those necessary purely for the volitional regulation of heart rate. If this were the case, the findings we observed within the brain (related to the task intention and correlating with task achievement) may reflect other psychological processes (e.g., mediating wakefulness or emotionality) that are incidental to, though not independent of, the participants' directed regulation of their physiological arousal. However, all participants were aware that they were only required to increase or decreased their heart rate in accordance with task instructions. Moreover from the outset we defined the nebulous terms arousal and relaxation to refer operationally to cardiovascular arousal and relaxation (i.e., increased and decreased heart rate). No instructions were given to change level of alertness, wakefulness, or direct attention to emotional events. Our study suggests that the volitional regulation of cardiovascular arousal, at least within the setting of a neuroimaging experiment, is relatively easy to attain with minimal practice and no need for active feedback. While we extrapolate our findings to suggest that these same brain regions associated with task success may be engaged in similar mechanisms to regulate physiological arousal contributing to anxiety states, this proposal will require direct empirical validation. At a technical level, coverage of ventromedial prefrontal cortex during the acquisition of echo planar T2* datasets was not always consistent across participants, diminishing our ability to infer the contribution of this region to the regulation of autonomic state: previous studies report inverse correlations between ventromedial/orbitofrontal cortex activity and sympathetic arousal (Nagai et al., 2004b).

To summarize, our data provide evidence for the role of specific brain regions, notably ventrolateral prefrontal cortex, in the volitional control of heart rate, with implications for understanding, and treating anxiety and stress-sensitive neuropsychiatric and physical conditions. These regions are linked to wider functional brain networks implicated in emotional regulation. Interestingly we did not provide strong evidence for our prediction that insula cortex was critical to the volitional regulation of heart rate through biofeedback. However, our participants were relatively naïve to the use of biofeedback techniques and their overall task performance was not shaped by the presence of veridical visual feedback, but reflects the employment of alternative strategies to implement the directed task objectives. Nevertheless, we highlight the cortical and subcortical networks mediating intentional autonomic cardiac control. Understanding these mechanisms has implications for management of clinical disorders of emotion regulation, and relevance to training self-management using biofeedback approaches, including neurofeedback with fMRI.

Acknowledgments

CJ was funded by an MRC studentship through the University of Sussex, HC, NM, and JW receive funding from the Dr. Mortimer and Dame T. Sackler Foundation through the Sackler Centre for Consciousness Science University of Sussex. We thank the reviewers for their helpful comments that enhanced the paper, particularly to Andrea Caria for discussion regarding the possible contribution of the task instructions to the observed brain activity associated with feedback-independent physiological regulation.

References

Asmundson, G. J., Sandler, L. S., Wilson, K. G., and Norton, G. R. (1993). Panic attacks and interoceptive acuity for cardiac sensations. *Behav. Res. Ther.* 31, 193–197. doi: 10.1016/0005-7967(93)90071-2

Beacher, F. D., Gray, M. A., Mathias, C. J., and Critchley, H. D. (2009). Vulnerability to simple faints is predicted by regional differences in brain anatomy. *Neuroimage* 47, 937–945. doi: 10.1016/j.neuroimage.2009.05.038

Beauregard, M., Levesque, J., and Bourgouin, P. (2001). Neural correlates of conscious self-regulation of emotion. *J. Neurosci.* 21, RC165.

Beck, A. T., and Clark, D. A. (1997). An information processing model of anxiety: automatic and strategic processes. *Behav. Res. Ther.* 35, 49–58. doi: 10.1016/S0005-7967(96)00069-1

Beck, A. T., Epstein, N., Brown, G., and Steer, R. A. (1988). An inventory for measuring clinical anxiety: psychometric properties. *J. Consult. Clin. Psychol.* 56, 893–897. doi: 10.1037/0022-006X.56.6.893

Beer, J. S., Knight, R. T., and D'Esposito, M. (2006). Controlling the integration of emotion and cognition: the role of frontal cortex in distinguishing helpful from hurtful emotional information. *Psychol. Sci.* 17, 448–453. doi: 10.1111/j.1467-9280.2006.01726.x

Bonn, J. A., Turner, P., and Hicks, D. C. (1972). Beta-adrenergic-receptor blockade with practolol in treatment of anxiety. *Lancet* 1, 814–815. doi: 10.1016/S0140-6736(72)90799-4

Borkovec, T. D., and Costello, E. (1993). Efficacy of applied relaxation and cognitive-behavioural therapy in the treatment of generalized anxiety disorder. *J. Consult. Clin. Psychol.* 61, 611–619. doi: 10.1037/0022-006X.61.4.611

Buhle, J. T., Silvers, J. A., Wager, T. D., Lopez, R., Onyemekwu, C., Kober, H., et al. (2014). Cognitive reappraisal of emotion: a meta-analysis of human neuroimaging studies. *Cereb. Cortex.* 24, 2981–2990. doi: 10.1093/cercor/bht154

Caldiroli, D., and Minati, L. (2007). Early experience with remote pressure sensor respiratory plethysmography monitoring sedation in the MR scanner. *Eur. J. Anaesthesiol.* 24, 761–769. doi: 10.1017/S0265021507000312

Caria, A., Veit, R., Sitaram, R., Lotze, M., Weiskopf, N., Grodd, W., et al. (2007). Regulation of anterior insular cortex activity using real-time fMRI. *Neuroimage* 35, 1238–46. doi: 10.1016/j.neuroimage.2007.01.018

Chang, C., Metzger, C. D., Glover, G. H., Duyn, J. H., Heinze, H. J., and Walter, M. (2013). Association between heart rate variability and fluctuations in resting-state functional connectivity. *Neuroimage* 68, 93–104. doi: 10.1016/j.neuroimage.2012.11.038

Clark, D. M. (1986). A cognitive approach to panic. *Behav. Res. Ther.* 24, 461–470. doi: 10.1016/0005-7967(86)90011-2

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655–666. doi: 10.1038/nrn894

Craig, A. D. (2009). How do you feel - now? The anterior insula and human awareness. *Nat. Rev. Neurosci.* 10, 59–70. doi: 10.1038/nrn2555

Craske, M. G., Lang, A. J., Tsao, J. C., Mystkowski, J. L., and Rowe, M. K. (2001). Reactivity to interoceptive cues in nocturnal panic. *J. Behav. Ther. Exp. Psychiatry* 32, 173–190. doi: 10.1016/S0005-7916(01)00033-7

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *J. Comp. Neurol.* 493, 154–166. doi: 10.1002/cne.20749

Critchley, H. D., Corfield, D. R., Chandler, M. P., Mathias, C. J., and Dolan, R. J. (2000a). Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. *J. Physiol.* 523, 259–270. doi: 10.1111/j.1469-7793.2000.t01-1-00259.x

Critchley, H. D., Elliott, R., Mathias, C. J., and Dolan, R. J. (2000b). Neural activity relating to generation and representation of galvanic skin conductance responses: a functional magnetic resonance imaging study. *J. Neurosci.* 20, 3033–3040.

Critchley, H. D., and Harrison, N. A. (2013). Visceral influences on brain and behavior. *Neuron* 77, 624–638. doi: 10.1016/j.neuron.2013.02.008

Critchley, H. D., Melmed, R. N., Featherstone, E., Mathias, C. J., and Dolan, R. J. (2001). Brain activity during biofeedback relaxation: a functional neuroimaging investigation. *Brain* 124, 1003–1012. doi: 10.1093/brain/124.5.1003

Critchley, H. D., Melmed, R. N., Featherstone, E., Mathias, C. J., and Dolan, R. J. (2002a). Volitional control of autonomic arousal: a functional magnetic resonance study. *Neuroimage* 16, 909–919. doi: 10.1006/nimg.2002.1147

Critchley, H. D., Mathias, C. J., and Dolan, R. J. (2002b). Fear conditioning in humans: the influence of awareness and autonomic arousal on functional neuroanatomy. *Neuron* 33, 653–663. doi: 10.1016/S0896-6273(02)00588-3

Critchley, H. D., Rotshtein, P., Nagai, Y., O'Doherty, J., Mathias, C. J., and Dolan, R. J. (2005). Activity in the human brain predicting differential heart rate responses to emotional facial expressions. *Neuroimage* 24, 751–762. doi: 10.1016/j.neuroimage.2004.10.013

Critchley, H., and Seth, A. (2012). Will studies of macaque insula reveal the neural mechanisms of self-awareness? *Neuron* 74, 423–426. doi: 10.1016/j.neuron.2012.04.012

Critchley, H. D., Wiens, S., Rotshtein, P., Öhman, A., and Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nat. Neurosci.* 7, 189–195. doi: 10.1038/nn1176

Damasio, A. R. (1994). *Descartes' Error: Emotion, Reason and the Human Brain.* New York, NY: Putnam Publishing.

Domschke, K., Stevens, S., Pfleiderer, B., and Gerlach, A. L. (2011). Interoceptive sensitivity in anxiety and anxiety disorders: an overview and integration of neurobiological findings. *Clin. Psychol. Rev.* 30, 1–11. doi: 10.1016/j.cpr.2009.08.008

Dunn, B. D., Stefanovitch, I., Evans, D., Oliver, C., Hawkins, A., and Dalgleish, T. (2010). Can you feel the beat? Interoceptive awareness is an interactive function of anxiety- and depression-specific symptom dimensions. *Behav. Res. Ther.* 48, 1133–1138. doi: 10.1016/j.brat.2010.07.006

Duvernoy, H. (1991). *The Human Brain: Surface Three Dimensional Sectional Anatomy and MRI*. New York, NY: Springer-Verlag.

Ehlers, A., Margraf, J., Roth, W. T., Taylor, C. B., and Birbaumer, N. (1988). Anxiety induced by false heart rate feedback in patients with panic disorder. *Behav. Res. Ther.* 26, 1–11. doi: 10.1016/0005-7967(88)90028-9

Eickhoff, S. B., Stephan, K. E., Mohlberg, H., Grefkes, C., Fink, G. R., Amunts, K., et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25, 1325–1335. doi: 10.1016/j.neuroimage.2004.12.034

Fink, G. R., Halligan, P. W., Marshall, J. C., Frith, C. D., Frackowiak, R. S., and Dolan, R. J. (1996). Where in the brain does visual attention select the forest and the trees? *Nature* 382, 626–628. doi: 10.1038/382626a0

Frank, S., Lee, S., Preissl, H., Schultes, B., Birbaumer, N., and Veit, R. (2012). The obese brain athlete: self-regulation of the anterior insula in adiposity. *PLoS ONE* 7:e42570. doi: 10.1371/journal.pone.0042570

Garfinkel, S. N., and Critchley, H. D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. *Soc. Cogn. Affect. Neurosci.* 8, 231–234. doi: 10.1093/scan/nss140

Garfinkel, S. N., Seth, A. K., Barrett, A. B., Suzuki, K., and Critchley, H. D. (2015). Knowing your own heart: distinguishing interoceptive accuracy from interoceptive awareness. *Biol. Psychol.* 104, 65–74. doi: 10.1016/j.biopsycho.2014.11.004

Gianaros, P. J., Onyewuenyi, I. C., Sheu, L. K., Christie, I. C., and Critchley, H. D. (2012). Brain systems for baroreflex suppression during stress in humans. *Hum. Brain Mapp.* 33, 1700–1716. doi: 10.1002/hbm.21315

Gianaros, P. J., Van Der Veen, F. M., and Jennings, J. R. (2004). Regional cerebral blood flow correlates with heart period and high-frequency heart period

variability during working-memory tasks: implications for the cortical and subcortical regulation of cardiac autonomic activity. *Psychophysiology* 41, 521–530. doi: 10.1111/1469-8986.2004.00179.x

Gray, M. A., Beacher, F. D., Minati, L., Nagai, Y., Kemp, A. H., Harrison, N. A., et al. (2012). Emotional appraisal is influenced by cardiac afferent information. *Emotion* 12, 180–191. doi: 10.1037/a0025083

Gray, M. A., Harrison, N. A., Wiens, S., and Critchley, H. D. (2007). Modulation of emotional appraisal by false physiological feedback during fMRI. *PLoS ONE* 2:e546. doi: 10.1371/journal.pone.0000546

Gray, M. A., Rylander, K., Harrison, N. A., Wallin, B. G., and Critchley, H. D. (2009). Following one's heart: cardiac rhythms gate central initiation of sympathetic reflexes. *J. Neurosci.* 29, 1817–1825. doi: 10.1523/JNEUROSCI.3363-08.2009

Harrison, N. A., Gray, M., Gianaros, P. J., and Critchley, H. D. (2010). The embodiment of emotional feelings in the brain. *J. Neurosci.* 30, 12878–12884. doi: 10.1523/JNEUROSCI.1725-10.2010

Hoffman, B. L., and Rasmussen, T. (1953). Stimulation studies of insular cortex of *Macaca mulatta*. *J. Neurophysiol.* 16, 343–351.

Ibanez, A., and Manes, F. (2012). Contextual social cognition and the behavioral variant of frontotemporal dementia. *Neurology* 78, 1354–1362. doi: 10.1212/WNL.0b013e3182518375

Jones, C. L., Minati, L., Harrison, N. A., Ward, J., and Critchley, H. D. (2011). Under pressure: response urgency modulates striatal and insula activity during decision-making under risk. *PLoS ONE* 6:e20942. doi: 10.1371/journal.pone.0020942

Jones, C. L., Ward, J., and Critchley, H. D. (2010). The neuropsychological impact of insular cortex lesions. *J. Neurol. Neurosurg. Psychiatry* 81, 611–618. doi: 10.1136/jnnp.2009.193672

Kaada, B. R., and Jasper, H. (1952). Respiratory responses to stimulation of temporal pole, insula, and hippocampal and limbic gyri in man. *AMA Arch. Neurol. Psychiatry* 68, 609–619. doi: 10.1001/archneurpsyc.1952.02320230035004

Lane, R. D., Reiman, E. M., Axelrod, B., Yun, L. S., Holmes, A., and Schwartz, G. E. (1998). Neural correlates of levels of emotional awareness. evidence of an interaction between emotion and attention in the anterior cingulate cortex. *J. Cogn. Neurosci.* 10, 525–535. doi: 10.1162/089892998562924

Lee, K. H., and Siegle, G. J. (2012). Common and distinct brain networks underlying explicit emotional evaluation: a meta analytic study. *Soc. Cogn. Affect. Neurosci.* 7, 521–534. doi: 10.1093/scan/nsp001

Limongi, R., Tomio, A., and Ibanez, A. (2014). Dynamical predictions of insular hubs for social cognition and their application to stroke. *Front. Behav. Neurosci.* 8:380. doi: 10.3389/fnbeh.2014.00380

Meyer, S., Strittmatter, M., Fischer, C., Georg, T., and Schmitz, B. (2004). Lateralization in autonomic dysfunction. common and distinct brain networks underlying explicit emotional in ischemic stroke involving the insular cortex. *Neuroreport* 15, 357–361. doi: 10.1097/00001756-200402090-00029

Micoulaud-Franchi, J. A., Kotwas, I., Lanteaume, L., Berthet, C., Bastien, M., Vion-Dury, J., et al. (2014). Skin conductance biofeedback training in adults with drug-resistant temporal lobe epilepsy and stress-triggered seizures: a proof-of-concept study. *Epilepsy Behav.* 41, 244–250. doi: 10.1016/j.yebeh.2014.10.017

Moravec, C. S., and McKee, M. G. (2011) Biofeedback in the treatment of heart disease. *Cleve. Clin. J. Med.* 78(Suppl. 1), S20–S23. doi: 10.3949/ccjm.78.s1.03

Nagai, Y., Cavanna, A., and Critchley, H. D. (2009). Influence of sympathetic autonomic arousal on tics: implications for a therapeutic behavioral intervention for Tourette syndrome. *J. Psychosom. Res.* 67, 599–605. doi: 10.1016/j.jpsychores.2009.06.004

Nagai, Y., Goldstein, L. H., Fenwick, P. B., and Trimble, M. R. (2004a). Clinical efficacy of galvanic skin response biofeedback training in reducing seizures in adult epilepsy: a preliminary randomized controlled study. *Epilepsy Behav.* 5, 216–223. doi: 10.1016/j.yebeh.2003.12.003

Nagai, Y., Critchley, H. D., Featherstone, E., Trimble, M. R., and Dolan, R. J. (2004b). Activity in ventromedial prefrontal cortex covaries with sympathetic skin conductance level: a physiological account of a "default mode" of brain function. *Neuroimage* 22, 243–251. doi: 10.1016/j.neuroimage.2004.01.019

Napadow, V., Dhond, R., Conti, G., Makris, N., Brown, E. N., and Barbieri, R. (2008). Brain correlates of autonomic modulation: combining heart rate variability with fMRI. *Neuroimage* 42, 169–177. doi: 10.1016/j.neuroimage.2008.04.238

Nunes, J. S., and Marks, I. M. (1975). Feedback of true heart rate during exposure in vivo. *Arch. Gen. Psychiatry* 32, 933–936. doi: 10.1001/archpsyc.1975.01760250125014

Oppenheimer, S. M., Gelb, A., Girvin, J. P., and Hachinski, V. C. (1992). Cardiovascular effects of human insular cortex stimulation. *Neurology* 42, 1727–1732. doi: 10.1212/WNL.42.9.1727

Pollatos, O., Traut-Mattausch, E., Schroeder, H., and Schandry, R. (2007). Interoceptive awareness mediates the relationship between anxiety and the intensity of unpleasant feelings. *J. Anxiety Disord.* 21, 931–943. doi: 10.1016/j.janxdis.2006.12.004

Paulus, M. P., and Stein, M. B. (2006). An insular view of anxiety. *Biol. Psychiatry* 60, 383–387. doi: 10.1016/j.biopsych.2006.03.042

Penfield, W., and Faulk, M. E. (1955). The insula; further observations on its function. *Brain* 78, 445–470. doi: 10.1093/brain/78.4.445

Phan, K. L., Fitzgerald, D. A., Nathan, P. J., Moore, G. J., Uhde, T. W., and Tancer, M. E. (2005). Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biol. Psychiatry* 57, 210–219. doi: 10.1016/j.biopsych.2004.10.030

Phelps, E. A., O'Connor, K. J., Gatenby, J. C., Gore, J. C., Grillon, C., and Davis, M. (2001). Activation of the left amygdala to a cognitive representation of fear. *Nat. Neurosci.* 4, 437–441. doi: 10.1038/86110

Radna, R. J., and MacLean, P. D. (1981). Vagal elicitation of respiratory-type and other unit responses in basal limbic structures of squirrel monkeys. *Brain Res.* 213, 45–61. doi: 10.1016/0006-8993(81)91247-6

Sakagami, M., and Pan, X. (2007). Functional role of the ventrolateral prefrontal cortex in decision making. *Curr. Opin. Neurobiol.* 17, 228–233. doi: 10.1016/j.conb.2007.02.008

Sedeño, L., Couto, B., Melloni, M., Canales-Johnson, A., Yoris, A., Baez, S., et al. (2014). How do you feel when you can't feel your body? Interoception, functional connectivity and emotional processing in depersonalization-derealization disorder. *PLoS ONE* 9:e98769. doi: 10.1371/journal.pone.0098769

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/JNEUROSCI.5587-06.2007

Seth, A. K. (2013). Interoceptive inference, emotion, and the embodied self. *Trends Cogn. Sci.* 17, 565–573. doi: 10.1016/j.tics.2013.09.007

Seth, A. K., and Critchley, H. D. (2013). Extending predictive processing to the body: emotion as interoceptive inference. *Behav. Brain Sci.* 36, 227–228. doi: 10.1017/S0140525X12002270

Singer, T., Critchley, H. D., and Preuschoff, K. (2009). A common role of insula in feelings, empathy and uncertainty. *Trends Cogn. Sci.* 13, 334–340. doi: 10.1016/j.tics.2009.05.001

Sridharan, D., Levitin, D. J., and Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12569–12574. doi: 10.1073/pnas.0800005105

Telch, M. J., Valentine, D. P., Ilai, D., Petruzzi, D., and Hehmsoth, M. (2000). The facilitative effects of heart-rate feedback in the emotional processing of claustrophobic fear. *Behav. Res. Ther.* 38, 373–387. doi: 10.1016/S0005-7967(99)00038-8

Terasawa, Y., Shibata, M., Moriguchi, Y., and Umeda, S. (2013). Anterior insular cortex mediates bodily sensibility and social anxiety. *Soc. Cogn. Affect Neurosci.* 8, 259–266. doi: 10.1093/scan/nss108

van Buuren, M., Gladwin, T. E., Zandbelt, B. B., van den Heuvel, M., Ramsey, N. F., Kahn, R. S., et al. (2009). Cardiorespiratory effects on default-mode network activity as measured with fMRI. *Hum. Brain Mapp.* 30, 3031–3042. doi: 10.1002/hbm.20729

Wager, T. D., Waugh, C. E., Lindquist, M., Noll, D. C., Fredrickson, B. L., and Taylor, S. F. (2009). Brain mediators of cardiovascular responses to social threat: part I: reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. *Neuroimage* 47, 821–835. doi: 10.1016/j.neuroimage.2009.05.043

Wilhelm, F. H., Trabert, W., and Roth, W. Y. (2001). Physiologic instability in panic disorder and generalized anxiety disorder. *Biol. Psychiatry* 49, 596–605. doi: 10.1016/S0006-3223(00)01000-3

Williams, L. M., Phillips, M. L., Brammer, M. J., Skerrett D., Lagopoulos, J., Rennie, C., et al. (2001). Arousal dissociates amygdala and hippocampal fear responses: evidence from simultaneous fMRI and skin conductance recording. *Neuroimage* 14, 1070–1079. doi: 10.1006/nimg.2001.0904

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in PSYCHOLOGY

ORIGINAL RESEARCH ARTICLE
published: 14 October 2014
doi: 10.3389/fpsyg.2014.01162

Neuroimaging and psychophysiological investigation of the link between anxiety, enhanced affective reactivity and interoception in people with joint hypermobility

Núria Mallorquí-Bagué[1,2,3]*, Sarah N. Garfinkel[1,4,5], Miriam Engels[1,6], Jessica A. Eccles[1,4], Guillem Pailhez[2,7,8], Antonio Bulbena[2,7,8] and Hugo D. Critchley[1,4,5]

[1] Psychiatry, Brighton and Sussex Medical School, University of Sussex, Falmer, UK
[2] Department of Psychiatry and Forensic Medicine, School of Medicine, Universitat Autonoma de Barcelona, Barcelona, Spain
[3] Department of Psychiatry, Psychology and Psychosomatics, Hospital Universitari Quirón Dexeus, Barcelona, Spain
[4] Mood and Anxiety Research in Sussex (MARS), Sussex Partnership NHS Foundation Trust, Sussex, UK
[5] Sackler Centre for Consciousness Science, University of Sussex, Falmer, UK
[6] Clinical Psychological Science, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, Netherlands
[7] Anxiety Unit, Institute of Neuropsychiatry and Addictions, Hospital del Mar, Universitat Autònoma de Barcelona, Barcelona, Spain
[8] Hospital del Mar Medical Research Institute, Barcelona, Spain

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Belinda Jayne Liddell, University of New South Wales, Australia
Mayuresh S Korgaonkar, University of Sydney, Australia

***Correspondence:**
Núria Mallorquí-Bagué, Anxiety Unit, Institute of Neuropsychiatry and Addictions, Hospital del Mar-Parc de Salut Mar, Passeig Marítim num. 25, Barcelona, BCN 08003, Spain
e-mail: nmallorqui@live.com

Objective: Anxiety is associated with increased physiological reactivity and also increased "interoceptive" sensitivity to such changes in internal bodily arousal. Joint hypermobility, an expression of a common variation in the connective tissue protein *collagen,* is increasingly recognized as a risk factor to anxiety and related disorders. This study explored the link between anxiety, interoceptive sensitivity and hypermobility in a sub-clinical population using neuroimaging and psychophysiological evaluation.

Methods: Thirty-six healthy volunteers undertook interoceptive sensitivity tests, a clinical examination for hypermobility and completed validated questionnaire measures of state anxiety and body awareness tendency. Nineteen participants also performed an emotional processing paradigm during functional neuroimaging.

Results: We confirmed a significant relationship between state anxiety score and joint hypermobility. Interoceptive sensitivity mediated the relationship between state anxiety and hypermobility. Hypermobile, compared to non-hypermobile, participants displayed heightened neural reactivity to sad and angry scenes within brain regions implicated in anxious feeling states, notably insular cortex.

Conclusions: Our findings highlight the dependence of anxiety state on bodily context, and increase our understanding of the mechanisms through which vulnerability to anxiety disorders arises in people bearing a common variant of collagen.

Keywords: anxiety, functional magnetic resonance imaging (fMRI), interoception, emotion, joint hypermobility, psychology

INTRODUCTION

Anxiety is associated with heightened physiological arousal and accompanying physical sensations. Interoception (i.e., sensitivity to changes in the internal physiological state of the body) is considered to be fundamental to such emotional feelings (Damasio, 1994). Interoceptive sensitivity is viewed as a constitutional trait that is stable over much of an individual's lifespan. People who can judge their bodily signals (e.g., the timing of their heartbeats) to a high level of accuracy experience emotions more intensely (Wiens et al., 2000; Pollatos et al., 2007a,b) and, in both the general population and clinical populations, are more likely to experience higher levels of anxiety (Mor and Winquist, 2002; Domschke et al., 2010).

Neuropsychological and neuroimaging studies implicate a set of related brain regions in the expression of anxiety. In particular, responses within insula, amygdala and anterior cingulate cortex are linked both to the development and maintenance of anxiety disorders (Damsa et al., 2009; Shurick and Gross, 2013). Hyperactivity within these regions is coupled to exaggerated autonomic arousal responses (Critchley and Harrison, 2013) and occurs during anxiety symptom provocation (Holzschneider and Mulert, 2011).

People with joint hypermobility are vulnerable to anxiety disorders. Joint hypermobility is a common inherited connective tissue condition that represents a qualitative variation in the fibrous structural protein *collagen*. Collagen is a protein component of bone, cartilage, tendon, blood vessels, and other body constituents. Hence joint hypermobility can present multiple clinical features which are associated with the collagen abnormality and can be either articular or extra-articular: widespread

musculoskeletal pain, multiple soft tissue lesions and fragility of supportive connective tissue and skin (Ross and Grahame, 2011). The estimated prevalence of joint hypermobility ranges between 10 and 20% in western countries and it is more frequent in women (3:1). Individuals with joint hypermobility often present autonomic abnormalities and stress-sensitive illnesses, including fibromyalgia, temporomandibular joint disorder and chronic fatigue syndrome (Smith et al., 2014). The strong link between anxiety disorder and joint hypermobility was first established in 1988 (Bulbena et al.) and this finding has been widely replicated, confirming that joint hypermobility is associated with the heightened expression of anxiety symptoms (Garcia-Campayo et al., 2011; Bianchi Sanches et al., 2012; Smith et al., 2014) and represents a risk factor trait for developing anxiety disorders (Bulbena et al., 2011). However, little is known about the underlying neural mechanisms through which joint hypermobility and anxiety symptoms interrelate. One neuroimaging study of healthy non-anxious individuals associated the expression of hypermobility to structural differences in emotional-processing brain regions; notably people with features of hypermobility manifest larger amygdala volume bilaterally compared to participants without any hypermobility (Eccles et al., 2012). Interestingly, the same study shows that the hypermobile participants scored higher on questionnaire ratings of body awareness (an index of interoceptive sensibility) than non-hypermobile people.

The aim of the present study was to clarify the mechanistic relationship between anxiety, interoceptive sensitivity and joint hypermobility. We combined the measurement of trait and state anxiety, interoceptive sensitivity (using established heartbeat detection methods) in participants from a non-clinical population, with and without hypermobility. Further, we recorded neural responses during emotional processing using functional magnetic resonance imaging (fMRI). We tested the hypothesis that interoceptive sensitivity and its underlying neural substrates meditate the relationship between affective reactivity and hypermobility.

MATERIALS AND METHODS

PARTICIPANTS

We recruited thirty-six healthy volunteers (16 male and 20 female; mean age ± SD 24.1 ± 6.5 years) who participated after informed consent and eligibility screening (where neurological or psychiatric disorders were excluded and hypermobility was considered by asking if they considered themselves flexible and if they were able to touch the floor with their hands without bending their knees). All participants underwent a structured clinical examination for hypermobility, undertook tests of interoceptive sensitivity and completed validated questionnaire measures of state anxiety and body awareness tendency. Twenty right-handed participants randomly selected from this sample also performed an emotional processing task during fMRI, lasting approximately 20 min that were divided into two functional runs of 10 min each. One participant was removed from the fMRI analyses due to non-compliance with experimental procedures and excessive movement in the scanner, resulting in 19 (9 male and 10 female) right handed participants. The study was approved by the Brighton and Sussex Medical School Research Governance and Ethics Committee RGEC. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two- sided test, 16 participants were necessary in each group to recognize as statistically significant a difference greater than or equal to 0.5 units in interoceptive sensitivity. The common standard deviation is assumed to be 0.5. For correlation analyses 37 participants were estimated necessary, with a correlation coefficient of 0.45.

MEASURES

Ratings of state anxiety were acquired using the Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970). Hypermobility symptoms were quantified using the Beighton clinical exploration of hypermobility (Beighton et al., 1989) which requires a physical examination that was conducted by a trained and experienced clinician (according to the basis of the clinical rheumatologists' standards, kappa inter-examiners reliability ranged from 0, 8 to 1). The Beighton scoring system consists of five items (describing nine movements), that explores the joint mobility range of 5 body areas: wrists/thumb, knees, spine/hips, paired elbows and fifth metacarpo-phalangeals. The highest score is nine and an accepted cut-off point is 4/5 (man/women). Interoceptive sensitivity was measured by objective means using adaptations of two different heartbeat detection tasks (mental tracking and heartbeat perception) (Schandry, 1981; Katkin et al., 2001). Participants' interoceptive sensibility (i.e., their score subjective questionnaire of body awareness) (Garfinkel and Critchley, 2013) was also inferred from answers on the Multidimensional Assessment of Interoceptive Awareness (MAIA) questionnaire (Mehling et al., 2012) which is composed of the following sub-scales: Noticing (awareness of uncomfortable, comfortable, and neutral body sensations), Not-Distracting (tendency not to ignore or distract oneself from sensations of pain or discomfort), Not-Worrying (tendency not to worry or experience emotional distress with sensations of pain or discomfort), Attention Regulation (ability to sustain and control attention to body sensations), Emotional Awareness (awareness of the connection between body sensations and emotional states), Self-Regulation (ability to regulate distress by attention to body sensations), Body Listening (active listening to the body for insight) and Trusting (experience of one's body as safe and trustworthy).

Heartbeat detection tasks. Interoceptive sensitivity was assessed using two tasks: these were modified versions of the heartbeat perception task (Katkin et al., 2001) and the mental tracking task (Schandry, 1981), run using in-house Matlab software (Mathworks Inc. Sherborn, M.A.). In the heartbeat perception task, each participant was asked to judge whether a tone was or was not synchronized with his/her heartbeat across 15 different blocks. Each block consisted of 10 tones presented at 440 Hz and having 100 ms duration, triggered by the participant's heartbeat. Presentation of the tones was timed to coincide with systole (i.e., the cardiac ejection period, when heartbeats are typically felt) or to occur later (i.e., delayed relative to the heartbeat). Tasks were run using a pulse-oximetry signal from the left index finger. Assuming an average delay of 250 ms between the R-wave and the arrival of the pulse wave (Payne et al., 2006), this task setup delivered tones at around 250 or 550 ms after the ECG R-wave,

corresponding respectively to the maximum and minimum synchronicity judgments reported in systematic studies of heartbeat detection (Wiens and Palmer, 2001). Mean score was calculated for the 15 blocks answers (1 point for being correctly detected, 0 for not being correctly detected). Resulting in a number ranging for 0 to 1, were 1 was the highest score. In the mental tracking task, the participant was asked to count silently each heartbeat felt (without manually checking) over cued intervals, i.e., from the time he/she heard "start" to when he/she heard "stop." This task procedure was repeated over six intervals, using time-windows of 25, 30, 35, 40, 45, and 50 s, presented in randomized order. Following each block of both tasks described, the participant responded with a judgment (synchronous/asynchronous or number of heartbeats). For each trial, an accuracy score was automatically computed, defined as; $1 - \frac{|nbeats_{\text{real}} - nbeats_{\text{reported}}|}{(nbeats_{\text{real}} + nbeats_{\text{reported}})/2}$ Resulting scores were subsequently averaged over the six presentations, yielding an average value for each participant. All data points and corresponding scores will be automatically calculated in a data file that is automatically generated.

FUNCTIONAL NEUROIMAGING PARADIGM

During fMRI acquisition, each participant was presented with images depicting neutral, angry or sad scenes projected on a screen in successive counterbalanced blocks. Images were derived from the International Affective Pictures System (IAPS) (Lang et al., 1993), supplemented with similar valence-matched images and categorized according to the specific characterized emotion (Mikels, 2005). Some example images in each category include anger (e.g., a soldier about to kill a child; a man trying to rape a woman; an angry person) and sadness (e.g., a person crying). Participants were trained on the paradigm before scanning. The functional imaging study was split into two different runs: each run started with a fixation cross that lasted 10 s. Images were blocked in fours by emotion-type and each image was displayed for 5 s, with 10 s separating each block. Each participant completed 40 randomized blocks, viewing 72 neutral pictures, 32 angry scenes, 32 anger eliciting and 32 sad pictures. During the task, the participant performed an incidental task, deciding whether pictures depicted animate or inanimate scenes.

IMAGE ACQUISITION

Neuroimaging data were acquired using a Siemens Avanto 1.5 Tesla MRI scanner (Siemens, Erlangen, Germany) with 32 channel headcoil and upgraded gradients. Functional imaging involved the acquisition of echo-planar datasets sensitive to BOLD (Blood Oxygen Level Dependent) contrast from axial slices (anterioposterior phase encode direction) tilted 30° from intercommissural plane to minimize T2* signal dropout from orbitofrontal and anterior temporal regions. Thirty-five 3 mm slices with a 0.75 mm interslice gap provided full brain coverage with an in-plane resolution of 3 × 3 mm (TE 42 ms, volume TR 2.620 ms). Following acquisition of the functional dataset, full brain T1-weighted structural scans were acquired from each participant (MPRAGE, 0.9 mm3 voxels, 192 slices, 1160/4.24 ms TR/TE, 300 ms inversion time, 230 × 230 mm2 FOV).

IMAGE PROCESSING

Images were pre-processed within SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) implemented in MATLAB 7.14 (Mathworks Inc. Sherborn, M.A.). The initial four functional volumes were discarded to allow for equilibration of net magnetization. Images were spatially realigned, unwrapped and spatially normalized to standard MNI space (Montreal Neurologic Institute) and movement regressors added. Normalized functional scans were smoothed with an 8 mm Gaussian smoothing kernel using Statistical Parametric Mapping (SPM8) software.

NEUROIMAGING ANALYSES

Within individual (first level) analyses, neural activity inferred from voxel-wise changes in BOLD response across conditions was assessed for each participant according to the general linear model. The regressors-of-interest were convolved with the canonical hemodynamic response function implemented in SPM8, and optimal parameter estimates were computed using a least squares function. The models at the first level were constructed coding for emotion type: angers scenes vs. neutral scenes, sad vs. neutral scenes (corresponding to an increased neural response for the sad or anger scenes, as compared to neutral scenes) was applied to estimate the effect size for each participant and generate the associated statistical parametric map. Second level analyses isolated brain activity pertaining to these emotional contrasts as a function of the variable of interest (hypermobility) by using functional voxel-wise *t*-test group comparisons for hypermobile group ($n = 9$; mean age ± SD 23,75 ± 3,19 years) vs. non hypermobile group ($n = 10$; mean age ± SD 25,27 ± 6,1 years). Correction for multiple comparisons was undertaken using the combination of voxel-wise and extent thresholds (Slotnick, 2004) 10,000 Monte Carlo simulations established the simultaneous requirement for a voxel level significance of $P < 0.001$ and activation clusters exceeding seven contiguous voxels for equivalency a family wise error correction of $P < 0.05$.

STATISTICAL ANALYSIS OF CLINICAL VARIABLES AND QUESTIONNAIRE MEASURES

Descriptive data (measures of data anxiety, joint hypermobility and interoception task performance) were analyzed using SPSS 20.0. Correlation tests were undertaken (Pearson correlation index and Point-Biserial according to the test application criteria). One-tailed analyses were performed based on positive associations established across all previous studies and literature, between state anxiety and joint hypermobility, and between state anxiety and interoception sensitivity (i.e., heart beat detection tasks). We also tested if the subgroup of individuals who underwent neuroimaging ($n = 19$) showed the same pattern of associations found on the whole sample ($n = 36$). Additionally, we conducted mediation analysis to infer causality regarding the relationship between joint hypermobility, interoceptive sensitivity and state anxiety. Specifically, we tested pathways linking joint hypermobility (as predictor) to state anxiety (as dependent variable) with interoceptive accuracy (as putative mediator). Mediation first requires that the predictor is significantly

and independently related to all mediators and to the dependent variable (Baron and Kenny, 1986). Joint hypermobility was therefore entered into a multiple regression model, along with interoceptive accuracy and state anxiety, to test for a mediating relationship.

RESULTS

CLINICAL VARIABLES AND QUESTIONNAIRE MEASURES

Across the thirty-six non-clinical volunteers (16 male and 20 female) who participated in the study (**Table 1**), there were no significant differences in anxiety or body awareness scores between male and female participants. According to standardized cut-off points (≥5 for women and ≥4 for men), fourteen (38.9%) of the sample participants had joint hypermobility. Neuroimaging was undertaken on a subset of nineteen (9 male and 10 female) participants who performed the emotional processing task during fMRI. Nine (42.1%) of this subsample met criteria for joint hypermobility. Hypermobile individuals scored significantly higher on measures of state anxiety than non-hypermobile individuals ($rpb = 0.318$, $p = 0.029$).

Across all participants, the MAIA attention regulation (i.e., ability to control the attention given to body sensations) and the trusting body sensations subscales, negatively correlated with state anxiety scores ($r = -0.370$, $p = 0.031$; $r = -0.340$, $p = 0.049$, respectively). These results were also extensive to the hypermobile group, where state anxiety scores negatively correlated with MAIA attention regulation ($r = -0.612$, $p = 0.02$) and with MAIA trusting body sensations subscales ($r = -0.503$, $p = 0.06$), although the observed tendency was not significant in this latter subscale. Among the non-hypermobile group, MAIA emotional awareness subscale (i.e., awareness of the connection between body sensations and emotional states) negatively correlated with state anxiety scores ($r = -0.481$, $p = 0.031$). No further associations were found between the body awareness subscales and state anxiety. No significant differences were found on the MAIA body awareness subscales when comparing hypermobile individuals to non-hypermobile.

INTEROCEPTION ACCURACY DATA

State anxiety positively correlated with a better performance in the mental tracking interoceptive sensitivity task ($r = 0.284$, $p = 0.046$). This association was particularly observed for those scoring in the higher range for state anxiety ($n = 9$, $rpb = 0.385$; $p = 0.010$) compared to those with lower state anxiety scores. Hypermobile individuals also showed a strong positive association with state anxiety scores ($rpb = 0.318$, $p = 0.029$) and a better performance in the mental tracking interoceptive sensitivity task ($rpb = 0.387$, $p = 0.020$) when compared to non-hypermobile individuals. No further associations were found between the heart beat detection tasks and joint hypermobility.

When exploring the relationship between joint hypermobility, state anxiety and interoceptive sensitivity (by means of heartbeat mental tracking task); interoception was observed to mediate the association between joint hypermobility and state anxiety (**Figure 1**).

FUNCTIONAL IMAGING DATA

We tested for neural substrates underlying the relationship between hypermobility and the expression of affective/anxiety symptoms by examining how regional brain responses to emotional (sad and anger vs. neutral) stimuli compared between participants with and without hypermobility. As in the larger group, hypermobile participants ($n = 9$) in the imaging subgroup ($n = 19$) showed significantly higher state anxiety scores ($rpb = 0.387$, $p = 0.050$) and a better performance in the mental tracking interoceptive sensitivity task ($rpb = 0.438$, $p = 0.030$) than non-hypermobile participants.

WHOLE BRAIN ANALYSIS

During the processing of sad vs. neutral imagery a discrete set of brain regions associated with emotion and anxiety manifested greater responses within the hypermobile group. These

Table 1 | Descriptive data for anxiety, body awareness and Joint Hypermobility measures ($n = 36$).

	Min	Max	Mean	*SD*	Joint hypermobility ($n = 14$)				Non-joint hypermobility ($n = 22$)			
					Min	Max	Mean	*SD*	Min	Max	Mean	*SD*
Age	20.00	42.00	24.83	5.04	21.00	30.00	24.07	3.03	20.00	42.00	24.18	8.00
Joint hypermobility* (0–9)	0.00	9.00	3.40	2.69	4.00	9.00	6.11	1.15	0.00	4.00	1.36	1.55
STAI-state (20–80)	20.00	54.00	33.86	10.72	20.00	54.00	38.07	12.83	20.00	49.00	31.18	8.38
MAIA noticing (0–5)	1.00	4.25	3.03	0.85	1.00	4.00	2.80	0.94	1.25	4.25	3.19	0.77
MAIA not-distracting (0–5)	0.00	3.67	2.04	0.86	0.33	3.67	2.31	0.96	0.00	3.00	1.85	0.76
MAIA not-worrying (0–5)	1.33	4.66	3.06	0.83	1.33	4.66	2.99	1.12	2.00	4.33	3.10	0.60
MAIA attention regulation (0–5)	0.71	5.00	3.02	1.05	1.57	4.71	2.93	0.88	0.71	5.00	3.03	1.18
MAIA emotional awareness (0–5)	1.60	4.80	3.09	0.83	1.60	4.80	3.17	0.86	1.60	4.40	3.04	0.83
MAIA self-regulation (0–5)	0.00	5.00	2.74	1.09	1.00	4.50	2.75	0.89	0.00	5.00	2.74	1.24
MAIA body listening (0–5)	0.00	4.00	1.77	1.02	0.60	3.67	1.78	0.87	0.00	4.00	1.76	1.14
MAIA trusting (0–5)	0.33	5.00	3.49	1.14	1.30	5.00	3.28	1.06	0.33	5.00	3.57	1.31

*STAI-State, state trait anxiety inventory-state subscale; MAIA, Multidimensional Assessment of Interoceptive Awareness; *Beighton Joint Hypermobility assessment.*

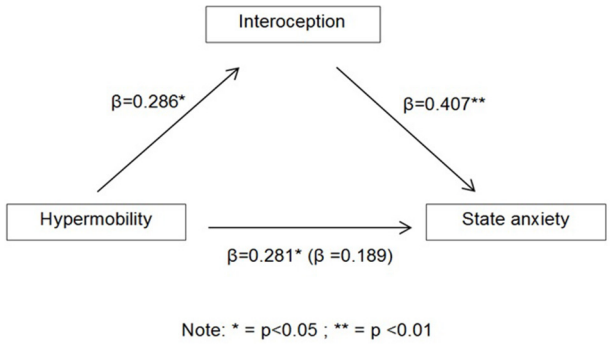


FIGURE 1 | Schematic showing the regression coefficients, with the coefficients (β) for the effect of hypermobility on state anxiety with the latter (when entering interoception into the model) in parentheses.

Table 2 | Activity seen in response to sad vs. neutral images when comparing hypermobility participants (high Beighton score) to non-hypermobility participants (low Beighton score) (cluster size >7; $p = 0.001$).

Brain area	Side	Cluster size	Coordinates*	*t*-Value
Insula	R	63	42 2 4	5.76
Insula	L	9	−40 6 −14	3.95
Rolandic operculum	R	118	64 8 14	5.34
Rolandic operculum	R	118	46 4 14	4.37
Rolandic operculum	R	63	46 −4 8	4.96
Frontal inferior operculum	R	118	52 10 14	3.95
Triangular part of frontal inferior gyrus	R	41	58 30 2	5.92
Brainstem		15	−16 −20 −26	4.67
Brainstem		7	6 −26 −18	4.24
Cerebellum (Crus1)	L	84	−46 −68 −26	4.89
Cerebellum (Crus1)	L	12	−20 −74 −30	3.86
Cerebellum		59	−24 44 −4	4.17
Cerebellum (8)	R	27	30 −42 46	4.32
Supra marginal gyrus	R	45	64 −26 30	5.04
Postcentral gyrus	R	19	60 −10 −30	3.91
Postcentral gyrus	R	16	58 −10 32	4.41
Precentral gyrus	L	9	−40 8 38	3.95
Middle temporal gyrus	R	19	64 −8 −22	3.82
Inferior temporal gyrus	L	21	−46 −6 −34	4.61
Inferior temporal gyrus	L	21	−54 −4 −32	3.86
Thalamus	L	7	−8 −26 12	4.37
Middle occipital gyrus	R	8	36 −64 34	4.09

MNI Coordinates.

included insular cortex, brainstem, parietal and sensorimotor cortices, inferolateral prefrontal cortex, temporal cortices and thalamus (**Table 2**, **Figure 2**). During the processing of anger vs. neutral images, a discrete set of brain regions also demonstrated enhanced activity within the hypermobile group including cerebellum, temporal cortices and thalamus (**Table 3**).

DISCUSSION

The present study investigated the relationship between anxiety, interoceptive sensitivity and joint hypermobility. Our findings link joint hypermobility to the presence of anxiety symptoms through the expression of enhanced interoceptive sensitivity. Results also display heightened reactivity of brain regions notably "interoceptive" insular cortex during the processing of emotional stimuli in joint hypermobility. Our findings highlight the dependence of emotional state on bodily context, and increase our understanding of the mechanisms through which vulnerability to anxiety disorders arises in people bearing a common heritable variant of collagen.

Our findings also suggest that maladaptive cognitions and appraisal tendencies toward body sensations may also be a determining factor: Not only did state anxiety correlate positively with objective measures of interoceptive sensitivity but in the questionnaire measure of body awareness, state anxiety correlated negatively with the attention regulation subscale (i.e., ability to control attention to body sensations) and the "trusting body sensations" subscale. These associations were amplified in individuals with greatest state anxiety. These observations are noteworthy; our results confirm a primary hypothesis that increased interoceptive sensitivity is associated with increased likelihood of anxiety [and increased vulnerability to developing anxiety disorders (Domschke et al., 2010)]. Yet there is, a growing interest with empirical support (Parkin et al., 2014) in how enhancing awareness of bodily processes, e.g., through mindfulness approaches, may be used therapeutically for managing anxiety. Our observations further highlight the relevance of "attributional models" wherein a capacity to control bodily changes is compromised in people with anxiety. Thus, the concurrence of increased emotional reactivity and enhanced perceptual sensitivity to physiological arousal, with a diminished confidence in their interpretation and control, is characteristic of individuals with high-state-anxiety (Paulus and Stein, 2006). Future studies may clarify whether a subset highly anxious individuals particularly benefit from psychotherapy approaches (e.g., mindfulness, CBT) that capitalize on heightened interoceptive sensitivity traits or related constitutional characteristics (including joint hypermobility), in terms of having a more adaptive and quick response to bodily signals.

Hypermobile participants experienced significantly higher state anxiety than the non-hypermobile participants. The association between joint hypermobility and the clinical expression of anxiety is now robustly established (Bulbena et al., 2011; Bianchi Sanches et al., 2012). In our non-clinical sample, hypermobile individuals demonstrated higher interoceptive accuracy when performing the heart beat detection task. Moreover, it was confirmed that interoceptive sensitivity particularly mediated the association between hypermobility and anxiety: this was suspected from an earlier observation of increased interoceptive sensitivity in individuals with hypermobile features (Eccles et al., 2012). One potential mechanism is autonomic: In people with joint hypermobility, there is variant collagen in both joints and vasculature. Many hypermobile people develop autonomic symptoms (e.g., racing heartbeat) related to problems with orthostatic vasoconstriction. In more severe cases this is expressed as

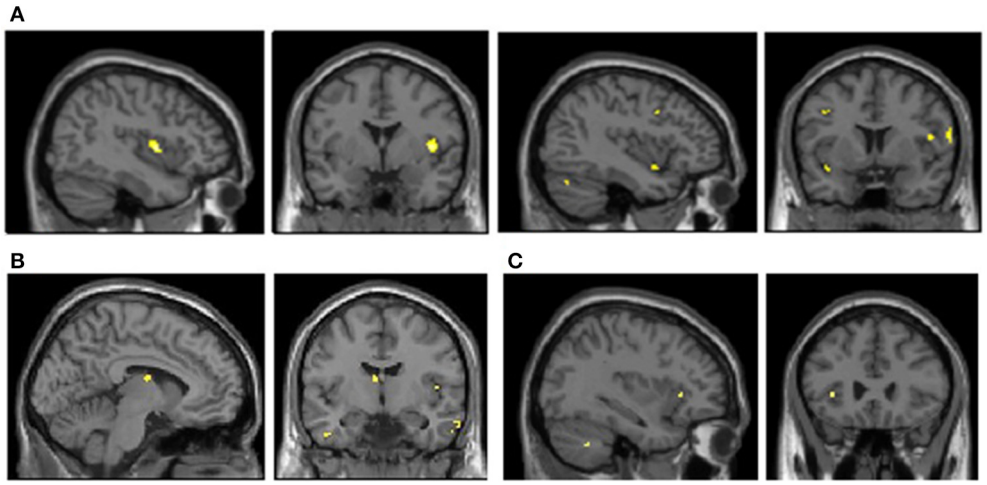


FIGURE 2 | Enhanced brain activation when presenting sad anger image paradigm in hypermobility compared to non-hypermobility (cluster size > 7; $p = 0.001$). (A) Right and left insula activayion in sad vs. neutral condition. **(B)** Left thalamus activation in sad vs. neutral condition. **(C)** Left insula activation in anger vs. neutral condition.

Table 3 | Activity seen in anger vs. neutral images when comparing hypermobility participants (high Beighton Score) to non-hypermobility participants (low Beighton score) (cluster size >7 $p = 0.001$).

Brain area	Side	Cluster size	Coordinates*			*t*-Value
Cerebellum (crus1)	L	49	−46	−60	−34	4.891736
Middle temporal gyrus	L	31	−66	−18	−20	4.686333
Inferior temporal gyrus	L		−54	−18	−26	4.406723
Middle temporal pole	L	14	36	2	−32	4.325048
Thalamus	R	15	10	−8	6	4.224494
Insula	L	8	−36	26	2	4.068141
Inferior parietal gyrus	L	8	−36	−54	−40	3.945567

**MNI coordinates.*

form of dysautonomia known as postural tachycardia syndrome (PoTS). In PoTS, heart rate significantly increases when standing upright to maintain blood pressure (Freeman et al., 2011). This uncontrolled increased cardiac response may result (through associative learning mechanisms) in increased interoceptive sensitivity (Pollatos et al., 2007a), which in turn may have emotional consequences. Nevertheless, this account emphasizes heart rate changes and it is known that both the interoceptive sensitivity in hypermobile individuals (and the dysautonomia in PoTS) extends beyond the cardiovascular system (Mathias et al., 2011).

With regards to the functional neuroimaging paradigm, hypermobile individuals manifest stronger neural reactivity to affective stimulation within brain regions known to be involved in emotional processing, particularly in anxiety (i.e., insula, brainstem, thalamus), when compared to non-hypermobile participants. Specifically, hypermobile participants presented higher activation to sad scenes in areas implicated in interoceptive representation, feeling states and self-representation (i.e., insular cortex and inferolateral prefrontal cortex) as well as in areas implicated in encoding socially salient visual information (i.e., temporal cortices) and executive control processes (i.e., inferolateral prefrontal cortex) (Adolphs, 2001; Critchley and Harrison, 2013). Hypermobile participants also revealed enhanced activity to anger scenes within insula, inferotemporal cortex and thalamus. In social interaction, insula is involved in emotional processing and empathy (Lamm and Singer, 2010) but it is fundamentally implicated in homeostasis; mapping and controlling autonomic functions and regulation of the sympathetic and parasympathetic systems (Gianaros et al., 2012; Critchley and Harrison, 2013). Furthermore, right anterior insula aids interoceptive awareness of body states, including as the ability to detect the timing of one's own heartbeat (Simmons et al., 2012). Thus, the enhanced activity within the insular cortex likely supports the association between hypermobility and interoceptive sensitivity (high accuracy in heartbeat detection) and, by extension, its association to anxiety. Our findings show a tendency to greater affective reactivity among hypermobile participants within emotion-related brain areas. Thus, hypermobile individuals do not only have greater interoceptive sensitivity but also higher emotional reactivity to visual stimuli with affective salience. Higher affective reactivity is described in people with anxiety disorders, particularly social anxiety disorder, where patients also display hyperreactivity within similar brain regions (Goldin et al., 2009).

The mechanisms underpinning the association between anxiety and hypermobility have not previously been explored in detail. Our results provide much needed insight to better understand the pathogenesis. Future studies will usefully replicate and extend these findings, with the aim of clarifying how high interoceptive accuracy and hypermobility might be exploited within psychotherapeutic approaches, for example to enhance more adaptive attitudes toward body signals.

Our study has some limitations, including the relatively small number of participants who underwent these detailed

assessments. Moreover, we infer associations with anxiety symptoms from data acquired in a non-clinical sample. Thus, the relevance to clinical anxiety populations is grounded on other literature, notably patient studies that also highlight associations between anxiety, interoceptive sensitivity and hypermobility. Future studies should nevertheless extend our findings to clinical patient groups. Lastly, while we used an accepted cut-off point of hypermobility among the scientific and clinical community, this still remains a point of discussion.

To conclude, we present the first functional laboratory and neuroimaging study of the relationship between anxiety and hypermobility that also examines interoceptive sensitivity (heartbeat detection). The interactions observed between anxiety, hypermobility and interoception, as well as the differences in the activity of particular emotional brain regions; provide an essential basis for future research constitutional factors underpinning anxiety disorders. Moreover, our findings have the potential to inform innovation in therapeutic approaches.

ACKNOWLEDGMENTS

Funding for this study came from the Brighton and Sussex Medical School and via the Dr. Mortimer and Theresa Sackler Foundation through the Sackler Centre for Consciousness Science. Many thanks to Nakulan Navaratnam and Emma Zorab for assistance with paradigm design and stimulus selection, and to Dr. Oscar Vilarroya for discussions. Hugo D. Critchley and Sarah N. Garfinkel are supported by an ERC Advanced grant to Hugo D. Critchley and through the Sackler Centre. Guillem Pailhez and Antonio Bulbena are supported by Fondo de Investigación Sanitaria ISCIII.

REFERENCES

Adolphs, R. (2001). The neurobiology of social cognition. *Curr. Opin. Neurobiol.* 11, 231–239. doi: 10.1016/S0959-4388(00)00202-6

Baron, R. M., and Kenny, D. A. (1986). The moderator-mediator variable distinction in social psychological research—conceptual, strategic, and statistical considerations. *J. Pers. Soc. Psychol.* 51, 1173–1182. doi: 10.1037/0022-3514.51.6.1173

Beighton, P., Grahame, R., and Bird, H. (1989). *Hypermobility of Joints, 2nd Edn*. Berlin: Springer Verlag.

Bianchi Sanches, S., De Lima Osório, F., Udina, M., Martin-Santos, R., and Crippa, J. A. (2012). Anxiety and joint hypermobility association: a systematic review. Rev. Bras. Psiquiatr. 34, 53–68. doi: 10.1590/S1516-44462012000500005

Bulbena, A., Duró, J. C., Mateo, A., Porta, M., and Vallejo, J. (1988). Joint hypermobility síndrome and anxiety disorders. *Lancet* 2, 694–670. doi: 10.1016/S0140-6736(88)90514-4

Bulbena, A., Gago, J., Pailhez, G., Sperry, L., Fullana, M., and Vilarroya, O. (2011). Joint hypermobility syndrome is a risk factor trait for anxiety disorders: a 15-year follow-up cohort study. *Gen. Hosp. Psychiatry* 33, 363–370. doi: 10.1016/j.genhosppsych.2011.03.004

Critchley, H., and Harrison, N. (2013). Visceral influences on brain and behavior. *Neuron* 77, 624–638. doi: 10.1016/j.neuron.2013.02.008

Damasio, A. R. (1994). *Descartes' Error: Emotion, Reason and the Human Brain*, New York, NY: Grosset/Putman.

Damsa, C., Kosel, M., and Moussally, J. (2009). Current status of brain imaging in anxiety disorders. *Curr. Opin. Psychiatry* 22, 96–110. doi: 10.1097/YCO.0b013e328319bd10

Domschke, K., Stevens, S., Pfleiderer, B., and Geralch, A.L. (2010). Interoceptive sensitivity in anxiety and anxiety disorders: an overview and integration of neurobiological findings. *Clin. Psychol. Rev.* 30, 1–11. doi: 10.1016/j.cpr.2009.08.008

Eccles, J. A., Beacher, F. D., Gray, M. A., Jones, C. L., Minati, L., Harrison, N. A., et al. (2012). Brain structure and joint hypermobility: relevance to the expression of psychiatric symptoms. *Br. J. Psychiatry* 200, 508–509. doi: 10.1192/bjp.bp.111.092460

Freeman, R., Wieling, W., Axelrod, F. B., Benditt, D. G., Be narroch, E., Biaggioni, I., et al. (2011). Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin. Auton. Res.* 21, 69–72. doi: 10.1007/s10286-011-0119-5

Garcia-Campayo, J., Asso, E., and Alda, M. (2011). Joint hypermobility and anxiety: the state of the art. *Curr. Psychiatry Rep.* 13, 18–25. doi: 10.1007/s11920-010-0164-0

Garfinkel, S. N., and Critchley, H. D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. Commentary on: "Anterior insular cortex mediates bodily sensibility and social anxiety" by Terasawa et al. (2012). *Soc. Cogn. Affect. Neurosci.* 8, 231–234. doi: 10.1093/scan/nss140

Gianaros, P. J., Onyewuenyi, I. C., Sheu, L. K., Christie, I., and Critchley, H. D. (2012). Brain systems for baroreflex suppression during stress in humans. *Hum. Brain Mapp.* 33, 1700–1716. doi: 10.1002/hbm.21315

Goldin, P. R., Manber, T., Hakimi, S., Canli, T., and Gross, J. J. (2009). Neural bases of social anxiety disorder: emotional reactivity and cognitive regulation during social and physical threat. *Arch. Gen. Psychiatry* 66, 170–180. doi: 10.1001/archgenpsychiatry.2008.525

Katkin, E., Wiens, S., and Ohman, A. (2001). Nonconsious fear condition, visceral perception, and the development of gut feelings. *Psychol. Sci.* 12, 366–370. doi: 10.1111/1467-9280.00368

Holzschneider, K., and Mulert, C. (2011). Neuroimaging in anxiety disorders. *Dialogues Clin. Neurosci.* 13, 453–461.

Lamm, C., and Singer, T. (2010). The role of anterior insular cortex in social emotions. *Brain Struct. Funct.* 214, 579–591. doi: 10.1007/s00429-010-0251-3

Lang, P. J., Greenwald, M. K., Bradley, M. M., and Hamm, A. O. (1993). Looking at pictures: affctive, facial, visceral, and behavioral reactions. *Psychophysiology* 30, 261–273. doi: 10.1111/j.1469-8986.1993.tb03352.x

Mathias, C. J., Low, D. A., Iodice, V., Owens, A. P., Kirbis, M., and Grahame, R. (2011). Postural tachycardia syndrome—current experience and concepts. *Nat. Rev. Neurol.* 8, 22–34. doi: 10.1038/nrneurol.2011.187

Mehling, W. E., Price, C., Daubenmier, J. J., Acree, M., Bartmess, E., and Stewart, A. (2012). The Multidimensional Assessment of Interoceptive Awareness (MAIA). *PLoS ONE* 7:e48230. doi: 10.1371/journal.pone.0048230

Mikels, J. A. (2005). Emotional category data on images from the International Affective Picture System. *Behav. Res. Methods*. 37, 626–630. doi: 10.3758/BF03192732

Mor, N., and Winquist, J. (2002). Self-focused attention and negative affect: a meta-analysis. *Psychol. Bull.* 128, 638–662. doi: 10.1037/0033-2909.128.4.638

Parkin, L., Morgan, R., Rosselli, A., Howard, M., Sheppard, A., Evans, D., et al. (2014). Exploring the relationship between mindfulness and cardiac perception. *Mindfulness* 5, 298–313. doi: 10.1007/s12671-012-0181-7

Paulus, M. P., and Stein, M. B. (2006). An insular view of anxiety. *Biol. Psychiatry* 60, 383–387. doi: 10.1016/j.biopsych.2006.03.042

Payne, R. A., Symeonides, C. N., Webb, D. J., and Maxwell, S. R. (2006). Pulse transit time measured from the ECG: an unreliable marker of beat- to-beat blood pressure. *J. Appl. Physiol.* 100, 136–141. doi: 10.1152/japplphysiol.00657.2005

Pollatos, O., Herbert, B. M., Matthias, E., and Schandry, R. (2007a). Heart rate response after emotional picture presentation is modulated by interoceptive awareness. *Int. J. Psychophysiol.* 63, 117–124. doi: 10.1016/j.ijpsycho.2006.09.003

Pollatos, O., Traut-Mattausch, E., Schroeder, H., and Schandry, R. (2007b). Interoceptive awareness mediates the relationship between anxiety and the intensity of unpleasant feelings. *J. Anxiety Disord.* 21, 931–943. doi: 10.1016/j.janxdis.2006.12.004

Ross, J., and Grahame, R. (2011). Easily mised? Joint hypermobility syndrome. *BMJ* 342:c7167. doi: 10.1136/bmj.c7167

Schandry, R. (1981). Heart beat perception and emotional experience. *Psychophysiology* 18, 483–488. doi: 10.1111/j.1469-8986.1981.tb02486.x

Shurick, A. A., and Gross, J. J. (2013). Emotional Reactivity and regulation in Panic Disorder: insights from a functional magnetic resonance imaging study of cognitive behavioral therapy. *Biol. Psychiatry* 73, 5–6. doi: 10.1016/j.biopsych.2012.10.008

Simmons, W. K., Avery, J. A., Barcalow, J. C., Bodurka, J., Drevets, W. C., and Bellgowan, P. (2012). Keeping the body in mind: insula functional organization and functional connectivity integrate interoceptive, exteroceptive, and emotional awareness. *Hum. Brain Mapp.* 34, 2944–2958. doi: 10.1002/hbm.22113

Slotnick, S. D. (2004). Visual memory and visual perception recruit common neural substrates. *Behav. Cogn. Neurosci. Rev.* 3, 207–221. doi: 10.1177/1534582304274070

Smith, T.O., Easton, V., Bacon, H., Jerman, E., Armon, K., Poland, F., et al. (2014). The relationship between benign joint hypermobility syndrome and psychological distress: a systematic review and meta-analysis. *Rheumatology (Oxford)* 53, 114–122. doi: 10.1093/rheumatology/ket317

Spielberger, C. D., Gorsuch, R. L., and Lushene, R. (1970). *STAI Manual for the State-Trait. Anxiety Inventory*. Palo alto, CA: Consuling Psychologist Press.

Wiens, S., Mezzacappa, E. S., and Katkin, E. S. (2000). Heartbeat detection and the experience of emotions. *Cogn. Emot.* 14, 417–427. doi: 10.1080/026999300378905

Wiens, S., and Palmer, S. N. (2001). Quadratic trend analysis and heartbeat detection. *Biol. Psychol.* 58, 159–175. doi: 10.1016/S0301-0511(01)00110-7

Conflict of Interest Statement: The research activity of Sarah N. Garfinkel and Hugo D. Critchley is supported by the Dr. Mortimer and Theresa Sackler Foundation through the Sackler Centre for Consciousness Science and by the European Research Council Advanced Grant CCFIB AG 234150 to Hugo D. Critchley. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 June 2014; accepted: 24 September 2014; published online: 14 October 2014.

*Citation: Mallorquí-Bagué N, Garfinkel SN, Engels M, Eccles JA, Pailhez G, Bulbena A and Critchley HD (2014) Neuroimaging and psychophysiological investigation of the link between anxiety, enhanced affective reactivity and interoception in people with joint hypermobility. Front. Psychol. **5**:1162. doi: 10.3389/fpsyg.2014.01162*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in Psychology

ORIGINAL RESEARCH
published: 01 May 2015
doi: 10.3389/fpsyg.2015.00503

Disentangling interoception: insights from focal strokes affecting the perception of external and internal milieus

Blas Couto[1,2,3†], *Federico Adolfi*[1†], *Lucas Sedeño*[1,2,3†], *Alejo Salles*[4], *Andrés Canales-Johnson*[2,5], *Pablo Alvarez-Abut*[1], *Indira Garcia-Cordero*[1], *Marcos Pietto*[1], *Tristan Bekinschtein*[6], *Mariano Sigman*[7], *Facundo Manes*[1,3,8] and *Agustin Ibanez*[1,2,3,8,9*]

[1] Laboratory of Experimental Psychology and Neuroscience, Institute of Cognitive Neurology, Favaloro University, Buenos Aires, Argentina, [2] UDP-INECO Foundation Core on Neuroscience, Diego Portales University, Santiago, Chile, [3] National Scientific and Technical Research Council, Buenos Aires, Argentina, [4] Physics Department, University of Buenos Aires, Buenos Aires, Argentina, [5] Medical Research Council-Cognition and Brain Sciences Unit, Cambridge, UK, [6] Department of Psychology, University of Cambridge, Cambridge, UK, [7] Laboratory of Neuroscience, Universidad Torcuato Di Tella, Buenos Aires, Argentina, [8] ARC Centre of Excellence in Cognition and its Disorders, Sidney, NSW, Australia, [9] Universidad Autónoma del Caribe, Barranquilla, Colombia

OPEN ACCESS

Edited by:
Andrew Kemp,
Universidade de São Paulo, Brazil

Reviewed by:
Jason Moser,
Michigan State University, USA
Hugo Critchley,
University of Sussex, UK

***Correspondence:**
Agustin Ibanez,
Laboratory of Experimental Psychology and Neuroscience, Institute of Cognitive Neurology and National Scientific and Technical Research Council, Pacheco de Melo 1860, Buenos Aires, Argentina
aibanez@ineco.org.ar

† *First Authors*

Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *28 December 2014*
Accepted: *08 April 2015*
Published: *01 May 2015*

Citation:
Couto B, Adolfi F, Sedeño L, Salles A, Canales-Johnson A, Alvarez-Abut P, Garcia-Cordero I, Pietto M, Bekinschtein T, Sigman M, Manes F and Ibanez A (2015) Disentangling interoception: insights from focal strokes affecting the perception of external and internal milieus. Front. Psychol. 6:503. doi: 10.3389/fpsyg.2015.00503

Interoception is the moment-to-moment sensing of the physiological condition of the body. The multimodal sources of interoception can be classified into two different streams of afferents: an internal pathway of signals arising from core structures (i.e., heart, blood vessels, and bronchi) and an external pathway of body-mapped sensations (i.e., chemosensation and pain) arising from peripersonal space. This study examines differential processing along these streams within the insular cortex (IC) and their subcortical tracts connecting frontotemporal networks. Two rare patients presenting focal lesions of the IC (insular lesion, IL) or its subcortical tracts (subcortical lesion, SL) were tested. Internally generated interoceptive streams were assessed through a heartbeat detection (HBD) task, while those externally triggered were tapped via taste, smell, and pain recognition tasks. A differential pattern was observed. The IC patient showed impaired internal signal processing while the SL patient exhibited external perception deficits. Such selective deficits remained even when comparing each patient with a group of healthy controls and a group of brain-damaged patients. These outcomes suggest the existence of distinguishable interoceptive streams. Results are discussed in relation with neuroanatomical substrates, involving a fronto-insulo-temporal network for interoceptive and cognitive contextual integration.

Keywords: interoception, interoceptive awareness, peripersonal space, lesion, stroke, interoceptive sensitivity, exteroception

Introduction

Interoception is the processing of the body's physiological condition (Craig, 2002), including varied multimodal signals sensed by internal baroreceptors and chemosensors, as well as by surface temperature receptors and nociceptors (Cameron, 2002; Craig, 2002; Garfinkel and Critchley,

Craig). The representation of the organism's internal state has been termed interoceptive awareness (Craig, 2009), as it drives goal-directed actions associated with homeostatic regulation (Craig, 2007). Converging neurobiological evidence points to the insular cortex (IC) as a critical hub underlying multimodal interoceptive integration (Saper, 1982; Critchley et al., 2004; Pollatos et al., 2007b; Kurth et al., 2010; Kelly et al., 2012; Farb et al., 2013; Simmons et al., 2013). Topographic and modality-specific signals are relayed by the posterior insula and integrated in the anterior insula, where they interact with information from other limbic and cortical areas (Craig, 2003b)—heartbeat and breathing rate signals being the core of internal information needed for survival.

The IC has been implicated in interoceptive processes, such as awareness of bodily sensations (Khalsa et al., 2009), and exteroceptive processes, such as perception of pain (Brooks et al., 2002; Gramsch et al., 2014), smell (Kurth et al., 2010), and taste (Gagnon et al., 2014; Iannilli et al., 2014; Parabucki and Netser, 2014; van den Bosch et al., 2014). The posterior and mid insular cortices (Kurth et al., 2010) are activated by these processes, especially interoceptive ones. Interoceptive, exteroceptive, and emotional domains overlap in the anterior insula (Kurth et al., 2010), suggesting an underlying commonality (Critchley et al., 2002). In fact, the insula has been proposed as a convergence point between internal and external milieus (Ibanez et al., 2010).

Though generated in the external environment, pain and chemical signals involve a certain degree of body-mapping. This entails cross-modal processing of peripersonal space (Andre et al., 2000; de Paepe et al., 2014; Senkowski et al., 2014), i.e., the immediate surroundings of our bodies (Rizzolatti et al., 1997), which are represented differently from extrapersonal space (Holmes and Spence, 2004). The capacity to encode and integrate information from peripersonal space is vital to behavior and social interactions (Kennedy et al., 2009; Herz, 2014), such as avoidance movements (Berlucchi and Aglioti, 1997; Graziano, 1999; Graziano et al., 2000) and complex behaviors contributing to survival (Greenspan et al., 1999; Verhagen et al., 2004; Farrell et al., 2006).

The anterior insula supports more abstract encoding of internal–external information which interacts with other processes, such as emotion (Paulus et al., 2003; Simmons et al., 2004, 2006). Thus, this structure may support the integration of interoceptive and exteroceptive signals and their contribution to emotional processing networks (Simmons et al., 2013). Such integrative mechanism may rely on smell, taste, and pain, all of which contribute to socio-emotional processes (Critchley and Harrison, 2013; Craig, 2014; van Stralen et al., 2014). This is well-supported by reports of insular activation during emotion and risk-related processing (Simmons et al., 2006) and by evidence highlighting the role of exteroceptive and body-mapped signals in the neural representation of the body and peripersonal space (Azanon and Soto-Faraco, 2008; Mazzola et al., 2009; Azanon et al., 2010). In sum, pain, taste, and smell information may be integrated by insular networks in a peripersonal-like fashion and then further processed by emotional awareness and social behavior mechanisms.

Hence, insular networks for body perception could presumably underlie sensing of (a) a core group of interoceptive sensations that are centered on internal viscera and blood composition; and (b) taste, smell, and pain sensations, which jointly trigger multimodal bodily sensations and interoceptive awareness. This study aims to test a model of multiple interoceptive signaling streams by disentangling the internal and external pathways of body awareness. We evaluated two patients, one with a focal lesion to the right insular cortex (IC), and another with a lesion to the right posterior putamen (including subcortical white matter connecting the posterior IC to the fronto-temporal nodes). The patients' performance in these perception domains was compared with that of healthy controls and other groups of brain-damaged patients.

External Perception

Following Sherrington's pioneering definition (1900), exteroception includes vision, audition, smell, taste, and touch. Interoception might involve signals related to at least three of these senses: smell, taste, and pain. Different IC regions were revealed as primary or secondary areas where these signals are initially processed and passed on for integration (Mufson and Mesulam, 1982; De Araujo et al., 2003). Since the IC constitutes a crucial hub for interoception (Verhagen, 2007; Craig, 2009), internal (visceral) and external (bodily) signals may be sub-served by hubs of the interoceptive network. In this regard, affective and motivational aspects inherent to thermal pain, taste, and olfaction (Greenspan et al., 1999; Wicker et al., 2003; Verhagen et al., 2004; Verhagen, 2007) differ from classical exteroceptive (e.g., visual, auditory) stimuli. The former depend more closely on bodily needs and correspond to primary evolutionary requirements. Accordingly, they are associated with emotional processes and their neural substrates have developed earlier in evolutionary time (Mesulam, 2000). In line with recent approaches that relate body feelings and visceral perception with embodied cognition (Herbert and Pollatos, 2012; Tajadura-Jimenez and Tsakiris, 2014), we propose that the external signals might also be considered as body-mapped signals of an interoceptive peripersonal space. In other words, taste, smell, and pain signals could be conceived as an extension of interoceptive processing to peripersonal space (Ferri et al., 2013).

In functional neuroanatomical terms, taste, smell, and pain sensations engage paralimbic (and mesocortical, including IC) areas and are transmitted through parallel pathways to cortical sites (Verhagen, 2007) involved in autonomic, emotional, and drive functions. This is supported by the functional topography of the IC (Mesulam and Mufson, 1982a,b; Mesulam, 2000) and its segregation into different functional and anatomical connectivity clusters (Kurth et al., 2010; Kelly et al., 2012). Moreover, chemosensation evolved alongside the hypothalamic structures that sense the internal milieu components pertinent to homeostasis (Mesulam, 2000). Taste and smell impairments have been observed in left IC lesions (Pritchard et al., 1999; Cereda et al., 2002), and taste stimuli were reported to activate the IC (Faurion et al., 1999; for a review see, Small et al., 1999). Additionally, heat pain sensation has been proposed as a body signal that motivates emotional behavior and contributes to

monitoring the body's physiological condition (Craig, 2002, 2014; Singer et al., 2009). Evidence for this function comes from IC lesion studies reporting pain symptoms (Cereda et al., 2002) and functional connectivity studies showing differential links between the IC and the affective/discriminative pain systems (Peltz et al., 2011). Such functional evidence indicates that taste, smell, and pain are closely related with the internal body signals fostered by IC networks. However, it remains unclear which qualities of taste and smell are simultaneously affected following an IC lesion. To date, no report has assessed these qualities in combination with heat pain thresholds in an evaluation of the body-related external signals.

Internal Interoception

A reliable measure of internal drive is cardiac interoception, which relies on different pathways conveyed to the insular, secondary somatosensory (S2), and anterior cingulate cortices (ACC). The self-heartbeat detection (HBD) is a valid method to quantitatively measure cardiac interoception (Craig, 2003a; Critchley et al., 2004). Functional evidence from electrophysiological studies (Pollatos et al., 2005), intracortical recordings in monkeys (Caruana et al., 2011), and functional magnetic resonance imaging (fMRI) in humans (Dosenbach et al., 2007; Seeley et al., 2007; Seeley, 2008; Sridharan et al., 2008; Taylor et al., 2009; Menon and Uddin, 2010; Deshpande et al., 2011; Kelly et al., 2012) has revealed the involvement of the IC in heartbeat sensitivity. Most studies have successfully used HBD tasks as behavioral measures of cardiac interoceptive sensitivity (Schandry, 1981). Thus, HBD assessment triggers the internally driven interoceptive signals. Interoception has been proposed to encompassed multiple dimensions (Garfinkel and Critchley, 2013) including: (i) interoceptive sensitivity (IS)—the objective detection of visceral sensations, via tasks such as HBD—, and (ii) metacognitive interoception (MI)—reflexive beliefs and thoughts about one's own body sensations. MI and IS represent different interoceptive processes (Garfinkel and Critchley, 2013) they are not necessarily associated (Antony et al., 1995; Zoellner and Craske, 1999) and it is the former the one to which we refer with the present results.

Disentangling the Internal and External Sources of Interoception

Given their similarities in functionality and gross neuroanatomical location within the IC, internal and external body perception can be functionally related. Here we aim to disentangle external (taste, smell, and pain) and internal (cardiac) body perception signals arriving to the IC by evaluating two rare patients with focal lesions of the (a) right IC and (b) right posterior IC connections to the fronto-temporal nodes. These patients—already evaluated by Couto et al. (2013c) regarding social cognition—were assessed for smell, taste, thermal-pain sensation, and cardiac interoception. Note that lesion studies, as a tool for inferring brain function, are powered by the use of a second control group including patients with damage to areas not implicated in the function of interest (Rorden and Karnath, 2004). Hence, we compared our IL and SL patients with both healthy subjects and non-insular brain-damaged patients. Note that the definition of interoception presently adopted is based on the one posited by Craig (2002). Current neurophysiological and neuroscientific research has not yet enabled a definite consensus on the classification and precise borders of this concept. Indeed, interoception remains as one of the open fields at the frontiers of the neuroscience.

Materials and Methods

Participants

Insular Lesion (IL) Patient

G.G. is a 51-year-old right-handed woman who suffered an ischemic IC stroke 18 months before the evaluation. Her initial symptoms were dysarthria, left hand hemiparesia, and left hemianesthesia. This symptomatology was transient and disappeared 3 days after the onset of the stroke, with no residual signs at neurological examination, despite complaints of a subjective change in taste perception and occasional mild pain in her left arm. Structural magnetic resonance imaging (MRI) of the brain, scanned between 6 and 12 months after the stroke, showed an ischemic focal lesion comprising the complete right anterior, mid, and posterior IC as well as the internal portion of the posterior part of the frontal opercula (fronto-opercular/insular) (Sridharan et al., 2008; Menon and Uddin, 2010; Cauda et al., 2011), with no impairment of the adjacent subcortical structures, as demonstrated by Couto et al. (2013c) (**Figures 1A,C,D**). Both patients completed general neuropsychological tests (measures

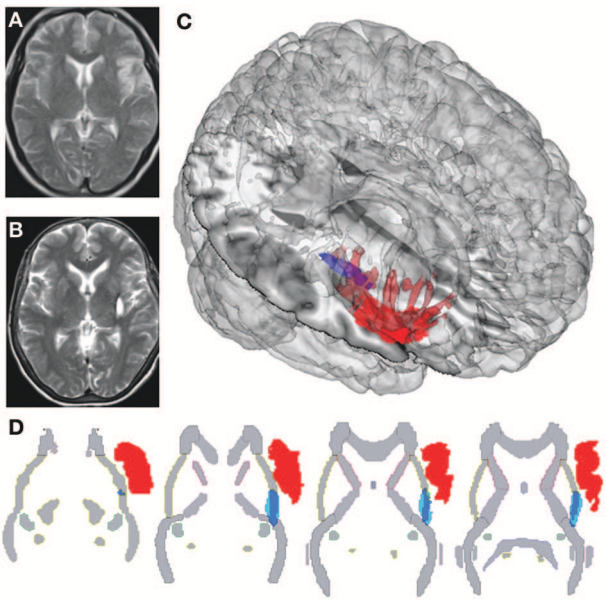


FIGURE 1 | Different plots of IL and SL brain damage localization. (A) Structural MRI of IL, with sequence T2 showing the right insular cortex lesion. **(B)** Structural MRI of SL, with sequence T2 showing damage to the right posterior putamen, capsula extrema, *claustrum*. **(C)** Three-dimensional rendering of lesion-traced MNI-normalized brain lesions of IL and SL plotted onto a standard T1 glass brain with the Mango software. **(D)** Overlap of lesion-traced MNI-normalized brain lesions of IL and SL and the JHU-Atlas of white matter, showing differential affectation of external capsule in both lesions.

TABLE 1 | Demographic and neuropsychological assessment.

(A) HEALTHY CONTROL GROUP													
	IL						**SL**						**Healthy controls**
		t		***p***		***Z-cc***		***t***		***p***		***Z-cc***	
SOCIODEMOGRAPHIC DATA													
Age	51	−1.21		0.14		−1.291	59	0.02		0.49		0.02	*M* = 58.86; *SD* = 6.09 (51–70)
Formal education #	17	0.32		0.38		0.347	7*	−3.45		0.01*		−3.69	*M* = 16.14; *SD* = 2.48 (12–18)
		t	***P***	***Z-cc***	***P***	***Z-ccc***		***t***	***p***	***Z-cc***	***P***	***Z-ccc***	
IFS													
Total score	26/30	0.66	0.27	0.70	0.02*	3.75	29/30*	4.61	<0.01*	4.93	0.02*	5.24	*M* = 25.50; *SD* = 0.71 (25–27)
AFFECTIVE SCREENING													
Depression (BDI)	3	−0.99	0.18	−1.06			24	2.74	0.02*	2.93			*M* = 8.57; *SD* = 5.26 (3–19)
Anxiety state (STAI-S)	21	−2.07	0.04*	−2.21	0.07	−2.21	28	−0.58	0.29	−0.62	0.36	−0.64	*M* = 30.71; *SD* = 4.39 (26–39)
Anxiety trait (STAI-T)	28	−1.12	0.15	−1.19	0.34	−0.55	55	1.43	0.10	1.52	0.13	−2.27	*M* = 39.86; *SD* = 9.94 (27–59)
(B) STROKE CONTROL GROUP													
	IL						**SL**						**Frontal damage**
		t		***p***		***Z-cc***		***t***		***p***		***Z-cc***	
SOCIODEMOGRAPHIC DATA													
Age	51	−0.16		0.44		−0.17	59	0.16		0.44		0.17	*M* = 55; *SD* = 23.05 (23–78)
Formal Education #	17	1.00		0.19		1.09	7	−1.49		0.10		−1.64	*M* = 13; *SD* = 3.67 (7–16)
IFS													
		t	***p***	***Z-ccc***	***p***	***Z-ccc***		***t***	***p***	***Z-ccc***	***p***	***Z-ccc***	
Total score	26/30	0.96	0.20	1.05	0.13	2.06	29/30	1.76	0.08	1.93	0.17	1.59	*M* = 22.40; *SD* = 3.42 (19–27)
AFFECTIVE SCREENING													
Depression (BDI)	3	−1.16	0.16	−1.27			24	1.00	0.19	1.10			*M* = 14.25; *SD* = 8.87 (5–25)
Anxiety state (STAI-S)	21	−1.86	0.07	−2.04	0.13	−2.05	28	−0.59	0.29	−0.65	0.34	−0.64	*M* = 31.25; *SD* = 5.02 (25–39)
Anxiety trait (STAI-T)	28	−1.17	0.15	−1.28	0.43	−0.27	55	1.76	0.08	1.93	0.09	2.52	*M* = 38.75; *SD* = 8.41 (31–52)

M, mean; SD, standard deviation, range in parentheses.
 # In years.
** Significantly different to controls.*

of cognitive screening, ACE-R; executive functions, IFS; and intelligence, WAT), as previously reported (see **Table 1** and Supplementary material in Couto et al., 2013c).

Subcortical Lesion (SL) Patient

N.F. is a 59-year-old, right-handed woman who presented with a stroke that had occurred 12 months before the evaluation. Her initial symptoms consisted of left-sided hemiparesia and hemianesthesia, both of which remained for 4 months and then disappeared. At the time of evaluation, she presented with no neurological deficits and complained only about some pain in her left arm, leg, and foot. Brain MRIs, scanned between 6 and 12 months after the stroke, showed a right subcortical hemorrhage. Once normalized to an MNI (Montreal Neurology Institute) standardized brain atlas, the lesion demonstrated engagement of the right putamen and claustrum and the white matter belonging to the external capsule. An additional overlap with the JHU-Atlas of white matter showed damage to the external capsule (Couto et al., 2013c) (**Figures 1B,C,D**).

Control Samples

Seven right-handed women with no history of neurological or psychiatric conditions were evaluated as controls (**Table 1A**). A second control group consisted of five patients presenting brain lesions in the frontal lobe and postcentral gyrus (see Figure S1 and **Table 1B**). Their demographic data were statistically controlled (see the socio-demographic and neuropsychological results below) in both controls groups.

All the participants signed an informed consent before the evaluation. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional ethics committee.

Assessment

The neuropsychological and clinical evaluations of the patients and healthy controls (including assessment of executive functions, depression, and anxiety) have been described by Couto et al. (2013c). They are briefly recapped in the Results and Supplementary Data sections. The patients' assessments included tasks and measures of olfaction, taste, thermal pain, and cardiac interoception (see below). The subjects were asked to refrain from smoking, eating or drinking anything other than water for 1 h prior to testing.

External Signals of Interoception

Smell testing

To establish odor sensitivity thresholds, we used eight solutions at increasing concentrations of phenyl ethyl alcohol in a staircase procedure based on the design of the commercial Sniffin' Sticks (©2014 US Neurologicals, Poulsbo, Washington, USA; Hummel et al., 1997). Odor identification skills were assessed through the commercial test of olfactory function Brief Smell Identification Test (Doty et al., 1984), consisting of 12 stimuli with a forced-choice answer. Finally, threshold and identification means were used to create a global score variable representing overall smell performance. Single *t*-tests between each patient and each control group were calculated using these variables.

Smell threshold

Individual odor sensitivity was assessed by acquiring thresholds for phenyl ethyl alcohol with an ascending double-forced choice staircase procedure. We used an eight-step geometric series, starting from a 4% phenyl ethyl alcohol solution (dilution ratio 1:2 in deionized water). Each subject was presented for 3 s at a distance of 3 mm from each nostril with two bottles in a randomized order: one contained only the deionized water, and the other contained the odorant at a certain dilution. While blindfolded, the subjects were asked to identify the odor-containing bottle. The threshold was defined as the trial in which the participant correctly identified five consecutive stimuli (Hummel et al., 1997) and this number was later transformed to percentage of intensity of perceived smell.

Smell identification

Odor identification abilities were further evaluated through the B-SIT (B-SIT, Sensonics Inc.). This test consisted of 12 stimuli, each presented for 3 s at 3 mm from each nostril. Each participant selected which odor was perceived from a forced-choice list with four options. The smell identification score was measured as the number of correct choices, ranging from 0 to 12, with higher scores indicating better identification. The 12 odors commonly used in commercially available tests were smoke, chocolate, onion, strawberry, gasoline, turpentine, banana, pineapple, cinnamon, soap, lemon, and rose. The number of correct responses was later transformed into an identification percentage.

Taste testing

Taste intensity

To evaluate taste intensity perception, each participant was given five sapid stimuli at four increasing concentrations: sucrose (0.03, 0.1, 0.3, 1.0 M), sodium chloride (NaCl; 0.03, 0.1, 0.3, 1.0 M), citric acid (0.001, 0.003, 0.01, 0.032 M), quinine hydrochloride (QHC1; 0.00003, 0.0001, 0.0003, 0.001 M), and monosodium glutamate (Glut; 0.006, 0.02, 0.06, 1.8 M). Each stimulus was dissolved in distilled water and presented at room temperature as part of an ascending concentration series (Bartoshuk et al., 1985). With the subject's tongue extended and stabilized between the lips, each stimulus was applied to both sides of the anterior tongue using a sterile, cotton-tipped applicator. Participants used a number line (range = 0–10) to report the intensity of the stimulus before retracting their tongue. Subjects were told that the first stimulus of each concentration series, distilled water, rated zero on the taste intensity scale. The output score was intensity feeling (from 1 to 50), which was later transformed to percentage of intensity of perceived taste.

Taste identification

To measure the subjects' ability to identify five basic tastants, the maximum concentrated stimuli from the previous task (0.3 M sucrose, 0.3 M NaCl, 0.01 M citric acid, 0.0003 M QHC1, and 1.8 M Glut) or distilled water was applied to the tongue using the same procedure described above. Each side of the tongue was tested two times for the five tastants. Participants indicated the perceived flavor by pointing to a labeled card in a six-option forced choice: salty, sweet, sour, bitter, umami, or non-flavor. This test was conducted twice for each stimulus following procedures described elsewhere (Pritchard et al., 1999). The output score was correct responses from 1 to 10, which was transformed into percentage of smell identification.

Finally, taste intensity and identification measures were used to create a global score variable representing overall taste performance. Single *t*-tests between each patient and each control group were calculated using these variables.

Thermal testing

Using a Peltier-driven thermo test device (probe size 3 × 3 cm; TSA-II NeuroSensory Analyzer, Medoc Advanced Medical Systems, Rimat Yishai, Israel), we assessed the subjects' threshold for detecting innocuous warmth and innocuous cold, as well as pain thresholds for noxious heat and noxious cold. The Peltier probe was fixed with a rubber band over the skin of the thenar region of each palm and the dorsomedial region of each foot. Temperature stimuli were applied with a slope of 1°C/s, following the method of limits previously described (Yarnitsky and Sprecher, 1994), in which the temperature detection thresholds and pain thresholds were determined as the average of four and three successive stimuli, respectively. The participant stopped these stimuli by pressing a button, with automatic safety limit temperatures of 0°C for the cool/cold and 50°C for the warmth/heat tasks, respectively. The resulting mean stimulation temperatures of the distinct conditions were 37.06 ± 1.52°C for innocuous warm, 23.96 ± 2.60°C for innocuous cold, 43.58 ± 1.93°C for noxious heat, and 11.60 ± 3.09°C for noxious cold. Finally, general scores were calculated for cool sensation, warm sensation, heat pain, and cold pain by averaging outputs of the four limbs. Moreover, we created three global score variables representing: (i) whole pain (the average of heat and cold scores); (ii) thermal sensation (the average of warm and cool scores);

and (iii) general thermal-pain sensation score, (the average of the previous two). Single *t*-tests between each patient and each control group were calculated using these variables.

Internal Stream of Interoception

Interoceptive measures

Heartbeat detection task

Two different HBD tasks have been used in the literature: (i) mental tracking paradigms, currently questioned because the working memory load of the task might affect cardiac perception; (Richards and Lorraine, 1996) and (ii) discrimination tasks, where an interference generated by attending simultaneously to cardiac sensation and external stimuli would constitute a confounding factor. We conducted a behavioral HBD task (Couto et al., 2013b; Melloni et al., 2013; Sedeno et al., 2014), in which the participants tracked their own heartbeats by pressing a key under different conditions. Compared with classical HBD tasks, this measure is more sensitive than traditional interoceptive paradigms given that it provides (i) a one-to-one EKG and motor response fitting and (ii) correct and incorrect response measures. Here we report the two most relevant measures. First, as a motor control condition, each patient was instructed to follow an audio recording of a sampled heartbeat. Next, in another block, they were asked to follow their own heartbeat with no external stimulation or feedback (interoceptive condition). To track the synchronization of the responses with the actual heartbeat, the EKG signal was recorded with an *ad-hoc* circuit composed of an AD620 amplifier and a band-pass filter (low 0.05 Hz, high 40 Hz) and then fed as an analog signal to a laptop computer's audio-card. Three Ag/Ag-Cl adhesive electrodes were placed on every participant in lead II positions, together with headphones for audio stimulus delivery. The signal was processed online with a PsychToolbox script, running on the Matlab platform (MathWorks). The two conditions offered (1) a control measure of audio-motoric performance (first condition) and (2) a cardiac interoceptive measure (second condition). Full instructions and data for the HBD task's validation and reliability have been detailed in previously published work from our group (Couto et al., 2013b; Melloni et al., 2013; Sedeno et al., 2014). The results were reported with an interoceptive accuracy index, which is calculated as follows:

$$\frac{\left(\text{Mean } |\text{RT}| - n \text{ of Incorrect Taps}\right)}{n \text{ of Total Taps}}$$

The continuous EKG signal was scanned by an *ad-hoc* matlab script which classified correct taps to those which were time-locked to the current heartbeat considering a fixed time window, which depended on the heart-rate of the participant (average -200 and +600 ms); RT (reaction times) were calculated within this time window with respect to the heartbeat, and their absolute values were used; *n* of Taps is the total amount of taps made by the participant during the whole 2-min-long experimental block. This interoceptive score can vary between 0 and 1, with lower scores indicating better interoceptive performance. Heart rate was also calculated, and included as a covariate in the analysis of interoception differences.

The tapping–tracking design used in this study avoids the cognitive overload of complex processes (such as attentional and working memory demands) involved in mental tracking and discrimination paradigms. For instance, the former imposes this burden as subjects must internally count numbers to keep track of heartbeats (Schandry et al., 1986). In the discrimination paradigms (Whitehead et al., 1977; Critchley et al., 2004), participants have to split their attention between their own heartbeats and an external train of stimuli to judge their synchronicity, which results in an interference affecting performance on the HBD task (Richards and Lorraine, 1996). By circumventing these cognitive demands, our methodology offers a more accurate measure of the ability to follow heartbeats sensations. Second, our method records each subject's answers and allows us to separate those synchronized with heartbeats from those not enabling us to calculate the mean reaction time (RT) and use it to calculate the accuracy index that reflects a participants' performance based on the ratio between correct RT and the total amount of heartbeats recorded.

Procedure

Two expert vascular neurologists (LS and PR) evaluated the patients via a neurological examination. Two other experts in clinical neuroimaging (FM and BC) analyzed the patients' MRI lesion data. Subsequently, the subjects were compared with the both control groups (**Tables 1A,B**) regarding age, gender, affective state (see Couto et al., 2013c), executive functions (through the INECO Frontal Screening battery Torralva et al., 2009), anxiety trait/state (see Spielberger et al., 1970), and mood state (Beck's Depression Scale (Beck et al., 1996). In addition, we used both sub-domains of sensory tests and additional global scores for the analyses. Note that clinical populations evince threshold changes in their response to heat and cold pain (Valmunen et al., 2009; Averbeck et al., 2013; Ahmad et al., 2014) and in their sensitivity to smell and taste (Mattes et al., 1995; Grossmann et al., 2005; Iranzo et al., 2013). On the assumption that heat/cold pain perception and smell/taste identification are substantially different processes, we analyzed them additionally to the global scores for each sensation. In addition, we described cardiac interoceptive performance by analyzing the HBD scores of a motor control condition and an interoceptive condition. These measures constitute the gold standard to describe interoceptive effects (Pollatos et al., 2009; Dunn et al., 2010; Elsenbruch et al., 2010; Kirk et al., 2011; Ferri et al., 2013).

Data Analysis

Behavioral Data Analysis

To compare the patients' performance with that of the control samples, we used a modified one-tailed *t*-test (Crawford and Howell, 1998; Crawford and Garthwaite, 2002, 2012; Crawford et al., 2009, 2011). This methodology allows an assessment of significance by comparing multiple individuals' test scores with norms derived from small samples (∼5 control subjects). This modified test is more robust for non-normal distributions. It effectively controls for Type I errors and proves robust in comparison with other methods (Crawford and Garthwaite, 2012). Additionally, it has been used in several

neuropsychological studies (Carlesimo et al., 2007; Hulleman and Humphreys, 2007; Garrido et al., 2009; Kennedy et al., 2009; Straube et al., 2010) to compare varied measurements of a single case with those of a control sample. We also performed inferences for significance of single case results using the BTD-Cov software (Crawford et al., 2011), including depression symptoms (BDI score) as a covariate. Furthermore, we used this same procedure to covariate out the heart rate measure from the interoceptive score. Because we are reporting case studies, only values with $p < 0.05$ were considered statistically significant in all comparisons (i.e., trends were not considered as significant differences). The effect sizes obtained using the same methods are reported as point estimates (z_{ccc} as effect size for the modified t-test with covariate analysis), as suggested by a previous study (Crawford et al., 2010). Therefore, the results are presented for a simple analysis (no covariates) and followed by the effect size and p-values for the BTD-Cov.

Physiological Data Analysis

Heartbeat detection task

EKG data were analyzed using *ad-hoc* scripts that included the following steps for each condition in each subject: (1) extracting heartbeat peaks (HB) from the EKG signal using the peakfinder function (Yoder, 2009); (2) tracking and assigning each EKG peak to the relevant keyboard tap (KT) of each participant using a time window that was dependent on heart rate (HR < 69, window = 650 ms; HR < 85, window = 700 ms; HR < 99, window = 750 ms); and (3) calculating the HB-KT, RT, and measures of accuracy from the assignments, as described elsewhere (Couto et al., 2013b; Melloni et al., 2013; Sedeno et al., 2014).

Results

Neither the IL nor the SL patients showed general cognitive impairments including the frontal lobe and executive functions (the SL patient even outperformed the healthy controls; see **Tables 1A,B**, as well as Supplementary Material). While their age and mood state were similar to those of controls from both groups (except for the IL patient, who scored lower for anxiety), the SL patient showed higher depressive symptoms score (BDI) (**Table 1A** and Supplementary Material). Hence, we assessed every single experimental measure entering BDI score as a covariate. Also, in our interoceptive score analyses, we introduced heart rate as a covariate (see Behavioral Data Analysis above). Note that the brain damaged control group did not show deficits relative to the healthy control group in overall variables.

Assessment of the External Stimuli

Smell Testing

The IL patient did not differ significantly from controls (**Figure 2A**) in smell thresholdsor smell (see Figure S2C). The SL patient did not differ significantly from controls in smell thresholds (see **Table 2** and Figure S2C) but showed significantly lower smell identification skills ($t = -5.37$, $p < 0.01$, $p\text{cov} < 0.01$, $Z_{ccc} = -5.54$). Global smell scores were lower for the IL patient than for healthy controls, but such a difference did not stand after covariation ($t = -2$; $p = 0.05$; $p\text{cov} = 0.1$; $Z_{cc} = -2.24$). The SL patient also showed significantly lower global smell scores, but her impairment remained even after covariation ($t = -6.21$; $p < 0.01$; $p\text{cov} < 0.01$; $Z_{cc} = -6.71$, see **Table 4**).

When compared with frontal stroke patients, the IL patient showed no differences in either smell threshold or identification. Relative to the same group, the SL patient showed no difference in smell threshold. However, she did evidence impaired smell identification ($t = -4.57$; $p = 0.01$; $p\text{cov} = 0.02$; $Z_{cc} = -5$, see **Table 3** and **Figure 2A**). In terms of global smell scores, the IL patient showed no significant differences while the SL patient showed significant impairment both with and without covariation ($t = -2.08$; $p = 0.05$; $p\text{cov} = 0.05$; $Z_{cc} = -2.28$, see **Table 5**).

In sum, as hypothesized at the outset, the IL patient showed no smell impairments relative to frontal damage patients, but she exhibited lower smell performance than healthy controls (this difference, however, disappeared after covariation). Instead, the SL patient was outperformed in smell tasks by both groups. This was the case before and after covariation, a result that also supports our hypothesis.

Taste Testing

Relative to healthy controls, the IL patient (**Figure 2B**) showed no impairments in taste sensitivity or recognition (see also Figure S2C). Compared with the same group, the SL patient showed no differences for intensity and a lower taste identification after covariation ($t = -1.60$; $p = 0.09$; $p\text{cov} = 0.01$; $Zcc = -1.73$). In

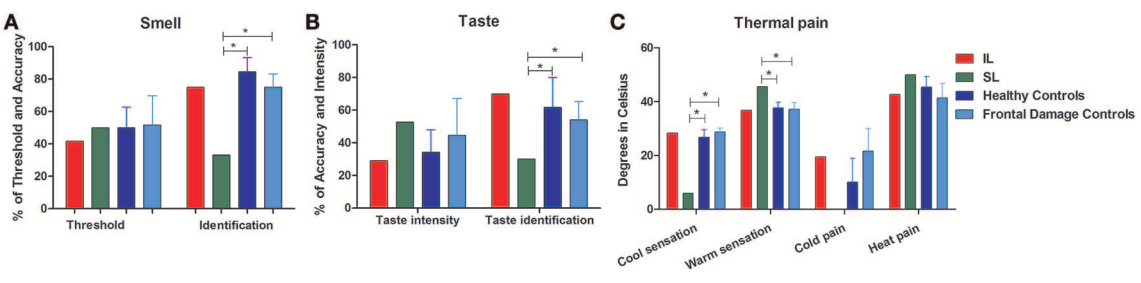


FIGURE 2 | External stream: smell, taste, and pain. The figure shows the results of tasks evaluating the external stream of interoception, demonstrating SL patient's impairment and IL patient's normal performance across measures. **(A)** Smell (threshold and identification). **(B)** Taste (intensity and identification). **(C)** Thermal pain (cold perception, warm perception, cold pain, heat pain). *Indicates statistically significant difference.

TABLE 2 | Results of comparing patient IL and SL with healthy age-matched controls.

	Patient IL						Patient SL						Healthy controls
	Score	*t*	*p*	*p*cov	*Z-cc*	*Z-ccc*	*Score*	*T*	*p*	*p*cov	*Z-cc*	*Z-ccc*	
BDI	3	−0.99	0.18		−1.06		24	2.74	**0.02***		2.93		*M* = 8.57; *SD* = 5.26 (3–19)
Smell threshold	41.6	−0.61	0.28	0.40	−0.65	−0.34	50	0.00	0.50	0.30	0.00	−0.96	*M* = 50; *SD* = 12.73 (42–75)
Smell identification	75	−1.00	0.18	0.20	−1.07	−1.15	33.3	−5.37	**<0.01***	**0.01***	−5.75	−5.54	*M* = 84.52; *SD* = 8.91 (67–92)
Taste intensity	29	−0.36	0.37	0.43	−0.39	0.25	52.7	1.25	0.13	0.40	1.35	−0.49	*M* = 34.33; *SD* = 13.68 (20–53)
Taste identification	70	0.42	0.35	0.09	0.45	2.39	30	−1.60	***0.09***	**0.01***	−1.73	−7.75	*M* = 61.67; *SD* = 18.35 (40–90)
Cool sensation	28.2	0.50	0.32	0.30	0.54	0.71	5.92	−6.70	**<0.01***	**<0.01***	−7.16	−7.72	*M* = 26.69; *SD* = 2.90 (21–29)
Warm sensation	36.7	−0.42	0.34	0.36	−0.45	−0.47	45.5	3.55	**0.01**	**0.04***	3.79	3.86	*M* = 37.66; *SD* = 2.09 (35–41)
Cold pain	19.4	1.00	0.18	0.20	1.07	1.19	0	−1.07	0.16	0.22	−1.14	−1.47	*M* = 10.06; *SD* = 8.79 (1–23)
Heat pain	42.6	−0.64	0.27	0.32	−0.69	−0.63	50	1.12	0.15	0.29	1.19	1.03	*M* = 45.36; *SD* = 3.89 (40–50)
M-score	0.05	−0.30	0.39	0.26	−0.33	−1.01	0.03	−0.70	0.26	0.38	−0.77	0.65	*M* = 0.06; *SD* = 0.03 (0.03–0.11)
Interoception	0.5	5.63	**<0.01***	**0.01***	6.17	5.71	0.09	−0.61	0.29	0.43	−0.67	−0.34	*M* = 0.13; *SD* = 0.06 (0.05–0.22)

BOLD font, significant results at p level <0.05; Italic Bold font, trends to significance; BDI, Beck Depression Scale; M, motor condition of heartbeat detection task.
**Indicates statistically significant difference.*

TABLE 3 | Results of comparing patient IL and SL with frontal damage patients.

	Patient IL						Patient SL						Frontal damage controls
	Score	*T*	*p*	*p*cov	*Z-cc*	*Z-ccc*	*Score*	*T*	*P*	*p*cov	*Z-cc*	*Z-ccc*	
BDI	3	−1.16	0.16		−1.27		24	1.00	0.19		1.10		*M* = 14.25; *SD* = 8.87 (5–25)
Smell threshold	41.67	−0.51	0.32	0.46	−0.55	−0.14	50	−0.08	0.47	0.38	−0.09	−0.48	*M* = 51.67; *SD* = 18.07 (42–83)
Smell identification	75	0.00	0.50	0.41	0.00	0.36	33.3	−4.57	**0.01***	**0.02***	−5.00	−5.52	*M* = 75; *SD* = 8.33 (67–83)
Taste intensity	29	−0.63	0.28	0.27	−0.69	−0.99	52.7	0.33	0.38	0.35	0.36	0.61	*M* = 44.60; *SD* = 22.61 (10–63)
Taste identification	70	1.28	0.13	0.21	1.40	1.37	30	−1.92	0.06	0.06	−2.11	−2.08	*M* = 54; *SD* = 11.40 (40–70)
Cool sensation	28.25	−0.32	0.38	0.33	−0.36	−0.73	5.92	−15.10	**<0.01***	**<0.01***	−16.5	−16.8	*M* = 28.74; *SD* = 1.38 (27-30)
Warm sensation	36.72	−0.17	0.44	0.35	−0.18	0.61	45.5	3.08	**0.02***	**0.05**	3.38	3.31	*M* = 37.17; *SD* = 2.49 (36-42)
Cold pain	19.43	−0.24	0.41	0.28	−0.26	−0.98	0	−2.36	**0.04***	0.10	−2.59	−2.35	*M* = 21.57; *SD* = 8.34 (11–29)
Heat pain	42.68	0.22	0.42	0.18	0.24	1.55	50	1.489	0.11	0.23	1.63	1.20	*M* = 41.39; *SD* = 5.28 (34–47)
M-score	0.054	−0.27	0.40	0.41	−0.29	0.37	0.03	−0.65	0.28	0.19	−0.71	−1.45	*M* = 0.066; *SD* = 0.041 (0.03–0.13)
Interoception	0.50	4.80	**<0.01***	**0.02***	5.26	5.23	0.09	−2.68	**0.03***	***0.07***	−2.94	−2.72	*M* = 0.237; *SD* = 0.05 (0.19–0.32)
Heart rate	65	−1.86	***0.07***		−1.98		92	1.45	0.11		1.59		*M* = 80; *SD* = 7.54 (67.5–83)

*BOLD font and *, significant results at p level <0.05;BDI, Beck Depression Scale; M, motor condition of heartbeat detection task.*
Bold italics indicate trends.

terms of global taste scores, the IL patient showed no significant impairments. Neither did the SL patient show any significant deficits (see **Table 4**).

When compared with the brain damaged group (**Figure 2B**), the IL patient did not present any impairment in taste intensity or taste recognition. Meanwhile, the SL patient showed impairment neither in taste intensity, nor in taste identification. Global taste scores revealed no significant differences in either the IL patient or the SL patient (see **Table 5**).

Therefore, our hypothesis is supported by the absence of impairment in the IL patient, although it does not account for spared performance in the SL patient. However, a qualitative analysis of this latter patient's responses indicated that she misidentified sweet as salty (3/4 times) or bitter (1/4 times), salty as sour (2/6 times), and bitter as salty (3/6 times) or sour (2/6 times), showing a disruption in her subjective taste experiences.

Thermal Sensation and Pain Testing

There were no differences between the IL patient and healthy controls (**Figure 2C**) for thermal cool sensation, warm sensation, heat pain or cold pain (see **Table 2**). Contrarily, the SL patient showed impairments in thermal cool sensation ($t = -6.70$; $p < 0.01$; $p\text{cov} < 0.01$; $Zcc = -7.16$) and warmth sensation ($t = 3.55$; $p = 0.01$; $p\text{cov} = 0.04$; $Zcc = 3.79$; see **Table 2** and **Figure 2C**) before and after covariation for depression score on BDI. No further differences were observed. In terms of global thermal sensation scores, the IL patient showed no significant differences while the SL patient exhibited significantly lower performance ($t = -5.6$; $p < 0.01$; $p\text{cov} < 0.01$; $Z_{cc} = -6.14$). Global pain scores revealed no impairments in the IL patient and no impairment in the SL patient. Finally, regarding global thermal-pain sensation score, the IL patient showed no significant differences but the SL patient showed significantly

TABLE 4 | Results of comparing patient IL and SL with healthy age-matched controls.

	Patient IL						Patient SL						Healthy controls
	Score	*t*	*p*	*p*cov	*Z-cc*	*Z-ccc*	*Score*	*t*	*p*	*p*cov	*Z-cc*	*Z-ccc*	
Smell	58.33	−2.07	**0.05***	0.10	−2.24	−2.01	41.67	−6.21	**<0.01***	**<0.01***	−6.71	−9.77	*M* = 66.67; *SD* = 3.73 (63–71)
Taste	49.5	0.08	0.47	0.19	0.09	1.38	41.38	−.37	0.37	**0.06**	−0.40	−4.08	*M* = 48.08; *SD* = 16.74 (34–71)
Thermal sensation	32.49	0.44	0.34	0.29	0.47	0.80	25.75	−5.68	**<0.01***	**<0.01***	−6.14	−7.19	*M* = 32.01; *SD* = 1.02 (30–33)
Pain	31.05	1.01	0.18	0.19	1.09	1.31	25	−1.02	0.18	0.21	−1.11	−1.64	*M* = 28.04; *SD* = 2.75 (25–32)
Thermal pain sensation	31.77	0.89	0.21	0.20	0.97	1.23	25.38	−2.38	**0.03***	***0.07***	−2.57	−3.25	*M* = 30.03; *SD* = 1.81 (28–32)
Interoception	0.5	5.63	**<0.01***	**0.01***	6.17	6.59	0.09	−.61	0.30	0.43	−0.67	1.33	*M* = 0.13; *SD* = 0.06 (0.05–0.22)

BOLD font, significant results at p level <0.05; Italic Bold font, trends to significance; BDI, Beck Depression Scale.
**Indicates statistically significant difference.*

TABLE 5 | Results of comparing patient IL and SL with frontal damage patients.

	Patient IL						Patient SL						Brain lesion controls
	Score	*t*	*P*	*p*cov	*Z-cc*	*Z-ccc*	*Score*	*t*	*p*	*p*cov	*Z-cc*	*Z-ccc*	
Smell	58.33	−0.48	0.33	0.48	−0.53	0.08	41.67	−2.08	**0.05***	**0.05***	−2.28	−3.27	*M* = 63.33; *SD* = 9.50 (54–79)
Taste	49.5	0.02	0.49	0.43	0.03	−0.28	41.38	−0.89	0.21	0.32	−0.98	−0.72	*M* = 49.30; *SD* = 8.12 (40–58)
Thermal sensation	32.49	−0.63	0.28	0.43	−0.69	0.26	25.75	−9.76	**<0.01***	**<0.01***	−10.69	−16.58	*M* = 32.96; *SD* = 0.67 (32–34)
Pain	31.05	−0.18	0.43	0.44	−0.20	−0.24	25	−2.77	**0.03***	***0.06***	−3.03	−3.00	*M* = 31.48; *SD* = 2.14 (29–34)
Thermal pain sensation	31.77	−0.45	0.34	0.45	−0.49	−0.21	25.38	−6.81	**<0.01***	**<0.01**	−7.46	−7.94	*M* = 32.22; *SD* = 0.92 (31–33)
Interoception	0.50	4.80	**<0.01***	**0.01***	5.26	6.90	0.09	−2.68	**0.03*#**	**0.05*#**	−2.94	−5.07	*M* = 0.237; *SD* = 0.05 (0.19–0.32)

*BOLD font and *, significant results at p level <0.05;Italic Bold font, trends to significance; BDI, Beck Depression Scale; M, motor condition of heartbeat detection task. # Note this effects evidence a better performance of SL regarding the lesion group.*

lower performance, a pattern that did not remain significant after covariation (t = -2.38; $p = 0.03$; $p\text{cov} = 0.07$; $Z_{cc} = -2.57$, see **Table 4**).

The IL patient and the frontal patients obtained similar scores for thermal cool sensation, warmth sensation, heat pain and cold pain (**Table 3** and Figure S2C). Conversely, the SL patient showed impairments in thermal cool sensation ($t = -15.10$; $p < 0.01$; $p\text{cov} < 0.01$; $Z_{cc} = -16.54$) and warmth sensation ($t = 3.08$; $p = 0.02$; $p\text{cov} = 0.05$; $Z_{cc} = 3.38$) before and after covariation for depression score on BDI scores. Relative to frontal patients, the SL patient showed no significant differences in heat pain and a difference that did not survive covariation for cold pain ($t = -2.36$; $p = 0.04$; $p\text{cov} = 0.10$; $Zcc = -2.59$). Global thermal cool performance was unimpaired in the IL patient but significantly compromised in the SL patient ($t = -9.76$; $p < 0.1$; $p\text{cov} < 0.1$; $Z_{cc} = -10.6$). In terms of global pain scores, the IL patient showed no significant differences but the SL patient showed significantly lower performance, which did not survive after covariation ($t = -2.77$; $p = 0.03$; $p\text{cov} = 0.06$; $Z_{cc} = -3.03$). Finally, global thermal-pain sensation scores showed no significant differences in the IL patient but were significantly affected in the SL patient ($t = -6.81$; $p < 0.1$; $p\text{cov} < 0.1$; $Z_{cc} = -7.46$, see **Table 5**).

Considering that cool/cold thresholds are higher as the temperature departs from baseline (diminishes from 32 to 0°C), these results represent a diminished sensitivity to all conditions in SL (cool and warmth sensations).

In sum, when compared with both control groups, the IL patient showed no impairments in taste or thermal-pain sensation, which confirms our hypothesis, but a lower smell performance than healthy controls, which did not survive covariation. Conversely, when compared with healthy controls, the SL patient exhibited impaired smell identification, and diminished sensitivity to cool and warm sensations as well as to global thermal-pain sensation. Such impairments remained

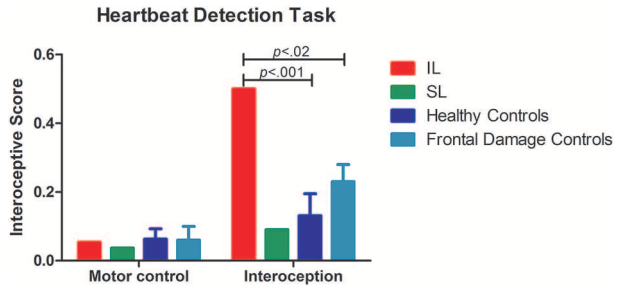


FIGURE 3 | Internal stream: HBD task. Results of the accuracy in the HBD task. Left side of the panel shows the motor control condition, where no differences where observed given that all participants performed the task accurately. The right side of the panel shows the performance on interoceptive sensitivity, where the IL patient exhibited impaired interoception. Conversely, the SL patient was spared.

in the comparison with the brain-damaged controls, also confirming the hypothesis.

Assessment of Internal Stream of Interoception

Heartbeat Detection Task (HBD)

Compared with healthy controls, the IL patient (**Figure 3**) showed impaired cardiac interoception ($t = 5.63$; $p < 0.01$; $p\text{cov} = 0.01$; $Zcc = 6.17$) with preserved performance in the control motor condition (see **Table 2**). Contrarily, the SL patient's performance was spared in both the interoceptive and the motor control (see **Table 2**) conditions. Even after covariation for HBD performance, the IL patient had significantly lower scores ($t = 5.63$; $p < 0.01$; $p\text{cov} = 0.01$; $Z_{cc} = 6.17$) whereas the SL patient performed similarly (see **Table 4**) to controls.

The patients' differential patterns were replicated following comparison with the brain-damaged group. The IL patient exhibited impairments in the interoceptive ($t = 4.80$; $p < 0.01$; $p\text{cov} = 0.02$; $Z_{cc} = 5.26$; see **Table 3** and **Figure 3**) but not in the control condition ($t = -0.27$; $p = 0.40$; $p\text{cov} = 0.41$; $Z_{cc} = -0.29$). Conversely, the SL patient showed preserved performance in both the motor and the interoceptive conditions (she even had better interoceptive performance than the brain-damaged group, but this result did not remain after covariation: $t = -2.68$; $p = 0.03$; $p\text{cov} = 0.07$; $Z_{cc} = -2.94$; see **Table 3** and **Figure 3**). Similarly, after covariation the IL patient performed significantly worse than brain-damaged patients ($t = 4.8$; $p < 0.1$; $p\text{cov} = 0.1$; $Z_{cc} = 5.26$). Conversely, relative to brain-damaged patients, the SL patient showed significantly better interoception (see **Table 5**).

Furthermore, we calculated for both IL and SL patients the heart-rate variability with three different methods and non-significant differences were found compared with healthy controls (see Table S2).

In summary, before and after covariation for HBD performance, and when compared to both healthy controls and the brain-damaged group, the IL patient presented disrupted interoceptive performance, while the SL patient showed no such disruption. Both of these results are in line with the general hypothesis that the internal stream of interoception depends on the insula as its putative basis.

Discussion

We presented two single cases with respective damage of the right insular cortex (IL) and of right putamen (affecting frontotemporal connections, SL). These patients showed a differential pattern of impairment regarding interoceptive-related behavior and body-mapped functions. The IL patient presented impaired internal (cardiac) interoception and preserved external perception (thermal pain, smell, and taste). A distinct pattern arose in SL, who displayed impaired processing via the external signals (smell identification and thermal-pain thresholds) with preserved cardiac interoception. Importantly, this partially opposite internal–external pattern was replicated when the patients' performance was compared to that of subjects with lesions in other regions. These results suggest that the deficits found in both patients relate to their specific focal lesions, as opposed to unspecific brain damage (Rorden and Karnath, 2004). Second, the pattern of results suggests differential disruption of internal cardiac interoception—affected mainly by focal insular damage (IL)—and external pain-smell—affected by specific subcortical and white matter damage of the fronto-temporo-insular connections (SL). Below we discuss these results in terms of internal and external signals of bodily stimuli and their possible relations with insular networks.

External Stimuli Related to Interoception

The existence of (external and internal) multimodal insular afferents and their differential requirements for processing (Cameron, 2002; Craig, 2002) supports the view of an external stream (smell, taste, and thermal pain deficits found in SL) involved in interoceptive and IC processing. Chemosensation and pain are typically processed by the paralimbic cortices (ICC, orbitofrontal cortex, ACC, and parahipoccampal cortex) nested between the limbic and higher-order multimodal association regions (Mesulam, 2000; Sewards and Sewards, 2001). Relevant neural pathways run contiguously until they reach the cortical areas, with gustatory pathways ending at the dorsal insula next to the thermal pain region (Verhagen, 2007). Such neurofunctional evidence aligns with the disconnection between IC and frontotemporal regions in the SL patient.

It has been suggested that the information carried by these external stream must first be integrated with stimulus saliency (Seeley et al., 2007; Singer et al., 2009) and hedonic value (Yin and Knowlton, 2006). Thus, disrupted connections in SL might compromise integrative contextual processing of external-internal signals via a fronto-insulo-temporal network including the IC as a critical hub. Damage to this network in the SL patient may underlie ongoing contextual embedding deficits (Mesulam and Mufson, 1982b; Amoruso et al., 2011; Ibáñez and Manes, 2012; Ibanez et al., 2014) leading to impairments in external domains which were spared in the IL patient (see also Couto et al., 2013c). This conjecture might be tested in future studies (Limongi et al., 2014).

Internal Stream of Interoceptive Afferents

The IL patient exhibited cardiac interoceptive deficits with preserved processing of external signals. Cardiac interoception is a basic modality of visceral perception that relies on an internal drive. It has proven to influence both homeostasis (Oppenheimer et al., 1991, 1992) and affective-cognitive domains (Singer et al., 2009; Garfinkel et al., 2013). Additionally, a wealth of neuroimaging and electrophysiological evidence shows the engagement of the right anterior IC in heartbeat awareness (Craig, 2002; Critchley et al., 2004; Pollatos et al., 2007b; Dunn et al., 2010) and the correlation of this activity with physical and cardio-dynamic variables (Pollatos et al., 2007a). These data point to a critical role of the right IC in sensing cardiac signatures, in line with the cardiac interoceptive impairment evinced by the IL patient. Additionally, the SL patient showed no interoceptive impairment, suggesting that right insula and not their frontotemporal connections running through the external capsule have a specific role in this domain. This is supported by the fact that she outperformed frontal patients, which is to be expected, given that frontal damage affects larger amounts of cortex and white matter than subcortical lesions, and leads to executive deficits (Miller and Cummings, 2007) (see Supplementary Table 1 for comparison between frontal damage and healthy controls, IFS compared with SL: $t = 1.5$; $p = 0.09$; zcc = 1.62).

Distinct Insular Networks for Processing Internal and External Streams of Bodily Signals

As proposed above, cardiovascular and respiratory reflexes (i.e., baroreflex and CO_2 concentration) that are sensed and processed in a beat-to-beat manner in the brainstem (Barrett et al., 2012) have a highly specific role in physiological modulation, are crucial for motor and affective behaviors (Mesulam and Mufson, 1982b; Garfinkel et al., 2014) and may constitute a privileged internal interoceptive stream. Further evidence suggests they are intrinsically related to the central autonomic regulation of the brainstem, amygdale, and insular cortex (Gray et al., 2009; Feinstein et al., 2013) with scarce signs of engagement from neocortical or higher-order associative structures. Thus, a single and focal right insular lesion might yield interoceptive impairments without compromising the body sensing of external signals.

As expected, the SL patient presented thermal-pain, taste and smell identification deficits. Other lesion studies (Pritchard et al., 1999; Cereda et al., 2002) have shown that IC disconnection from olfactory areas (piriform and mid temporal cortices) is associated with loss of smell. Similarly, cortical thickness of the right insula has been related to odor discrimination, mostly in women (Frasnelli et al., 2010). We also observed spared taste identification in SL that became a significant impairment after covariation with depression symptoms. This can be related with the strong negative correlation ($r = -0.85$, not reported in results) between depression symptoms and taste functions in the healthy control sample. This impairment was even observed when compared to healthy controls (see **Table 4**). Thus, our results indicate that two patients presenting selective damage to different areas of the IC body-sensing networks have a differential pattern of disruption of internal and external perception.

Interoceptive Relevance on Models of Perceptual Processing

The possible existence of internal and external subdivisions of interoceptive afferents could reflect a distinction between high and low cognitive processing. The lower level may consist of internal organ signals or proper interoception, such as vegetative cardiac and respiratory rhythms serving vital processes (Oppenheimer et al., 1991). These signals are integrated and represented in the IC (Mesulam and Mufson, 1982b; Brannan et al., 2001; Porges, 2009), shaping cognition in a very direct fashion. For example the activity in IC depends on evoked autonomic response (Critchley et al., 2002). It correlates with performance accuracy in the HBD task (Critchley, 2005) and with changes in peripheral electrodermal activity during a gambling task (Critchley et al., 2000). In addition, the insula is involved in shaping the anticipation and experience of pain and empathetic reproduction of pain experience (Singer et al., 2004). Moreover, the higher level may implicate further connections between the insula and multimodal cognitive association sites (Mesulam, 2000; Couto et al., 2013c) enabling the insula to integrate bottom-up interoceptive signals with top-down predictions from high-order brain regions (i.e., ACC and PFC). This results in the generation of real-time awareness of bodily emotional state (Gu et al., 2013), and contributes to the emergence of complex processes such as moral cognition (Moll et al., 2008), empathy (Decety et al., 2012), or theory of mind (Keysers and Gazzola, 2007).

Conversely, external afferents would involve body-mapped sensory inputs (smell, pain, taste). These may indirectly modulate complex behaviors only after a contextual updating that occurs in the IC just before being projected to cognitive sites (Limongi et al., 2014). This intermediate process may rely on fronto-temporal networks based on their contextual integration to high-level spheres of cognition (Ibáñez and Manes, 2012; Couto et al., 2013a; Baez et al., 2014; Ibanez et al., 2014).

Here we show that the same SL patient who presented emotional awareness deficits (Couto et al., 2013c) is impaired in the external domains of interoceptive processing (chemosensation and thermal-pain). This is consistent with the view that at least some negative emotions, such as disgust, may have emerged from adaptive needs throughout phylogenesis. Note, in this sense, that recent fMRI studies showed insular network activation both when feeling disgust and during observation of another person experiencing this aversive emotion (Wicker et al., 2003). Nevertheless, from the neuroanatomical point of view the affectation of different portions of the insular networks can lead to different patterns of behavioral impairment. Additionally, punctual injury to its white matter connections impact more notably on overall network functionality than damage in one isolated node of the network (Duffau, 2008). This indicates that, within a network, different groups of neurons work together in order to process the same information through designed wad of pathways' connections. Therefore, damage in the subcortical white matter would result in a more consistent

affectation of general network functionality relative to the affectation caused by damage to a given gray matter node.

Interoceptive afferent information arriving to the insular cortex, through the lamina I spino-thalamocortical system (lamina 1–solitary tract nucleus–parabrachial nucleus–periaqueductal gray–VMPo thalamus-insula) constitutes the basic information for the elaboration of higher cognitive domains (Craig, 2002) such as verbal memory (Garfinkel et al., 2013), social cognition (Couto et al., 2013b), and emotions (Garfinkel et al., 2014). In particular chemosensation and thermal-pain information are represented by the activity of a fronto-insulo-temporal network and may be anatomo-functionally dissociated through the study of focal lesions in different anatomical points of the network.

Limitations and Further Results and Future Research

This work presents important limitations that should be tackled in future studies.

Interoceptive performance in the brain-damaged group was better than in the IL patient but worse than in the SL patient. These patients' extended damage of the frontal cortex and other regions (reaching adjacent cortical areas and white matter) would explain their intermediate cardiac interoceptive performance. Further studies could assess whether frontal patients present subtle interoceptive deficits and whether these are secondary to other cognitive deficits (e.g., executive dysfunction).

The SL patient showed unimpaired taste abilities, as attested by our methodological strategy. We first used a covariation method to report the variance of the patient's performance beyond the depression covariate. This is a meaningful result in light of the strong negative correlation ($r = -0.85$, not reported in results) between depression symptoms and taste functions in healthy controls. Moreover, results from the four global scores of exteroception evidenced that the patient's impairment is present in thermal-pain processing, especially in heat pain, leaving only taste as a spared domain of external sensation. We then analyzed smell threshold and smell identification separately, and found deficits in the latter. Finally, the SL is not located in the primary gustatory cortex (dorsal anterior IC and dorsal mid-IC, Ogawa et al., 2005; Kurth et al., 2010), which indicates that damage to the taste brain network beyond this critical hub does not compromise the function. In sum, our methodology and results does not enable us to fully rule out a deficit smell domain despite the presence of other exteroceptive impairments.

The IL patient presented spared taste perception, which may seem to contradict the primary role of the IC in gustatory processing (Rolls et al., 2009). Nevertheless, similar findings were observed in the IL patient assessed by Mesulam (2000) and in three out of four focal insular patients evaluated by Cereda et al. (2002). Moreover, gustatory processing relies on a distributed network, with orbitofrontal hubs sub-serving multimodal integration (with visual and olfactory signals) and representation previous to subjective report (Rolls et al., 2003, 2009). In this sense, if gustatory process pertain to the external stream of interoception (as suggested here), it would be more dependent on extended fronto-temporal nodes.

Although we made a covariation by depression scores, we cannot rule out their possible effect on interoception. In particular, smell sensitivity (but not smell identification) is reduced in Major Depressive Disorder (MDD) (Pause et al., 2001; Thomas et al., 2002). Nevertheless, research on mood disorders and olfaction has yielded inconsistent results. In a recent review on olfactory perception and depression (Schablitzky and Pause, 2014), a number of studies showed that reduced performance on smell tasks is disorder-specific (Postolache et al., 1999; Swiecicki et al., 2009; Schablitzky and Pause, 2014). Moreover, negative association (Scinska et al., 2008) and even increased olfactory discrimination during depressive mood states (Goel and Grasso, 2004; Pollatos et al., 2007a) have been reported. Furthermore, studies assessing odor *identification* in MDD patients have shown no differences with healthy controls (Amsterdam et al., 1987; Warner et al., 1990; Kopala et al., 1994; Pause et al., 2003; Lombion-Pouthier et al., 2006; Swiecicki et al., 2009; Negoias et al., 2010; Naudin et al., 2012). However, the performance of our SL patient deviates from previous reports (i.e., reduced olfactory *sensitivity* but preserved *identification* in MDD). In fact, the pattern observed in our SL patient is the exact opposite (compromised *identification* and preserved *sensitivity*). Furthermore, the patient does not present a depression diagnosis, but only some depressive symptoms which were also covariate. Thus, although we cannot rule out the possibility of these symptoms affecting the results in the olfactory tasks, they do not represent the most plausible explanation for the patients' deficit pattern.

The absence of impairments in pain, taste and smell identification in the IC patient could reflect the action of compensatory functions provided by an intact left insula. In fact, this structure has been implicated in pain, taste and smell recognition (Pritchard et al., 1999; Brooks et al., 2002; Cereda et al., 2002). While relatively unexpected, this result also would be explained by the patient's use of explicit compensatory strategies. In addition, enhanced neuroplasticity and a successful functional remapping of the fronto-insular-temporal network after IC stroke would enable correct pain, smell and taste recognition. In fact, this interoceptive information are processed ultimately in the right anterior insula. Such interpretations are in line with our findings in patients with damage in the right network particularly of extra-insular connections.

Finally, the SL is not located in the primary gustatory cortex (dorsal anterior IC), which indicates that damage to the taste brain network beyond this critical hub does not compromise the function. In addition, this interpretation is reinforced by evidence that damage to the left insula causes a bilateral affectation in taste recognition (Pritchard et al., 1999).

Since all perceptive tests require preserved language abilities, our deficits would not be explained by a lack of transmission between the right insular network and neural substrates of language. Indeed, there is evidence that absence of the more important bundle of inter-hemispheric communication does not affect the verbal report of chemosensation awareness (Aglioti et al., 2001).

Conclusion

Most previous reports of the insular patients (Calder et al., 2000; Adolphs, 2002; Adolphs et al., 2003; Bar-On et al., 2003) included extended neural damage to the amygdala and frontal-parietal-temporal opercula. Focal cerebrovascular accidents represent a gold-standard model for brain injury studies (Rorden and Karnath, 2004). A particular strength of this work is that we compare only patients with very rare focal lesions of the insula and adjacent sites. A differential pattern of behavioral disruption as evidenced by the internal stream's affectation of IC lesion in IL and the external one in SL may shed light on the distinct neuroanatomical signatures of body perception. These disparate deficits would imply a hypothetical stratification of the multimodal bodily signals which surround the body in a peripersonal space, contribute to interoception and engage different aspect of insular networks for coordinating the internal and external milieus with higher functions such as emotional awareness.

Acknowledgments

This research was partially supported by Grants CONICYT/FONDECYT Regular (1130920 and 1140114), FONCyT-PICT 2012-0412 and 2012-1309, CONICET, and the INECO Foundation.

Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2015.00503/abstract

References

Adolphs, R. (2002). Neural systems for recognizing emotion. *Curr. Opin. Neurobiol.* 12, 169–177. doi: 10.1016/S0959-4388(02)00301-X

Adolphs, R., Tranel, D., and Damasio, A. R. (2003). Dissociable neural systems for recognizing emotions. *Brain Cogn.* 52, 61–69. doi: 10.1016/S0278-2626(03)00009-5

Aglioti, S. M., Tassinari, G., Fabri, M., Del Pesce, M., Quattrini, A., Manzoni, T., et al. (2001). Taste laterality in the split brain. *Eur. J. Neurosci.* 13, 195–200. doi: 10.1046/j.0953-816X.2000.01378.x

Ahmad, S., de Oliveira, G. S. Jr., Bialek, J. M., and McCarthy, R. J. (2014). Thermal quantitative sensory testing to predict postoperative pain outcomes following gynecologic surgery. *Pain Med.* 15, 857–864. doi: 10.1111/pme.12374

Amoruso, L., Couto, B., and Ibanez, A. (2011). Beyond Extrastriate Body Area (EBA) and Fusiform Body Area (FBA): context integration in the meaning of actions. *Front. Hum. Neurosci.* 5:124. doi: 10.3389/fnhum.2011.00124

Amsterdam, J. D., Settle, R. G., Doty, R. L., Abelman, E., and Winokur, A. (1987). Taste and smell perception in depression. *Biol. Psychiatry* 22, 1481–1485. doi: 10.1016/0006-3223(87)90108-9

Andre, J. M., Beis, J. M., Morin, N., and Paysant, J. (2000). Buccal hemineglect. *Arch. Neurol.* 57, 1734–1741. doi: 10.1001/archneur.57.12.1734

Antony, M., Brown, T., Craske, M., Barlow, D., Mitchell, W., and Meadows, E. (1995). Accuracy of heartbeat perception in panic disorder, social phobia, and nonanxious subjects. *J. Anxiety Disord.* 9, 355–371. doi: 10.1016/0887-6185(95)00017-I

Averbeck, B., Rucker, F., Laubender, R. P., and Carr, R. W. (2013). Thermal grill-evoked sensations of heat correlate with cold pain threshold and are enhanced by menthol and cinnamaldehyde. *Eur. J. Pain* 17, 724–734. doi: 10.1002/j.1532-2149.2012.00239.x

Azanon, E., Longo, M. R., Soto-Faraco, S., and Haggard, P. (2010). The posterior parietal cortex remaps touch into external space. *Curr. Biol.* 20, 1304–1309. doi: 10.1016/j.cub.2010.05.063

Azanon, E., and Soto-Faraco, S. (2008). Changing reference frames during the encoding of tactile events. *Curr. Biol.* 18, 1044–1049. doi: 10.1016/j.cub.2008.06.045

Baez, S., Couto, B., Torralva, T., Sposato, L. A., Huepe, D., Montanes, P., et al. (2014). Comparing moral judgments of patients with frontotemporal dementia and frontal stroke. *JAMA Neurol.* 71, 1172–1176. doi: 10.1001/jamaneurol.2014.347

Bar-On, R., Tranel, D., Denburg, N. L., and Bechara, A. (2003). Exploring the neurological substrate of emotional and social intelligence. *Brain* 126, 1790–1800. doi: 10.1093/brain/awg177

Barrett, K. E., Barman, S. M., Boitano, S., and Brooks, H. (2012). *Ganong's Review of Medical Physiology.* New York, NY: McGraw-Hill.

Bartoshuk, L. M., Desnoyers, S., O'brien, M., Gent, J. F., and Catalanotto, F. A. (1985). Taste stimulation of localized tongue areas: the Q-tip test. *Chem. Senses* 10, 453.

Beck, A. T., Steer, R. A., and Brown, G. K. (eds.). (1996). *Manual For The Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation.

Berlucchi, G., and Aglioti, S. (1997). The body in the brain: neural bases of corporeal awareness. *Trends Neurosci.* 20, 560–564. doi: 10.1016/S0166-2236(97)01136-3

Brannan, S., Liotti, M., Egan, G., Shade, R., Madden, L., Robillard, R., et al. (2001). Neuroimaging of cerebral activations and deactivations associated with hypercapnia and hunger for air. *Proc. Natl. Acad. Sci. U.S.A.* 98, 2029–2034. doi: 10.1073/pnas.98.4.2029

Brooks, J. C., Nurmikko, T. J., Bimson, W. E., Singh, K. D., and Roberts, N. (2002). fMRI of thermal pain: effects of stimulus laterality and attention. *Neuroimage* 15, 293–301. doi: 10.1006/nimg.2001.0974

Calder, A. J., Keane, J., Manes, F., Antoun, N., and Young, A. W. (2000). Impaired recognition and experience of disgust following brain injury. *Nat. Neurosci.* 3, 1077–1078. doi: 10.1038/80586

Cameron, O. G. (2002). *Visceral Sensory Neuroscience: Interoception.* New York, NY: Oxford University Press.

Carlesimo, G. A., Serra, L., Fadda, L., Cherubini, A., Bozzali, M., and Caltagirone, C. (2007). Bilateral damage to the mammillo-thalamic tract impairs recollection but not familiarity in the recognition process: a single case investigation. *Neuropsychologia* 45, 2467–2479. doi: 10.1016/j.neuropsychologia.2007.03.025

Caruana, F., Jezzini, A., Sbriscia-Fioretti, B., Rizzolatti, G., and Gallese, V. (2011). Emotional and social behaviors elicited by electrical stimulation of the insula in the macaque monkey. *Curr. Biol.* 21, 195–199. doi: 10.1016/j.cub.2010.12.042

Cauda, F., D'agata, F., Sacco, K., Duca, S., Geminiani, G., and Vercelli, A. (2011). Functional connectivity of the insula in the resting brain. *Neuroimage* 55, 8–23. doi: 10.1016/j.neuroimage.2010.11.049

Cereda, C., Ghika, J., Maeder, P., and Bogousslavsky, J. (2002). Strokes restricted to the insular cortex. *Neurology* 59, 1950–1955. doi: 10.1212/01.WNL.0000038905.75660.BD

Couto, B., Manes, F., Montanes, P., Matallana, D., Reyes, P., Velasquez, M., et al. (2013a). Structural neuroimaging of social cognition in progressive non-fluent aphasia and behavioral variant of frontotemporal dementia. *Front. Hum. Neurosci.* 7:467. doi: 10.3389/fnhum.2013.00467

Couto, B., Salles, A., Sedeno, L., Peradejordi, M., Barttfeld, P., Canales-Johnson, A., et al. (2013b). The man who feels two hearts: the different pathways of interoception. *Soc. Cogn. Affect. Neurosci.* 9, 1253–1260. doi: 10.1093/scan/nst108

Couto, B., Sedeno, L., Sposato, L. A., Sigman, M., Riccio, P. M., Salles, A., et al. (2013c). Insular networks for emotional processing and social cognition: comparison of two case reports with either cortical or subcortical involvement. *Cortex* 49, 1420–1434. doi: 10.1016/j.cortex.2012.08.006

Craig, A. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655–666. doi: 10.1038/nrn894

Craig, A. D. (2003a). Interoception: the sense of the physiological condition of the body. *Curr. Opin. Neurobiol.* 13, 500–505. doi: 10.1016/S0959-4388(03)00090-4

Craig, A. D. (2003b). A new view of pain as a homeostatic emotion. *Trends Neurosci.* 26, 303–307. doi: 10.1016/S0166-2236(03)00123-1

Craig, A. D. (2007). "Interoception and emotion: a neuroanatomical perspective," in *Handbook of Emotions, 3rd Edn*., eds M. Lewis, J. M. Haviland-Jones, and L. Feldman Barrett (New York, NY: Guilford Press), 272–290.

Craig, A. D. (2009). How do you feel–now? The anterior insula and human awareness. *Nat. Rev. Neurosci.* 10, 59–70. doi: 10.1038/nrn2555

Craig, A. D. (2014). *How Do You Feel? An Interoceptive Moment with Your Neurobiological Self*. Princeton, NJ: Princeton University Press.

Crawford, J. R., and Garthwaite, P. H. (2002). Investigation of the single case in neuropsychology: confidence limits on the abnormality of test scores and test score differences. *Neuropsychologia* 40, 1196–1208. doi: 10.1016/S0028-3932(01)00224-X

Crawford, J. R., and Garthwaite, P. H. (2012). Single-case research in neuropsychology: a comparison of five forms of t-test for comparing a case to controls. *Cortex* 48, 1009–1016. doi: 10.1016/j.cortex.2011.06.021

Crawford, J. R., Garthwaite, P. H., and Howell, D. C. (2009). On comparing a single case with a control sample: an alternative perspective. *Neuropsychologia* 47, 2690–2695. doi: 10.1016/j.neuropsychologia.2009.04.011

Crawford, J. R., Garthwaite, P. H., and Porter, S. (2010). Point and interval estimates of effect sizes for the case-controls design in neuropsychology: rationale, methods, implementations, and proposed reporting standards. *Cogn. Neuropsychol.* 27, 245–260. doi: 10.1080/02643294.2010.513967

Crawford, J. R., Garthwaite, P. H., and Ryan, K. (2011). Comparing a single case to a control sample: testing for neuropsychological deficits and dissociations in the presence of covariates. *Cortex* 47, 1166–1178. doi: 10.1016/j.cortex.2011.02.017

Crawford, J. R., and Howell, D. C. (1998). Comparing an individual's test score against norms derived from small samples. *Clin. Neuropsychol.* 12, 482–486. doi: 10.1076/clin.12.4.482.7241

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *J. Comp. Neurol.* 493, 154–166. doi: 10.1002/cne.20749

Critchley, H. D., Elliott, R., Mathias, C. J., and Dolan, R. J. (2000). Neural activity relating to generation and representation of galvanic skin conductance responses: a functional magnetic resonance imaging study. *J. Neurosci.* 20, 3033–3040.

Critchley, H. D., and Harrison, N. A. (2013). Visceral influences on brain and behavior. *Neuron* 77, 624–638. doi: 10.1016/j.neuron.2013.02.008

Critchley, H. D., Mathias, C. J., and Dolan, R. J. (2002). Fear conditioning in humans: the influence of awareness and autonomic arousal on functional neuroanatomy. *Neuron* 33, 653–663. doi: 10.1016/S0896-6273(02)00588-3

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., and Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nat. Neurosci.* 7, 189–195. doi: 10.1038/nn1176

De Araujo, I. E., Rolls, E. T., Kringelbach, M. L., McGlone, F., and Phillips, N. (2003). Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain. *Eur. J. Neurosci.* 18, 2059–2068. doi: 10.1046/j.1460-9568.2003.02915.x

Decety, J., Michalska, K. J., and Kinzler, K. D. (2012). The contribution of emotion and cognition to moral sensitivity: a neurodevelopmental study. *Cereb. Cortex* 22, 209–220. doi: 10.1093/cercor/bhr111

de Paepe, A. L., Crombez, G., Spence, C., and Legrain, V. (2014). Mapping nociceptive stimuli in a peripersonal frame of reference: evidence from a temporal order judgment task. *Neuropsychologia* 56, 219–228. doi: 10.1016/j.neuropsychologia.2014.01.016

Deshpande, G., Santhanam, P., and Hu, X. (2011). Instantaneous and causal connectivity in resting state brain networks derived from functional MRI data. *Neuroimage* 54, 1043–1052. doi: 10.1016/j.neuroimage.2010.09.024

Dosenbach, N. U., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A., et al. (2007). Distinct brain networks for adaptive and stable task control in humans. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11073–11078. doi: 10.1073/pnas.0704320104

Doty, R. L., Shaman, P., Applebaum, S. L., Giberson, R., Siksorski, L., and Rosenberg, L. (1984). Smell identification ability: changes with age. *Science* 226, 1441–1443. doi: 10.1126/science.6505700

Duffau, H. (2008). The anatomo-functional connectivity of language revisited. New insights provided by electrostimulation and tractography. *Neuropsychologia* 46, 927–934. doi: 10.1016/j.neuropsychologia.2007.10.025

Dunn, B. D., Stefanovitch, I., Evans, D., Oliver, C., Hawkins, A., and Dalgleish, T. (2010). Can you feel the beat? Interoceptive awareness is an interactive function of anxiety- and depression-specific symptom dimensions. *Behav. Res. Ther.* 48, 1133–1138. doi: 10.1016/j.brat.2010.07.006

Elsenbruch, S., Rosenberger, C., Bingel, U., Forsting, M., Schedlowski, M., and Gizewski, E. R. (2010). Patients with irritable bowel syndrome have altered emotional modulation of neural responses to visceral stimuli. *Gastroenterology* 139, 1310–1319. doi: 10.1053/j.gastro.2010.06.054

Farb, N. A., Segal, Z. V., and Anderson, A. K. (2013). Attentional modulation of primary interoceptive and exteroceptive cortices. *Cereb. Cortex* 23, 114–126. doi: 10.1093/cercor/bhr385

Farrell, M. J., Egan, G. F., Zamarripa, F., Shade, R., Blair-West, J., Fox, P., et al. (2006). Unique, common, and interacting cortical correlates of thirst and pain. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2416–2421. doi: 10.1073/pnas.0511019103

Faurion, A., Cerf, B., van de Moortele, P. F., Lobel, E., Mac Leod, P., and le Bihan, D. (1999). Human taste cortical areas studied with functional magnetic resonance imaging: evidence of functional lateralization related to handedness. *Neurosci. Lett.* 277, 189–192. doi: 10.1016/S0304-3940(99)00881-2

Feinstein, J. S., Buzza, C., Hurlemann, R., Follmer, R. L., Dahdaleh, N. S., Coryell, W. H., et al. (2013). Fear and panic in humans with bilateral amygdala damage. *Nat. Neurosci.* 16, 270–272. doi: 10.1038/nn.3323

Ferri, F., Ardizzi, M., Ambrosecchia, M., and Gallese, V. (2013). Closing the gap between the inside and the outside: interoceptive sensitivity and social distances. *PLoS ONE* 8:e75758. doi: 10.1371/journal.pone.0075758

Frasnelli, J., Lundstrom, J. N., Boyle, J. A., Djordjevic, J., Zatorre, R. J., and Jones-Gotman, M. (2010). Neuroanatomical correlates of olfactory performance. *Exp. Brain Res.* 201, 1–11. doi: 10.1007/s00221-009-1999-7

Gagnon, L., Vestergaard, M., Madsen, K., Karstensen, H. G., Siebner, H., Tommerup, N., et al. (2014). Neural correlates of taste perception in congenital olfactory impairment. *Neuropsychologia* 62, 297–305. doi: 10.1016/j.neuropsychologia.2014.07.018

Garfinkel, S. N., Barrett, A. B., Minati, L., Dolan, R. J., Seth, A. K., and Critchley, H. D. (2013). What the heart forgets: cardiac timing influences memory for words and is modulated by metacognition and interoceptive sensitivity. *Psychophysiology* 50, 505–512. doi: 10.1111/psyp.12039

Garfinkel, S. N., and Critchley, H. D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. Commentary on:: "Anterior insular cortex mediates bodily sensibility and social anxiety" by Terasawa et al. (2012). *Soc. Cogn. Affect. Neurosci.* 8, 231–234. doi: 10.1093/scan/nss140

Garfinkel, S. N., Minati, L., Gray, M. A., Seth, A. K., Dolan, R. J., and Critchley, H. D. (2014). Fear from the heart: sensitivity to fear stimuli depends on individual heartbeats. *J. Neurosci.* 34, 6573–6582. doi: 10.1523/JNEUROSCI.3507-13.2014

Garrido, L., Eisner, F., McGettigan, C., Stewart, L., Sauter, D., Hanley, J. R., et al. (2009). Developmental phonagnosia: a selective deficit of vocal identity recognition. *Neuropsychologia* 47, 123–131. doi: 10.1016/j.neuropsychologia.2008.08.003

Goel, N., and Grasso, D. J. (2004). Olfactory discrimination and transient mood change in young men and women: variation by season, mood state, and time of day. *Chronobiol. Int.* 21, 691–719. doi: 10.1081/CBI-200025989

Gramsch, C., Kattoor, J., Icenhour, A., Forsting, M., Schedlowski, M., Gizewski, E. R., et al. (2014). Learning pain-related fear: neural mechanisms mediating rapid differential conditioning, extinction and reinstatement processes in human visceral pain. *Neurobiol. Learn. Mem.* 116C, 36–45. doi: 10.1016/j.nlm.2014.08.003

Gray, M. A., Rylander, K., Harrison, N. A., Wallin, B. G., and Critchley, H. D. (2009). Following one's heart: cardiac rhythms gate central initiation of sympathetic reflexes. *J. Neurosci.* 29, 1817–1825. doi: 10.1523/JNEUROSCI.3363-08.2009

Graziano, M. S. (1999). Where is my arm? The relative role of vision and proprioception in the neuronal representation of limb position. *Proc. Natl. Acad. Sci. U.S.A.* 96, 10418–10421. doi: 10.1073/pnas.96.18.10418

Graziano, M. S., Cooke, D. F., and Taylor, C. S. (2000). Coding the location of the arm by sight. *Science* 290, 1782–1786. doi: 10.1126/science.290.5497.1782

Greenspan, J. D., Lee, R. R., and Lenz, F. A. (1999). Pain sensitivity alterations as a function of lesion location in the parasylvian cortex. *Pain* 81, 273–282. doi: 10.1016/S0304-3959(99)00021-4

Grossmann, N., Brin, I., Aizenbud, D., Sichel, J. Y., Gross-Isseroff, R., and Steiner, J. (2005). Nasal airflow and olfactory function after the repair of cleft palate (with and without cleft lip). *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 100, 539–544. doi: 10.1016/j.tripleo.2005.02.074

Gu, X., Hof, P. R., Friston, K. J., and Fan, J. (2013). Anterior insular cortex and emotional awareness. *J. Comp. Neurol.* 521, 3371–3388. doi: 10.1002/cne.23368

Herbert, B. M., and Pollatos, O. (2012). The body in the mind: on the relationship between interoception and embodiment. *Top. Cogn. Sci.* 4, 692–704. doi: 10.1111/j.1756-8765.2012.01189.x

Herz, R. S. (2014). Verbal priming and taste sensitivity make moral transgressions gross. *Behav. Neurosci.* 128, 20–28. doi: 10.1037/a0035468

Holmes, N. P., and Spence, C. (2004). The body schema and the multisensory representation(s) of peripersonal space. *Cogn. Process.* 5, 94–105. doi: 10.1007/s10339-004-0013-3

Hulleman, J., and Humphreys, G. W. (2007). Maximizing the power of comparing single cases against a control sample: an argument, a program for making comparisons, and a worked example from the Pyramids and Palm Trees Test. *Cogn. Neuropsychol.* 24, 279–291. doi: 10.1080/02643290601181890

Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E., and Kobal, G. (1997). 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem. Senses* 22, 39–52. doi: 10.1093/chemse/22.1.39

Iannilli, E., Noennig, N., Hummel, T., and Schoenfeld, A. M. (2014). Spatio-temporal correlates of taste processing in the human primary gustatory cortex. *Neuroscience* 273, 92–99. doi: 10.1016/j.neuroscience.2014.05.017

Ibanez, A., Gleichgerrcht, E., and Manes, F. (2010). Clinical effects of insular damage in humans. *Brain Struct. Funct.* 214, 397–410. doi: 10.1007/s00429-010-0256-y

Ibanez, A., Kotz, S. A., Barrett, L., Moll, J., and Ruz, M. (2014). Situated affective and social neuroscience. *Front. Hum. Neurosci.* 8:547. doi: 10.3389/fnhum.2014.00547

Ibáñez, A., and Manes, F. (2012). Contextual social cognition and the behavioral variant of frontotemporal dementia. *Neurology* 78, 1354–1362. doi: 10.1212/WNL.0b013e318251837

Iranzo, A., Serradell, M., Vilaseca, I., Valldeoriola, F., Salamero, M., Molina, C., et al. (2013). Longitudinal assessment of olfactory function in idiopathic REM sleep behavior disorder. *Parkinsonism Relat. Disord.* 19, 600–604. doi: 10.1016/j.parkreldis.2013.02.009

Kelly, C., Toro, R., Di Martino, A., Cox, C. L., Bellec, P., Castellanos, F. X., et al. (2012). A convergent functional architecture of the insula emerges across imaging modalities. *Neuroimage* 61, 1129–1142. doi: 10.1016/j.neuroimage.2012.03.021

Kennedy, D. P., Glascher, J., Tyszka, J. M., and Adolphs, R. (2009). Personal space regulation by the human amygdala. *Nat. Neurosci.* 12, 1226–1227. doi: 10.1038/nn.2381

Keysers, C., and Gazzola, V. (2007). Integrating simulation and theory of mind: from self to social cognition. *Trends Cogn. Sci.* 11, 194–196. doi: 10.1016/j.tics.2007.02.002

Khalsa, S. S., Rudrauf, D., Feinstein, J. S., and Tranel, D. (2009). The pathways of interoceptive awareness. *Nat. Neurosci.* 12, 1494–1496. doi: 10.1038/nn.2411

Kirk, U., Downar, J., and Montague, P. R. (2011). Interoception drives increased rational decision-making in meditators playing the ultimatum game. *Front. Neurosci.* 5:49. doi: 10.3389/fnins.2011.00049

Kopala, L. C., Good, K. P., and Honer, W. G. (1994). Olfactory hallucinations and olfactory identification ability in patients with schizophrenia and other psychiatric disorders. *Schizophr. Res.* 12, 205–211. doi: 10.1016/0920-9964(94)90030-2

Kurth, F., Zilles, K., Fox, P. T., Laird, A. R., and Eickhoff, S. B. (2010). A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Struct. Funct.* 214, 519–534. doi: 10.1007/s00429-010-0255-z

Limongi, R., Tomio, A., and Ibanez, A. (2014). Dynamical predictions of insular hubs for social cognition and their application to stroke. *Front Behav Neurosci* 8:380. doi: 10.3389/fnbeh.2014.00380

Lombion-Pouthier, S., Vandel, P., Nezelof, S., Haffen, E., and Millot, J. L. (2006). Odor perception in patients with mood disorders. *J. Affect. Disord.* 90, 187–191. doi: 10.1016/j.jad.2005.11.012

Mattes, R. D., Wysocki, C. J., Graziani, A., and Macgregor, R. R. (1995). Chemosensory function and diet in HIV-infected patients. *Laryngoscope* 105, 862–866. doi: 10.1288/00005537-199508000-00018

Mazzola, L., Isnard, J., Peyron, R., Guenot, M., and Mauguiere, F. (2009). Somatotopic organization of pain responses to direct electrical stimulation of the human insular cortex. *Pain* 146, 99–104. doi: 10.1016/j.pain.2009.07.014

Melloni, M., Sedeno, L., Couto, B., Reynoso, M., Gelormini, C., Favaloro, R., et al. (2013). Preliminary evidence about the effects of meditation on interoceptive sensitivity and social cognition. *Behav. Brain Funct.* 9, 47. doi: 10.1186/1744-9081-9-47

Menon, V., and Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct. Funct.* 214, 655–667. doi: 10.1007/s00429-010-0262-0

Mesulam, M. M. (2000). *Principles of Behavioral and Cognitive Neurology*. Oxford: Oxford University Press.

Mesulam, M. M., and Mufson, E. J. (1982a). Insula of the old world monkey. I. Architectonics in the insulo-orbito-temporal component of the paralimbic brain. *J. Comp. Neurol.* 212, 1–22. doi: 10.1002/cne.902120102

Mesulam, M. M., and Mufson, E. J. (1982b). Insula of the old world monkey. III: efferent cortical output and comments on function. *J. Comp. Neurol.* 212, 38–52. doi: 10.1002/cne.902120104

Miller, B. L., and Cummings, J. (2007). *The Human Frontal Lobes.* New York, NY: The Guilford Press.

Moll, J., de Oliveira-Souza, R., and Zahn, R. (2008). The neural basis of moral cognition: sentiments, concepts, and values. *Ann. N.Y. Acad. Sci.* 1124, 161–180. doi: 10.1196/annals.1440.005

Mufson, E. J., and Mesulam, M. M. (1982). Insula of the old world monkey. II: afferent cortical input and comments on the claustrum. *J. Comp. Neurol.* 212, 23–37. doi: 10.1002/cne.902120103

Naudin, M., El-Hage, W., Gomes, M., Gaillard, P., Belzung, C., and Atanasova, B. (2012). State and trait olfactory markers of major depression. *PLoS ONE* 7:e46938. doi: 10.1371/journal.pone.0046938

Negoias, S., Croy, I., Gerber, J., Puschmann, S., Petrowski, K., Joraschky, P., et al. (2010). Reduced olfactory bulb volume and olfactory sensitivity in patients with acute major depression. *Neuroscience* 169, 415–421. doi: 10.1016/j.neuroscience.2010.05.012

Ogawa, H., Wakita, M., Hasegawa, K., Kobayakawa, T., Sakai, N., Hirai, T., et al. (2005). Functional MRI detection of activation in the primary gustatory cortices in humans. *Chem. Senses* 30, 583–592. doi: 10.1093/chemse/bji052

Oppenheimer, S. M., Gelb, A., Girvin, J. P., and Hachinski, V. C. (1992). Cardiovascular effects of human insular cortex stimulation. *Neurology* 42, 1727–1732. doi: 10.1212/WNL.42.9.1727

Oppenheimer, S. M., Wilson, J. X., Guiraudon, C., and Cechetto, D. F. (1991). Insular cortex stimulation produces lethal cardiac arrhythmias: a mechanism of sudden death? *Brain Res.* 550, 115–121. doi: 10.1016/0006-8993(91)90412-O

Parabucki, A., and Netser, S. (2014). Origin of palatability coding in medial prefrontal cortex. *J. Neurosci.* 34, 4121–4122. doi: 10.1523/JNEUROSCI.0362-14.2014

Paulus, M. P., Rogalsky, C., Simmons, A., Feinstein, J. S., and Stein, M. B. (2003). Increased activation in the right insula during risk-taking decision making is related to harm avoidance and neuroticism. *Neuroimage* 19, 1439–1448. doi: 10.1016/S1053-8119(03)00251-9

Pause, B. M., Miranda, A., Goder, R., Aldenhoff, J. B., and Ferstl, R. (2001). Reduced olfactory performance in patients with major depression. *J. Psychiatr. Res.* 35, 271–277. doi: 10.1016/S0022-3956(01)00029-2

Pause, B. M., Raack, N., Sojka, B., Goder, R., Aldenhoff, J. B., and Ferstl, R. (2003). Convergent and divergent effects of odors and emotions in depression. *Psychophysiology* 40, 209–225. doi: 10.1111/1469-8986.00023

Peltz, E., Seifert, F., Decol, R., Dorfler, A., Schwab, S., and Maihofner, C. (2011). Functional connectivity of the human insular cortex during

noxious and innocuous thermal stimulation. *Neuroimage* 54, 1324–1335. doi: 10.1016/j.neuroimage.2010.09.012

Pollatos, O., Albrecht, J., Kopietz, R., Linn, J., Schoepf, V., Kleemann, A. M., et al. (2007a). Reduced olfactory sensitivity in subjects with depressive symptoms. *J. Affect. Disord.* 102, 101–108. doi: 10.1016/j.jad.2006.12.012

Pollatos, O., Kirsch, W., and Schandry, R. (2005). Brain structures involved in interoceptive awareness and cardioafferent signal processing: a dipole source localization study. *Hum. Brain Mapp.* 26, 54–64. doi: 10.1002/hbm.20121

Pollatos, O., Schandry, R., Auer, D. P., and Kaufmann, C. (2007b). Brain structures mediating cardiovascular arousal and interoceptive awareness. *Brain Res.* 1141, 178–187. doi: 10.1016/j.brainres.2007.01.026

Pollatos, O., Traut-Mattausch, E., and Schandry, R. (2009). Differential effects of anxiety and depression on interoceptive accuracy. *Depress. Anxiety* 26, 167–173. doi: 10.1002/da.20504

Porges, S. W. (2009). The polyvagal theory: new insights into adaptive reactions of the autonomic nervous system. *Cleve Clin. J. Med.* 76(Suppl. 2), S86–90. doi: 10.3949/ccjm.76.s2.17

Postolache, T. T., Doty, R. L., Wehr, T. A., Jimma, L. A., Han, L., Turner, E. H., et al. (1999). Monorhinal odor identification and depression scores in patients with seasonal affective disorder. *J. Affect. Disord.* 56, 27–35. doi: 10.1016/S0165-0327(99)00027-0

Pritchard, T. C., Macaluso, D. A., and Eslinger, P. J. (1999). Taste perception in patients with insular cortex lesions. *Behav. Neurosci.* 113, 663–671. doi: 10.1037/0735-7044.113.4.663

Richards, J. C. E., and Lorraine, V. (1996). Cardiac acuity in panic disorder. *Cognit. Ther. Res.* 20, 361. doi: 10.1007/BF02228039

Rizzolatti, G., Fadiga, L., Fogassi, L., and Gallese, V. (1997). The space around us. *Science* 277, 190–191. doi: 10.1126/science.277.5323.190

Rolls, E. T., Critchley, H. D., Verhagen, J. V., and Kadohisa, M. (2009). The representation of information about taste and odor in the orbitofrontal cortex. *Chemosens. Percept.* 3, 16–33. doi: 10.1007/s12078-009-9054-4

Rolls, E. T., Verhagen, J. V., and Kadohisa, M. (2003). Representations of the texture of food in the primate orbitofrontal cortex: neurons responding to viscosity, grittiness, and capsaicin. *J. Neurophysiol.* 90, 3711–3724. doi: 10.1152/jn.00515.2003

Rorden, C., and Karnath, H. O. (2004). Using human brain lesions to infer function: a relic from a past era in the fMRI age? *Nat. Rev. Neurosci.* 5, 813–819. doi: 10.1038/nrn1521

Saper, C. B. (1982). Convergence of autonomic and limbic connections in the insular cortex of the rat. *J. Comp. Neurol.* 210, 163–173. doi: 10.1002/cne.902100207

Schablitzky, S., and Pause, B. M. (2014). Sadness might isolate you in a non-smelling world: olfactory perception and depression. *Front. Psychol.* 5:45. doi: 10.3389/fpsyg.2014.00045

Schandry, R. (1981). Heart beat perception and emotional experience. *Psychophysiology* 18, 483–488. doi: 10.1111/j.1469-8986.1981.tb02486.x

Schandry, R., Sparrer, B., and Weitkunat, R. (1986). From the heart to the brain: a study of heartbeat contingent scalp potentials. *Int. J. Neurosci.* 30, 261–275. doi: 10.3109/00207458608985677

Scinska, A., Wrobel, E., Korkosz, A., Zatorski, P., Sienkiewicz-Jarosz, H., Lojkowska, W., et al. (2008). Depressive symptoms and olfactory function in older adults. *Psychiatry Clin. Neurosci.* 62, 450–456. doi: 10.1111/j.1440-1819.2008.01824.x

Sedeno, L., Couto, B., Melloni, M., Canales-Johnson, A., Yoris, A., Baez, S., et al. (2014). How do you feel when you can't feel your body? interoception, functional connectivity and emotional processing in depersonalization-derealization disorder. *PLoS ONE* 9:e98769. doi: 10.1371/journal.pone.0098769

Seeley, W. W. (2008). Selective functional, regional, and neuronal vulnerability in frontotemporal dementia. *Curr. Opin. Neurol.* 21, 701–707. doi: 10.1097/WCO.0b013e3283168e2d

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/JNEUROSCI.5587-06.2007

Senkowski, D., Hofle, M., and Engel, A. K. (2014). Crossmodal shaping of pain: a multisensory approach to nociception. *Trends Cogn. Sci.* 18, 319–327. doi: 10.1016/j.tics.2014.03.005

Sewards, T. V., and Sewards, M. A. (2001). Cortical association areas in the gustatory system. *Neurosci. Biobehav. Rev.* 25, 395–407. doi: 10.1016/S0149-7634(01)00021-5

Sherrington, C. S. (1900). "The muscular sense," in *Text-Book of Physiology,* ed E. A. Schäfer (Edinburgh: Pentland), 247–263.

Simmons, A., Matthews, S. C., Stein, M. B., and Paulus, M. P. (2004). Anticipation of emotionally aversive visual stimuli activates right insula. *Neuroreport* 15, 2261–2265. doi: 10.1097/00001756-200410050-00024

Simmons, A., Strigo, I., Matthews, S. C., Paulus, M. P., and Stein, M. B. (2006). Anticipation of aversive visual stimuli is associated with increased insula activation in anxiety-prone subjects. *Biol. Psychiatry* 60, 402–409. doi: 10.1016/j.biopsych.2006.04.038

Simmons, W. K., Avery, J. A., Barcalow, J. C., Bodurka, J., Drevets, W. C., and Bellgowan, P. (2013). Keeping the body in mind: insula functional organization and functional connectivity integrate interoceptive, exteroceptive, and emotional awareness. *Hum. Brain Mapp.* 34, 2944–2958. doi: 10.1002/hbm.22113

Singer, T., Critchley, H. D., and Preuschoff, K. (2009). A common role of insula in feelings, empathy and uncertainty. *Trends Cogn. Sci.* 13, 334–340. doi: 10.1016/j.tics.2009.05.001

Singer, T., Seymour, B., O'doherty, J., Kaube, H., Dolan, R. J., and Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science* 303, 1157–1162. doi: 10.1126/science.1093535

Small, D. M., Zald, D. H., Jones-Gotman, M., Zatorre, R. J., Pardo, J. V., Frey, S., et al. (1999). Human cortical gustatory areas: a review of functional neuroimaging data. *Neuroreport* 10, 7–14. doi: 10.1097/00001756-199901180-00002

Spielberger, C. D., Gorsuch, R. L., and Lushene, R. E. (eds.). (1970). *Manual for the State-Trait Inventory*. Palo Alto, CA: Consulting Psychological Press.

Sridharan, D., Levitin, D. J., and Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12569–12574. doi: 10.1073/pnas.0800005105

Straube, T., Weisbrod, A., Schmidt, S., Raschdorf, C., Preul, C., Mentzel, H. J., et al. (2010). No impairment of recognition and experience of disgust in a patient with a right-hemispheric lesion of the insula and basal ganglia. *Neuropsychologia* 48, 1735–1741. doi: 10.1016/j.neuropsychologia.2010.02.022

Swiecicki, L., Zatorski, P., Bzinkowska, D., Sienkiewicz-Jarosz, H., Szyndler, J., and Scinska, A. (2009). Gustatory and olfactory function in patients with unipolar and bipolar depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 827–834. doi: 10.1016/j.pnpbp.2009.03.030

Tajadura-Jimenez, A., and Tsakiris, M. (2014). Balancing the "inner" and the "outer" self: interoceptive sensitivity modulates self-other boundaries. *J. Exp. Psychol. Gen.* 143, 736–744. doi: 10.1037/a0033171

Taylor, K. S., Seminowicz, D. A., and Davis, K. D. (2009). Two systems of resting state connectivity between the insula and cingulate cortex. *Hum. Brain Mapp.* 30, 2731–2745. doi: 10.1002/hbm.20705

Thomas, H. J., Fries, W., and Distel, H. (2002). Evaluation of olfactory stimuli by depressed patients. *Nervenarzt* 73, 71–77. doi: 10.1007/s115-002-8150-9

Torralva, T., Roca, M., Gleichgerrcht, E., Lopez, P., and Manes, F. (2009). INECO Frontal Screening (IFS): a brief, sensitive, and specific tool to assess executive functions in dementia. *J. Int. Neuropsychol. Soc.* 15, 777–786. doi: 10.1017/S1355617709990415

Valmunen, T., Pertovaara, A., Taiminen, T., Virtanen, A., Parkkola, R., and Jaaskelainen, S. K. (2009). Modulation of facial sensitivity by navigated rTMS in healthy subjects. *Pain* 142, 149–158. doi: 10.1016/j.pain.2008.12.031

van den Bosch, I., Dalenberg, J. R., Renken, R., van Langeveld, A. W., Smeets, P. A., Griffioen-Roose, S., et al. (2014). To like or not to like: neural substrates of subjective flavor preferences. *Behav. Brain Res.* 269, 128–137. doi: 10.1016/j.bbr.2014.04.010

van Stralen, H. E., van Zandvoort, M. J., Hoppenbrouwers, S. S., Vissers, L. M., Kappelle, L. J., and Dijkerman, H. C. (2014). Affective touch modulates the rubber hand illusion. *Cognition* 131, 147–158. doi: 10.1016/j.cognition.2013.11.020

Verhagen, J. V. (2007). The neurocognitive bases of human multimodal food perception: consciousness. *Brain Res. Rev.* 53, 271–286. doi: 10.1016/j.brainresrev.2006.09.002

Verhagen, J. V., Kadohisa, M., and Rolls, E. T. (2004). Primate insular/opercular taste cortex: neuronal representations of the viscosity, fat texture, grittiness, temperature, and taste of foods. *J. Neurophysiol.* 92, 1685–1699. doi: 10.1152/jn.00321.2004

Warner, M. D., Peabody, C. A., and Csernansky, J. G. (1990). Olfactory functioning in schizophrenia and depression. *Biol. Psychiatry* 27, 457–458. doi: 10.1016/0006-3223(90)90557-I

Whitehead, W. E., Drescher, V. M., Heiman, P., and Blackwell, B. (1977). Relation of heart rate control to heartbeat perception. *Appl. Psychophysiol. Biofeedback* 2, 371–392. doi: 10.1007/BF00998623

Wicker, B., Keysers, C., Plailly, J., Royet, J. P., Gallese, V., and Rizzolatti, G. (2003). Both of us disgusted in My insula: the common neural basis of seeing and feeling disgust. *Neuron* 40, 655–664. doi: 10.1016/S0896-6273(03)00679-2

Yarnitsky, D., and Sprecher, E. (1994). Thermal testing: normative data and repeatability for various test algorithms. *J. Neurol. Sci.* 125, 39–45. doi: 10.1016/0022-510X(94)90239-9

Yin, H. H., and Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nat. Rev. Neurosci.* 7, 464–476. doi: 10.1038/nrn1919

Yoder, N. (2009). *PeakFinder*. Matlab, MathWorks.

Zoellner, L. A., and Craske, M. G. (1999). Interoceptive accuracy and panic. *Behav. Res. Ther.* 37, 1141–1158. doi: 10.1016/S0005-7967(98)00202-2

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Public Health

OPINION
published: 29 April 2016
doi: 10.3389/fpubh.2016.00081

Diet and Common Mental Disorders: The Imperative to Translate Evidence into Action

*Sarah R. Dash[1], Adrienne O'Neil[2,3] and Felice N. Jacka[1,4,5,6]**

[1] IMPACT Strategic Research Centre, School of Medicine, Deakin University, Geelong, VIC, Australia, [2] School of Public Health and Preventive Medicine, Monash University, Prahran, VIC, Australia, [3] Alfred Hospital, School of Public Health and Preventive Medicine, Prahran, VIC, Australia, [4] Centre for Adolescent Health, Murdoch Children's Research Centre, Parkville, VIC, Australia, [5] Department of Psychiatry, Royal Melbourne Hospital, University of Melbourne, Melbourne, VIC, Australia, [6] Black Dog Institute, Prince of Wales Hospital, Randwick, NSW, Australia

Keywords: diet, mental health, public health, prevention, epidemiology

INTRODUCTION

The globalization of the food industry has lead to substantial dietary changes across developed and developing economies, comprising a shift toward the consumption of higher energy, less nutritious foods at the expense of traditional, more healthful, dietary patterns (1). These dietary changes have led to clear public health challenges as the burden of obesity and other diet-related non-communicable disorders (NCDs) continue to rise. In 2015, the Global Burden of Disease study identified unhealthy diet as the leading cause of early mortality worldwide (2). At the same time, mental and substance use disorders are recognized as the leading contributors to global disability (3). Of these, the common mental disorders (CMDs) – depression and anxiety – contribute the greatest proportion of disability, accounting for 40.5 and 14.6% of disease burden respectively. Only recently has it been recognized that unhealthy diet and CMDs are related: unhealthy diet is a significant risk factor not only for NCDs, such as cardiovascular diseases, some cancers, and diabetes, but also for CMDs (4). Dietary interventions may, thus, provide a far-reaching and low risk public health opportunity for the prevention and treatment of CMDs.

Traditionally, psychiatric epidemiology has directed much of its research efforts into understanding the etiology of psychiatric conditions and has lagged behind in the development of public health strategies for primary prevention (5). While the past decade has given rise to public health campaigns directed at mental illness, such campaigns are often focused on raising awareness and reducing stigma rather than on specific actions (6). Moreover, while several critical windows of opportunity for mental disorder prevention have been presented (7), there currently exists no clear or specific prevention strategy or recommendations for mental illness akin to that which exists for other common NCDs. Funding resources allocated to primary prevention of CMDs are greatly disproportionate to its disease burden, and resources for mental health prevention are not equitable to the priority placed on them by major stakeholders (8, 9). This paper argues the necessity of translating the new knowledge regarding the diet–depression paradigm into the development and implementation of public health and clinical intervention strategies at a population level.

CMDs AND DIET

There is now consistent epidemiological evidence for associations between measures of habitual diet quality and depression, globally (10–14) and across the lifespan (15–18), which do not appear to be explained by socioeconomic circumstances (4) or reverse causality (11, 19, 20). In fact, dietary habits have now been identified as a modifiable risk factor for depression and anxiety in

OPEN ACCESS

Edited by:
Andrew Kemp,
Swansea University, UK

Reviewed by:
Julian Koenig,
Heidelberg University, Germany
Laura Louise Wilkinson,
Swansea University, UK
Hayley Anne Young,
Swansea University, UK

****Correspondence:***
Felice N. Jacka
f.jacka@deakin.edu.au

Specialty section:
This article was submitted to Public Mental Health, a section of the journal Frontiers in Public Health

Received: *04 February 2016*
Accepted: *13 April 2016*
Published: *29 April 2016*

Citation:
Dash SR, O'Neil A and Jacka FN (2016) Diet and Common Mental Disorders: The Imperative to Translate Evidence into Action. Front. Public Health 4:81. doi: 10.3389/fpubh.2016.00081

several recent systematic reviews (21–23). The literature suggests that a good quality diet is characterized by high consumption of fruits, vegetables, whole grains, nuts, seeds, and fish while limiting intake of processed foods (24). Evidence at the clinical trial level indeed provide promising data; two new interventions indicate that preventing depression using dietary improvement as a strategy is possible (25, 26). Another systematic review has identified preliminary evidence for dietary improvement as a treatment strategy for symptoms of CMDs (24). There are also extensive animal and human data pointing to the biological underpinnings of CMDs that are modulated by diet, with gut microbiota and inflammatory pathways gaining particularly attention (27, 28), in addition to brain plasticity pathways in humans (29). While the link between individual nutrients, supplements, and mental disorder treatment has been studied, this discussion aims to focus on prevention and treatment through a whole-of-diet approach to mental health (30–32). Results from ongoing and future intervention studies are essential to continuing to strengthen this evidence base, as well as advancing the diet–depression paradigm, and must be a funding and research priority (5).

WHY ACT NOW?

Given the strength and consistency of the epidemiological and animal evidence, coupled with the emerging evidence from intervention studies, we contend that diet should be considered a risk factor for the onset of CMDs, with public health messages and strategies developed that build on this new understanding. These will have the added benefit of targeting the non-communicable conditions that are so commonly comorbid with CMDs and which are responsible for a significant proportion of premature deaths worldwide (33). Moreover, dietary recommendations that focus on mental health may have more salience for the public, given that the possible consequences of unhealthy diets – heart disease, diabetes, and cancer – may be perceived as distal, while mental health is a far more proximal consequence for many. This is particularly the case for young people who are especially affected by both detrimental dietary changes (34) and mental health issues (35). Although obesity may be considered a key indicator of lifestyle, most epidemiological studies in this field demonstrated that the relationship between diet and depression exists independently of body mass index (11, 20, 36). Furthermore, improvements in mood may precede weight loss and may also provide more tangible and immediate benefits that encourage sustaining health behaviors, with weight or obesity management a possible downstream benefit (37–39).

Despite advances in aspects of mental health care, researchers and clinicians have highlighted the shortfalls of pharmacological or individualized clinical to patient care for CMDs (40). While intervention studies on the diet–depression paradigm remain a priority, the current evidence base meets key Bradford Hill Criteria, with few perceivable risks to impede action (41). Given the high prevalence and burden of CMDs, even slight improvements in depression through dietary intervention or prevention strategies may translate to large gains at the population level given that diet is a variable with 100% exposure.

IMPLEMENTING DIETARY IMPROVEMENT AS A PUBLIC HEALTH STRATEGY FOR MENTAL HEALTH

There is much to be learned from previous public health movements that serve to guide the effective implementation of dietary improvement as a mental health strategy. While there is no country that has successfully managed to reverse its rising obesity trends (42), other public health campaigns that have been successful in improving health outcomes at a population level – such as folic acid supplementation during pregnancy or smoking cessation – have made changes using a top-down approach, supported by political changes to policies, practices, and taxation (43, 44). Implementing prevention strategies for mental health, and specific recommendations for how to proceed at community, academic, and government levels has been discussed in more detail elsewhere (5, 45, 46). Previous behavior change models have highlighted important periods for intervention, where targeted strategies may be more successful (47, 48). Previous research has demonstrated that *in utero*, early life, and adolescence are particularly important periods in determining future health and, thus, may be valuable target for public health campaigns. Given that pregnancy provides a unique "teachable moment," where mothers are particularly open to receiving health advice and making behavior change, such a targeted approach to prevention of the CMDs may prove to be feasible (49) and have important implications for the mental health of offspring.

Poor quality diet should be included within the risk assessment for major depressive disorder (MDD) within clinical care settings, with dietary recommendations and dietetic services as central components of primary care for individuals diagnosed with depressive symptoms and MDD. Moreover, given the robust evidence base for physical activity as a protective and treatment factor in depression (50, 51), and the emerging evidence for the mental health benefits of smoking cessation (52), exercise recommendations and smoking cessation services should also be standard components of clinical care for at-risk patients and those with established CMDs. In other words, treatment for CMD should regard physical health as of equal importance as a treatment target when considering the mental health of a patient.

BARRIERS TO IMPLEMENTATION

There are several key considerations for implementing public health dietary interventions for mental health. One imperative is to demonstrate clear, objective benefits to motivate both policy, local and individual health behavior change. For this, a large-scale, worldwide, case-control study that calculates the percentage population attributable risk of poor diet to the incidence of CMDs will be required; akin to those that exist for heart disease, for example, INTERHEART study (2001). Additionally, well-enacted, robust interventions demonstrating efficacy and cost-effectiveness will be critical. At least one clinical trial is currently underway (53); however, more are needed. Importantly, public health interventions aimed at reducing NCD risk factors should also assess mental health outcomes and utilize findings to assess

the effectiveness and cost-effectiveness of such interventions. As noted by many strategies in the past, single-approach or single-disciplinary approaches to complex, multi-modal problems have limited success, and there are demands for both systems-based interventions and monitoring systems and laws, regulations, and taxation changes to achieve traction in improving health at the population level (54).

While some studies have demonstrated the affordability of a healthy diet (55), financial incentives may be particularly important in groups with lower socioeconomic status (56). Finally, ensuring access to healthful foods for those in remote and highly disadvantaged areas is a critical challenge that should be prioritized, particularly given the burden of physical and mental health problems in such areas in addition to issues of food access.

CONCLUSION

Despite increased awareness and attention, we have yet to effectively and consistency address the high prevalence and burden of CMDs. Improving diet quality has the important yet neglected potential for prevention and treatment of CMDs. Here, we contend that while intervention studies will be an essential component of this evidence base, the current understanding of the diet–CMD association calls for "consequentialist epidemiology," with focus on the translation of diet-mental health research to action (57). Recognition of diet as a risk factor for CMDs and the development of public health strategies focused on dietary improvement are likely to positively address the global burden of both CMDs and NCDs. Effectively implementing dietary improvement as a public health strategy for mental health will require a multi-sectorial, multi-stakeholder approach (58), but offers substantial promise for improving outcomes for individuals and the wider community.

AUTHOR CONTRIBUTIONS

SD, AO, and FJ conceptualized the paper. SD wrote the first draft of the manuscript. AO and FJ made key contributions and revisions to the final version of the manuscript.

REFERENCES

1. Logan AC, Jacka FN. Nutritional psychiatry research: an emerging discipline and its intersection with global urbanization, environmental challenges and the evolutionary mismatch. *J Physiol Anthropol* (2014) **33**:22. doi:10.1186/1880-6805-33-22
2. Collaborators GBDRF, Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* (2015) **386**(10010):2287–323. doi:10.1016/S0140-6736(15)00128-2
3. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet* (2013) **382**:1575–86. doi:10.1016/S0140-6736(13)61611-6
4. Jacka FN, Cherbuin N, Anstey KJ, Butterworth P. Dietary patterns and depression symptoms over time: examining the relationships with socioeconomic position, health behaviours and cardiovascular risk. *PLoS One* (2014) **9**(1):e87657. doi:10.1371/journal.pone.0087657
5. Jacka FN, Reavley NJ, Jorm AF, Toumbourou JW, Lewis AJ, Berk M. Prevention of common mental disorders: what can we learn from those who have gone before and where do we go next? *Aust N Z J Psychiatry* (2013) **47**(10):920–9. doi:10.1177/0004867413493523
6. Dumesnil H, Verger P. Public awareness campaigns about depression and suicide: a review. *Psychiatr Serv* (2009) **60**(9):1203–13. doi:10.1176/appi.ps.60.9.1203
7. Shonkoff JP, Boyce WT, McEwen BS. Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention. *JAMA* (2009) **301**(21):2252–9. doi:10.1001/jama.2009.754
8. Christensen H, Batterham PJ, Griffiths KM, Gosling J, Hehir KK. Research priorities in mental health. *Aust N Z J Psychiatry* (2013) **47**(4):355–62. doi:10.1177/0004867412474072
9. Prince M, Patel V, Saxena S, Maj M, Maselko J, Phillips MR, et al. No health without mental health. *Lancet* (2007) **370**(9590):859–77. doi:10.1016/S0140-6736(07)61238-0
10. Nanri A, Kimura Y, Matsushita Y, Ohta M, Sato M, Mishima N, et al. Dietary patterns and depressive symptoms among Japanese men and women. *Eur J Clin Nutr* (2010) **64**(8):832–9. doi:10.1038/ejcn.2010.86
11. Jacka FN, Kremer PJ, Leslie ER, Berk M, Patton GC, Toumbourou JW, et al. Associations between diet quality and depressed mood in adolescents: results from the Australian Healthy Neighbourhoods Study. *Aust N Z J Psychiatry* (2010) **44**(5):435–42.
12. Belal NM. The relation between diet and behavioural problems in Kuwaiti adolescents. *J Appl Sci Res* (2012) **8**(8):4051–7.
13. Muñoz MA, Fíto M, Marrugat J, Covas MI, Schröder H, REGICOR and HERMES Investigators. Adherence to the Mediterranean diet is associated with better mental and physical health. *Br J Nutr* (2009) **101**(12):1821–7. doi:10.1017/S0007114508143598
14. Jacka FN, Kremer PJ, Berk M, de Silva-Sanigorski AM, Moodie M, Leslie ER, et al. A prospective study of diet quality and mental health in adolescents. *PLoS One* (2011) **6**(9):e24805. doi:10.1371/journal.pone.0024805
15. Oellingrath IM, Svendsen MV, Hestetun I. Eating patterns and mental health problems in early adolescence – a cross-sectional study of 12-13-year-old Norwegian schoolchildren. *Public Health Nutr* (2014) **17**(11):2554–62. doi:10.1017/S1368980013002747
16. Steenweg-de Graaff J, Tiemeier H, Steegers-Theunissen RP, Hofman A, Jaddoe VW, Verhulst FC, et al. Maternal dietary patterns during pregnancy and child internalising and externalising problems. The Generation R Study. *Clin Nutr* (2014) **33**(1):115–21. doi:10.1016/j.clnu.2013.03.002
17. Wiles NJ, Northstone K, Emmett P, Lewis G. 'Junk food' diet and childhood behavioural problems: results from the ALSPAC cohort. *Eur J Clin Nutr* (2009) **63**(4):491–8. doi:10.1038/sj.ejcn.1602967
18. Payne ME, Steck SE, George RR, Steffens DC. Fruit, vegetable, and antioxidant intakes are lower in older adults with depression. *J Acad Nutr Diet* (2012) **112**(12):2022–7. doi:10.1016/j.jand.2012.08.026
19. Jacka FN, Cherbuin N, Anstey KJ, Butterworth P. Does reverse causality explain the relationship between diet and depression? *J Affect Disord* (2015) **175**:248–50. doi:10.1016/j.jad.2015.01.007
20. Sánchez-Villegas A, Delgado-Rodríguez M, Alonso A, Schlatter J, Lahortiga F, Serra Majem L, et al. Association of the Mediterranean dietary pattern with the incidence of depression: the Seguimiento Universidad de Navarra/University of Navarra follow-up (SUN) cohort. *Arch Gen Psychiatry* (2009) **66**(10):1090–8. doi:10.1001/archgenpsychiatry.2009.129
21. Lai JS, Hiles S, Bisquera A, Hure AJ, McEvoy M, Attia J. A systematic review and meta-analysis of dietary patterns and depression in community-dwelling adults. *Am J Clin Nutr* (2014) **99**(1):181–97. doi:10.3945/ajcn.113.069880
22. Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N. Mediterranean diet, stroke, cognitive impairment, and depression: a meta-analysis. *Ann Neurol* (2013) **74**(4):580–91. doi:10.1002/ana.23944
23. Quirk SE, Williams LJ, O'Neil A, Pasco JA, Jacka FN, Housden S, et al. The association between diet quality, dietary patterns and depression in adults: a systematic review. *BMC Psychiatry* (2013) **13**:175. doi:10.1186/1471-244X-13-175

24. Opie RS, O'Neil A, Itsiopoulos C, Jacka FN. The impact of whole-of-diet interventions on depression and anxiety: a systematic review of randomised controlled trials. *Public Health Nutr* (2015) **18**(11):2074–93. doi:10.1017/S1368980014002614
25. Sánchez-Villegas A, Martínez-González MA, Estruch R, Salas-Salvadó J, Corella D, Covas MI, et al. Mediterranean dietary pattern and depression: the PREDIMED randomized trial. *BMC Med* (2013) **11**:208. doi:10.1186/1741-7015-11-208
26. Stahl ST, Albert SM, Dew MA, Lockovich MH, Reynolds CF III. Coaching in healthy dietary practices in at-risk older adults: a case of indicated depression prevention. *Am J Psychiatry* (2014) **171**(5):499–505. doi:10.1176/appi.ajp.2013.13101373
27. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* (2013) **11**:200. doi:10.1186/1741-7015-11-200
28. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* (2012) **13**(10):701–12. doi:10.1038/nrn3346
29. Jacka FN, Cherbuin N, Anstey KJ, Sachdev P, Butterworth P. Western diet is associated with a smaller hippocampus: a longitudinal investigation. *BMC Med* (2015) **13**:215. doi:10.1186/s12916-015-0461-x
30. Bloch MH, Hannestad J. Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis. *Mol Psychiatry* (2012) **17**(12):1272–82. doi:10.1038/mp.2011.100
31. Lai J, Moxey A, Nowak G, Vashum K, Bailey K, McEvoy M. The efficacy of zinc supplementation in depression: systematic review of randomised controlled trials. *J Affect Disord* (2012) **136**(1–2):e31–9. doi:10.1016/j.jad.2011.06.022
32. Sarris J, Logan AC, Akbaraly TN, Paul Amminger G, Balanzá-Martínez V, Freeman MP, et al. International Society for Nutritional Psychiatry Research consensus position statement: nutritional medicine in modern psychiatry. *World Psychiatry* (2015) **14**(3):370–1. doi:10.1002/wps.20223
33. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* (2012) **380**(9859):2095–128. doi:10.1016/S0140-6736(12)61728-0
34. Nielsen SJ, Siega-Riz AM, Popkin BM. Trends in food locations and sources among adolescents and young adults. *Prev Med* (2002) **35**(2):107–13. doi:10.1006/pmed.2002.1037
35. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* (2005) **62**(6):593–602. doi:10.1001/archpsyc.62.6.617
36. Akbaraly TN, Brunner EJ, Ferrie JE, Marmot MG, Kivimaki M, Singh-Manoux A. Dietary pattern and depressive symptoms in middle age. *Br J Psychiatry* (2009) **195**(5):408–13. doi:10.1192/bjp.bp.108.058925
37. McMillan L, Owen L, Kras M, Scholey A. Behavioural effects of a 10-day Mediterranean diet. Results from a pilot study evaluating mood and cognitive performance. *Appetite* (2011) **56**(1):143–7. doi:10.1016/j.appet.2010.11.149
38. Asmundson GJ, Fetzner MG, Deboer LB, Powers MB, Otto MW, Smits JA. Let's get physical: a contemporary review of the anxiolytic effects of exercise for anxiety and its disorders. *Depress Anxiety* (2013) **30**(4):362–73. doi:10.1002/da.22043
39. Fabricatore AN, Wadden TA, Higginbotham AJ, Faulconbridge LF, Nguyen AM, Heymsfield SB, et al. Intentional weight loss and changes in symptoms of depression: a systematic review and meta-analysis. *Int J Obes (Lond)* (2011) **35**(11):1363–76. doi:10.1038/ijo.2011.2
40. Kazdin AE, Blase SL. Rebooting psychotherapy research and practice to reduce the burden of mental illness. *Perspect Psychol Sci* (2011) **6**(1):21–37. doi:10.1177/1745691610393527
41. Lucas RM, McMichael AJ. Association or causation: evaluating links between "environment and disease". *Bull World Health Organ* (2005) **83**(10):792–5.
42. Lang T, Rayner G. Overcoming policy cacophony on obesity: an ecological public health framework for policymakers. *Obes Rev* (2007) **8**(Suppl 1):165–81. doi:10.1111/j.1467-789X.2007.00338.x
43. Obican SG, Finnell RH, Mills JL, Shaw GM, Scialli AR. Folic acid in early pregnancy: a public health success story. *FASEB J* (2010) **24**(11):4167–74. doi:10.1096/fj.10-165084
44. Holford TR, Meza R, Warner KE, Meernik C, Jeon J, Moolgavkar SH, et al. Tobacco control and the reduction in smoking-related premature deaths in the United States, 1964-2012. *JAMA* (2014) **311**(2):164–71. doi:10.1001/jama.2013.285112
45. Patel V, Belkin GS, Chockalingam A, Cooper J, Saxena S, Unützer J. Grand challenges: integrating mental health services into priority health care platforms. *PLoS Med* (2013) **10**(5):e1001448. doi:10.1371/journal.pmed.1001448
46. O'Neil A, Jacka FN, Quirk SE, Cocker F, Taylor CB, Oldenburg B, et al. A shared framework for the common mental disorders and non-communicable disease: key considerations for disease prevention and control. *BMC Psychiatry* (2015) **15**:15. doi:10.1186/s12888-015-0394-0
47. Sawyer SM, Afifi RA, Bearinger LH, Blakemore SJ, Dick B, Ezeh AC, et al. Adolescence: a foundation for future health. *Lancet* (2012) **379**(9826):1630–40. doi:10.1016/S0140-6736(12)60072-5
48. Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. *Environ Health* (2012) **11**:42. doi:10.1186/1476-069X-11-42
49. O'Neil A, Itsiopoulos C, Skouteris H, Opie RS, McPhie S, Hill B, et al. Preventing mental health problems in offspring by targeting dietary intake of pregnant women. *BMC Med* (2014) **12**:208. doi:10.1186/s12916-014-0208-0
50. Rosenbaum S, Tiedemann A, Sherrington C, Curtis J, Ward PB. Physical activity interventions for people with mental illness: a systematic review and meta-analysis. *J Clin Psychiatry* (2014) **75**(9):964–74. doi:10.4088/JCP.13r08765
51. Mammen G, Faulkner G. Physical activity and the prevention of depression: a systematic review of prospective studies. *Am J Prev Med* (2013) **45**(5):649–57. doi:10.1016/j.amepre.2013.08.001
52. Taylor G, McNeill A, Girling A, Farley A, Lindson-Hawley N, Aveyard P. Change in mental health after smoking cessation: systematic review and meta-analysis. *BMJ* (2014) **348**:g1151. doi:10.1136/bmj.g1151
53. O'Neil A, Berk M, Itsiopoulos C, Castle D, Opie R, Pizzinga J, et al. A randomised, controlled trial of a dietary intervention for adults with major depression (the "SMILES" trial): study protocol. *BMC Psychiatry* (2013) **13**:114. doi:10.1186/1471-244X-13-114
54. Jacka FN, Sacks G, Berk M, Allender S. Food policies for mental and physical health. *BMC Psychiatry* (2014) **14**:132. doi:10.1186/1471-244X-14-132
55. Opie RS, Segal L, Jacka FN, Nicholls L, Dash S, Pizzinga J, et al. Assessing healthy diet affordability in a cohort with major depressive disorders. *Publ Health Epidemiol* (2015) **7**(5):159–69. doi:10.5897/JPHE2014.0668
56. Wong KC, Coveney J, Ward P, Muller R, Carter P, Vertiy F, et al. Availability, affordability and quality of a healthy food basket in Adelaide, South Australian. *Nutr Diet* (2011) **68**:8–14. doi:10.1111/j.1747-0080.2010.01490.x
57. Galea S. An argument for a consequentialist epidemiology. *Am J Epidemiol* (2013) **178**(8):1185–91. doi:10.1093/aje/kwt172
58. Waxman A. Why a global strategy on diet, physical activity and health? *World Rev Nutr Diet* (2005) **95**:162–6. doi:10.1159/000088302

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
PSYCHOLOGY

ORIGINAL RESEARCH ARTICLE
published: 17 February 2015
doi: 10.3389/fpsyg.2015.00135

Exploratory multivariate analysis of the effect of fatty fish consumption and medicinal use on heart rate and heart rate variability data

Bjørn Grung[1]*, Anita L. Hansen[2,3], Mari Berg[1], Maria P. Møen-Knudseth[1], Gina Olson[4], David Thornton[4], Lisbeth Dahl[5] and Julian F. Thayer[6]

[1] Department of Chemistry, University of Bergen, Bergen, Norway
[2] Department of Psychosocial Science, University of Bergen, Bergen, Norway
[3] Centre for Research and Education in Forensic Psychiatry, Haukeland University Hospital, Bergen, Norway
[4] Sand Ridge Secure Treatment Centre, Mauston, WI, USA
[5] National Institute of Nutrition and Seafood Research, Bergen, Norway
[6] Department of Psychology, The Ohio State University, Columbus, OH, USA

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Daniel S. Quintana, University of Oslo, Norway
Kathleen M. Gustafson, University of Kansas Medical Center, USA

***Correspondence:**
Bjørn Grung, Department of Chemistry, University of Bergen, Allégaten 41, 5007 Bergen, Norway
e-mail: bjorn.grung@kj.uib.no

The overall aim of the present study was to explore the relationship between medicinal use and fatty fish consumption on heart rate variability (HRV) and heart rate (HR) in a group of forensic inpatients on a variety of medications. A total of 49 forensic inpatients, randomly assigned to a fish group ($n = 27$) or a control group ($n = 22$) were included in the present study. Before and by the end of the food intervention period HR and HRV were measured during an experimental test procedure. An additional aim of this paper is to show how multivariate data analysis can highlight differences and similarities between the groups, thus being a valuable addition to traditional statistical hypothesis testing. The results indicate that fish consumption may have a positive effect on both HR and HRV regardless of medication, but that the influence of medication is strong enough to mask the true effect of fish consumption. Without correcting for medication, the fish group and control group become indistinguishable ($p = 0.0794$, Cohen's $d = 0.60$). The effect of medication is demonstrated by establishing a multivariate regression model that estimates HR and HRV in a recovery phase based on HR and HRV data recorded during psychological tests. The model performance is excellent for HR data, but yields poor results for HRV when employed on participants undergoing the more severe medical treatments. This indicates that the HRV behavior of this group is very different from that of the participants on no or lower level of medication. When focusing on the participants on a constant medication regime, a substantial improvement in HRV and HR for the fish group compared to the control group is indicated by a principal component analysis and t-tests ($p = 0.00029$, Cohen's $d = 2.72$). In a group of psychiatric inpatients characterized by severe mental health problems consuming different kinds of medication, the fish diet improved HR and HRV, indices of both emotional regulation and physical health.

Keywords: heart rate, heart rate variability, fatty fish, medicine, multivariate data analysis

INTRODUCTION

Psychiatric inpatients are often on a variety of medications, and it is known that the life expectancy for this group is lower than the expectancy for the general population (Nome and Holsten, 2012). Studies have also shown that people suffering from psychiatric disorders often have deficiencies in key nutrients such as omega-3 fatty acids and vitamin D (Partonen, 1998; Perica and Delas, 2011). Diet has shown to have a profound effect on both physical health, e.g., cardiovascular diseases (CVDs; Van Horn et al., 2008), and the mental health (Timonen et al., 2004). Self-reporting survey studies and nutrient supplement studies dominate this research field, due to the difficulties in conducting a well-controlled diet intervention study. Because of the low life expectancy in psychiatric inpatients on a variety of medications more knowledge about effective health care interventions is needed. Due to the fact that fatty fish is an important source of essential fatty acids and vitamin D, nutrients important for both physical and mental health (Lansdowne and Provost, 1998; Christensen et al., 2001a,b; Ross et al., 2007; Dobnig et al., 2008; Giovannucci et al., 2008), results from controlled fish intervention studies may have important implications.

Although many studies report on the effects a steady diet of fatty fish may have on the physical and mental health, the number of studies examining the underlying mechanisms are much rarer. Heart rate variability (HRV), which is an objective and important index of both physical and mental health (Thayer and Lane, 2007), has been mentioned as one such mechanism. HRV is a measure of beat-to-beat changes in the heart rate (HR), and is a measure of the interplay of the sympathetic and parasympathetic branches of the autonomic nervous system. In itself HRV is a

predictor of sudden death in patients with coronary heart disease (Kleiger et al., 2005), which makes the relationship between fatty fish consumption and HRV interesting to investigate. A reduction in HRV is regarded as an indicator of future fatal or near-fatal cardiac arrhythmia (Huikuri et al., 2009), and it is thus an important measure of an individual's physical health. Thayer and Lane developed a model of neurovisceral integration (Thayer and Lane, 2000, 2007, 2009) that connects prefrontal cortex activity with that of the heart. This theory provides a physiological explanation as to why HRV not only predicts physical, but also emotion- and self-regulation. Several later studies (Hansen et al., 2003, 2004, 2009) have underpinned the model by associating HRV with executive functions, the underlying mechanism involved in emotion- and self-regulation (Shimamura, 2000; Thayer and Lane, 2000). Mozaffarian et al. (2008) found that changes in various HRV parameters were related to increased fatty fish intake. This was done by controlling for factors such as age, gender, ethnic background, education, smoking, alcohol intake, body mass index, diabetes mellitus, prevalent coronary heart disease, β-blocker use, physical activity, and intakes of beef, pork, fried fish and total calories. However, it is not well established whether the effect of fish consumption on HRV is direct or indirect (Mozaffarian et al., 2008). It has been speculated whether serotonin may be involved in the effect (Hansen et al., 2010) due to the fact that both vitamin D and omega-3, which are found in fatty fish, are important for the regulation of serotonin and serotonin is further important for the regulation of HRV (Stumpf and Privette, 1989; Hibbeln et al., 2006).

The use of HRV as an indicator of mental and physical health status is not problem free. A recent paper by Quintana and Heathers (Quintana and Heathers, 2014) highlights the impact of respiration on HRV. Several external factors, such as stress, age, physical shape and sickness affect the HRV, and may thus disturb the results. CVD may of course disturb the beat pattern of the heart, and persons suffering from depression tend to have lower HRV than the normal population (Kemp et al., 2010). There is a strong link between depression and CVDs, as a substantial number of CVD patients suffer from depression (Carney et al., 1987; Gonzalez et al., 1996), and persons diagnosed with depression run a higher risk of certain CVDs (Anda et al., 1993; Barefoot et al., 1996; Pratt et al., 1996; Penninx et al., 2001). Another major influence on HRV is medication. Blood pressure medications (Bonaduce et al., 1997; Pinar et al., 1998), cholesterol medication (Riahi et al., 2002; Welzig et al., 2003; Gomes et al., 2010), β (Cook et al., 1991; Mølgaard et al., 1993; Niemelä et al., 1994; Sandrone et al., 1994; Ebbehøj et al., 2002) - and α- blockers and ACE inhibitors (Guedon-Moreau et al., 1997; Shehab et al., 2008) are all examples of heart medications with either known or suspected effects on HR and HRV. Among antidepressants, the most commonly used are selective serotonin reuptake inhibitors (SSRI). Serotonin itself plays a role in the HRV regulation, and an effect of SSRI on HRV can be expected. Still, various studies come to different conclusions as to whether SSRI influences HRV. In their recent review and meta-analysis, Kemp et al. (2010), concluded that some antidepressants, although successful in easing the symptoms of depression, did not improve HRV significantly. As patients with depression have a higher risk for developing CVD, a treatment that remedies the psychological symptoms, but leaves the physiological ones untreated, may imply a lower longevity for this group. Some studies (Balogh et al., 1993; Khaykin et al., 1998) demonstrate a positive effect, whereas others (Licht et al., 2010) show a negative effect on HRV. For other types of antidepressants the situation is similar, and no consensus seems to have been arrived at (van Zyl et al., 2008) on their possible effect on HRV.

Recently it was demonstrated that fatty fish consumption improved sleep quality and resting HRV (Hansen et al., 2014a). However, in that study only the effect of high frequency (HF) HRV was investigated and other HRV parameters such as low frequency (LF) and the LF/HF ratio were not investigated. Thus, a combined effect of different HRV parameters was not investigated. Moreover, no attempts were made to investigate the effect of various medications used by the participants on their psychophysiological reactivity to different conditions, such as an experimental mild-stress procedure consisting of a baseline period (resting), a variety of different cognitive tasks taxing executive functioning and a recovery (resting) period. Executive functioning tasks require focused attention over prolonged periods of time in order to be performed accurately. Carrying out these tasks can be a stressful experience. Both increased HR and decreased HRV have been found during the performance of such tasks (Porges and Raskin, 1969). Since HRV is associated with mortality, and psychiatric inpatients on a variety of medications are known to have a lower life expectancy than the general population (Nome and Holsten, 2012), investigation of HRV reactivity to different conditions while controlling for medication may yield important information of general daily autonomic activity patterns.

A major aim of this study was to generate hypotheses concerning the effects of a fatty fish diet on the HRV and HR reactivity in a group of psychiatric patients characterized by severe mental health problems consuming many different kinds of medications. Here, we focus on heart medication and anti-depressants administered on a regular basis. In order to generate new hypotheses the investigation is carried out in an exploratory way using multivariate data analysis (Principal Component Analysis and Partial Least Squares), with focus on easily interpretable plots. This technique represents a valuable addition to the battery of statistical tests normally carried out. Because of the different findings in the literature, it was also investigated whether any effect on HRV and HR could be detected prior to intervention and assigned to the usage of anti-depressants. Finally, it was investigated whether the data indicated that users of such medication experienced a different effect of the fatty fish intake compared to the participants who did not use such medication.

MATERIALS AND METHODS

PARTICIPANTS

The present study was part of a larger research project concerning the effects of fatty fish consumption (Hansen et al., 2014a), where 95 male forensic inpatients characterized by severe emotional disturbances (e.g., antisocial personality disorders, borderline personality disorders, generalized anxiety and major depression) had been randomly assigned into a Fish group and a Control group. In the current study a subset of 49 participants were suitable for an investigation of whether the intake of medications had an impact

on the effect of the fish consumption. Details about the study progress are reported in Hansen et al. (2014a). The number of subjects in the fish group was 27. The number of subjects in the control group was 22. During the test period five subjects withdrew from the fish group, and six from the control group. Details on then randomization procedure can be found in Hansen et al. (2014b).

APPARATUS

Physiological activity was measured by recording HR and HRV using the Actiheart System (Cambridge Neurotechnology Ltd; Brage et al., 2005), a compact lightweight device that records HR and variability of R-R inter-beat intervals. The Actiheart clips onto a single ECG electrode (M-00-S/50 Blue Sensor) with a short ECG lead to another electrode that detects the ECG signal. The Actiheart was placed on the upper chest.

The Actiheart provides interbeat intervals with a resolution of 1 ms, which are used to calculate HR and HRV. HR was defined as the average HR in beats per minute for an analysis epoch of 1 min. In the frequency domain, HRV was measured as absolute high frequency power (HF; 0.15–0.4 Hz), absolute low frequency power (LF; 0.04–0.15 Hz) and their ratio (LF/HF). HF and LF were derived by fast Fourier transform of the spectrum. The HF component is known to reflect primarily parasympathetic influences. However, concerning the LF there is some controversy. It has been argued that LF reflects both sympathetic and parasympathetic activity; others argue that LF reflects only sympathetic activity (Malliani et al., 1991; Appelhans and Luecken, 2006; Xhyheri et al., 2012). In the time domain, HRV was measured as the root mean of the squared successive differences (RMSSD) of the inter-beat interval. The RMSSD reflects parasympathetic activity. The data was log transformed prior to analysis (Malik and Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). Artifacts were manually cleaned by visual inspection using the Actiheart program.

PROCEDURE

The study protocol and all experimental procedures were approved by the Ethics Committee at the facility in Mauston, WI, USA, and were in compliance with the Helsinki declaration for research ethics. Participants were recruited by both written and oral information about the study. Thus, the patients were invited to participate in a research project concerning nutrition and mental health. The participants were informed that the purpose of the study was to investigate if nutrition (fatty fish or an alternative meal like chicken, pork, or beef) would have any effects on mental health. They were also informed that they would be randomly assigned into two groups; one group that would eat *fatty fish* (portion size 150–300 g) three times a week and one group that would eat *meat* (e.g., chicken, pork, beef) meals three times a week for a period for 6 months (September–February). For information about the fish used in the intervention study, see Hansen et al. (2014a). The participants signed an informed consent form, and they were informed about their rights to withdraw from the study at any time for any reason without penalty. Thus, the study design was fully disclosed to all participants, and all participants provided informed consent.

Prior to (July) and toward the end (February) of the intervention period the participants went through a test procedure. Since the present study is part of a larger project concerning fatty fish consumption (Hansen et al., 2014a) these procedures involved collections of various kinds of data such as fasting blood sample, sleep data, self-report questionnaires as well as an experimental test procedure measuring psychophysiological activity during exposure to different executive function tasks. In order to obtain a broad measure of reactivity, psychophysiological activity was registered for 5 min of baseline (rest condition), during exposure to the different executive function tasks (i.e., Iowa Gambling Task, Tower of Hanoi, and N-back tasks; 0–3 back), and during 5 min of recovery (rest condition). The length of executive tasks varies from task to task, and person to person. Thus, the HRV was registered for a total period of 50–60 min. During these periods of time, one measurement epoch lasts for 1 min. This means that there are five measurements for baseline and recovery (five epochs of 1 min during a 5 min interval), and a varying number of measurements for the tasks. All participants were exposed to exactly the same experimental procedure before (pre) and after (post) the food intervention period. All participants were tested individually.

MEDICINAL USE

Copies of all medical records, from 1 month prior to 1 month after intervention, were available for a subset of participants. The various drugs administered were classified according to the Anatomic Therapeutic Chemical Classification System, which is controlled by the World Health Organization. For each participant, daily dosages of each pharmaceutical used continuously for at least 1 week prior to the pre-test period were registered. This was done to avoid inclusion of drugs to which the participants had not yet properly responded. The same procedure was carried out for the post-test. For the forthcoming data analysis, only categories C (cardiovascular drugs) and N06A (anti-depressants) were used actively in the analysis.

Some participants were excluded from the data analysis. Participation in the project was voluntary, and three participants withdrew for various reasons during the intervention period. Participants released from the facility or transferred to a different facility during the study were included in the analysis of the pre-test, but are of course not present in the post-data. Participants with partially missing journals were excluded from further analysis. For some participants, a substantial amount of missing data was observed in the HR and HRV data. Participants with more than 20% missing data were excluded from further analysis.

THE DATA TABLES

A data matrix was constructed from the pre-test data. It contained HR and HRV data for 49 participants. For each participant there were 40 variables recorded. These were the HR and the four HRV measures (LF, HF, LF/HF, and RMSSD) recorded during the eight stages of the experiment: baseline, the six psychological tests and recovery. A similar matrix was created from the post-intervention test data. To highlight the effect of the intervention on the HR and HRV, the pre-test data was subtracted from the post-test data,

thus yielding a matrix reflecting the change in HR and HRV measures. The resulting table is hereafter referred to as the change data. The creation of this matrix necessitated a full set of data from both before and after the intervention, and thus only 38 participants are included in this matrix. Five of the 11 participants lost to follow up belonged to the fish group, and six belonged to the control group. Data was logarithmically (base 10) transformed prior to analysis. Due to the variables being measured using different units of measurements, autoscaling was carried out whenever HR and HRV data was used in the same analysis. For analysis using only HR or HRV data, mean centering of the variables was carried out.

THE DATA ANALYTICAL TOOL

The data analytical tool used in the investigation here may be unfamiliar to many readers. Principal Component Analysis (PCA; Joliffe, 2002) and Partial Least Squares (Manne, 1987) belong to a group of methods which may be referred to as latent variable techniques. They are methods frequently employed in chemometrics and related fields. The strength of these methods comes from their ability to deal with collinear data structures, in which the variables exhibit correlations. Another advantage of these methods is the easily interpreted plots that result from such analysis, and a presentation of this is to be found in the Results section.

While a more detailed mathematical explanation can be found in, e.g., Manne (1987) and Joliffe (2002), a brief explanation is given here. Latent variable methods take a set of correlated, measured variables (like the HR and HRV data in this study) and create a new, smaller set of orthogonal latent variables. The latent variables are linear combinations of the measured variables, and may be constructed using different criteria. In PCA, the latent variables (called principal components) are constructed using the maximum variance criterion. This means that the set of principal components are the best lower-dimensional approximation of the data, and that they represent the major sources of variation in the data. This makes PCA well suited for exploratory analysis.

Just like any object (participant in our study) has a value for each measured variable, all objects have a *score* for each principal component. Similarly, the contribution of each measured variable to the latent variable is expressed as the variable's *loading*. Bivariate scatter plots of the first two score or loading vectors give the best two-dimensional presentation of the major correlation structures in the data, and reveal similarities and differences among the objects and variables in a data matrix. This soft modeling approach can be regarded as a hypothesis generator, since it visualizes relationships in the data that may otherwise go unnoticed. Latent variables can also be used to confirm or verify preconceived assumptions and hypotheses, since it is possible to carry out statistical tests on the latent variables. In the present work, the emphasis has been on data exploration, and less on quantification and hypothesis testing.

For the creation of regression models, other latent variable techniques are preferred. Rather than focusing on the variance in the predictors (the independent variables), PLS creates latent variables focusing on the covariance between the predictors and one or several response variables (the dependent variables). Because of the orthogonalization inherent in PLS, this regression technique does not suffer from the problem of collinear data that, e.g., multiple linear regression does.

The multivariate analyses were carried out using Sirius 9.0 from Pattern Recognition Systems AS, Bergen, Norway (www.prs.no).

RESULTS

Table 1 displays summary statistics for the HR and HRV data at pre- and post-test. For the executive functioning tasks (EF tasks) the average HRV and HR values for all cognitive tasks (Iowa Gambling Task, Tower of Hanoi, and N-back tasks; 0–3 back) are reported.

PRE-TEST

Plots of data are often useful conveyors of information. The data matrix from the pre-test period contains 40 measurements for each of the 49 participants. A graphical display of the information content in such a large data matrix may seem difficult to obtain. One alternative is to plot each variable separately (thus creating 40 plots), or to produce all possible combinations of bivariate plots of one variable against another. This creates 780 plots, which of course is impossible to carry out. Through PCA a two-dimensional projection capturing most of the variation in the data can be obtained, resulting in a bivariate plot to be used for interpretation. For this particular data set, a two component (two latent variables) PCA model captures nearly 70% of the variance in the data. A bivariate plot of these two latent variables is shown in **Figure 1**, and it is referred to as a score plot. Since the axes in **Figure 1** are latent variables, information from all 40 measured variables is used to create this plot showing information on all 49 participants. This multivariate nature of the axes makes the plot an excellent tool for detecting relationships among the individual participants. In **Figure 1**, members of the fish group are plotted in blue, while red has been used for the control group. This information has only been used to color code the plots, and is not included in the data material.

There is no discernable trace of any grouping of the samples; the future fish and control group members are spread evenly throughout the plot. This indicates that the randomization strategy has been successful, as there is no visible group separation prior to intervention. A t-test on the scores on the first principal component shows that there is no significant difference in the mean value of the score values of the fish group compared to the control group ($p = 0.38$, effect size Cohen's $d = 0.25$).

By changing the color coding (but not the underlying model) clustering can be revealed. **Figure 2** is the result of changing the color coding so that patients on anti-depressants are plotted in blue and those not using anti-depressants in red. The data analyzed and the resulting model is the same as in **Figure 1**, only the color coding has changed. **Figure 2** shows that patients using anti-depressants differ from the other patients by having a lower score on the first principal component. The findings from the PCA are confirmed by a t-test on the mean values of the scores on the first principal component. This results in a clear identification of a difference between the groups ($p = 0.0037$, effect size Cohen's $d = 1.01$).

While the score plot shows clustering, the corresponding loading plot is necessary to understand why groupings occur. **Figure 3**

Table 1 | Mean values (*M*) and SD for the heart rate (HR), the root mean square of successive differences (RMSSD), low frequency power (LF), high frequency power (HF), and the LF/HF ratio for both groups during pre- and post-test.

	Fish group			Control group		
	M	**SD**	***n***	***M***	**SD**	***n***
Pre-test						
Baseline HR	72.96	13.15	27	72.81	13.13	22
Baseline RMSSD	1.44	0.28	27	1.45	0.27	22
Baseline LF	3.02	0.40	27	2.90	0.40	22
Baseline HF	2.22	0.59	27	2.29	0.49	22
Baseline LF/HF	0.82	0.33	27	0.64	0.26	22
EF tasks HR	71.48	13.06	27	70.12	11.82	22
EF tasks RMSSD	7.23	0.34	26	7.05	3.87	22
EF tasks LF	2.82	0.59	26	2.86	0.37	22
EF tasks HF	2.14	0.75	26	2.36	0.46	22
EF tasks LF/HF	0.69	0.27	26	0.53	0.22	22
Recovery HR	70.47	12.08	26	69.71	12.14	22
Recovery RMSSD	1.62	0.31	27	1.63	0.27	22
Recovery LF	3.30	0.43	27	3.32	0.39	22
Recovery HF	2.61	0.61	27	2.69	0.46	22
Recovery LF/HF	0.79	0.41	27	0.72	0.28	22
Post-test						
Baseline HR	71.08	12.50	22	76.11	9.87	16
Baseline RMSSD	1.56	0.28	22	1.47	0.32	16
Baseline LF	3.11	0.40	22	2.99	0.59	16
Baseline HF	2.39	0.54	22	2.36	0.54	16
Baseline LF/HF	0.78	0.40	22	0.68	0.17	16
EF tasks HR	70.37	11.33	22	73.96	11.08	16
EF tasks RMSSD	1.54	0.36	22	1.52	0.26	16
EF tasks LF	2.92	0.47	22	2.81	0.49	16
EF tasks HF	2.37	0.70	22	2.38	0.49	16
EF tasks LF/HF	0.57	0.30	22	0.47	0.28	16
Recovery HR	69.75	9.98	22	75.17	10.81	16
Recovery RMSSD	1.63	0.30	22	1.49	0.23	16
Recovery LF	3.26	0.38	22	2.97	0.44	16
Recovery HF	2.61	0.62	22	2.44	0.40	16
Recovery LF/HF	0.73	0.33	22	0.60	0.27	16

The HRV data are log-transformed. The number of participants in each group is in the column n.

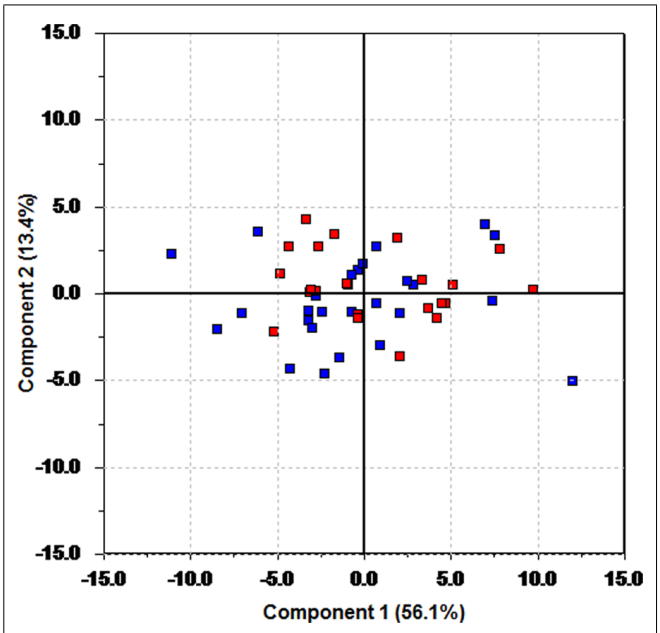


FIGURE 1 | Score plot of the first two principal components of the pre-test data. Blue color is used for the fish group, and red for the control group.

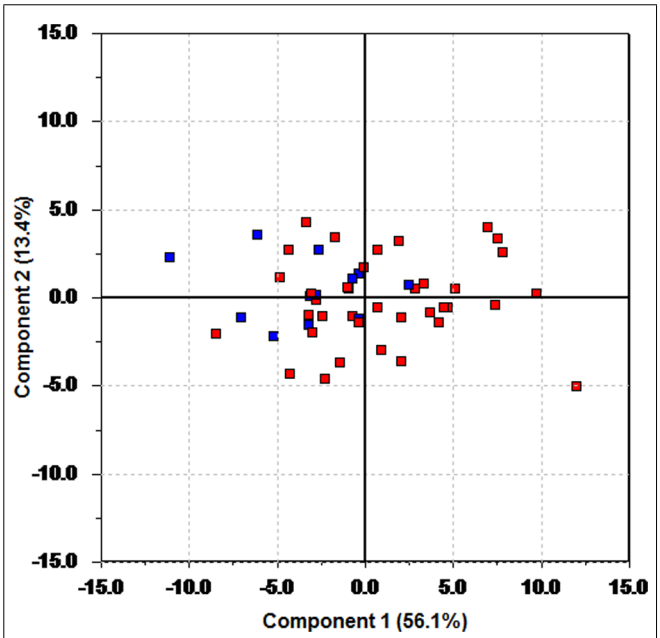


FIGURE 2 | Score plot of the first two principal components of the pre-test data. Blue color is used for participants using anti-depressants, and red for those without such usage.

shows the loading plot. Again, color coding has been used. HR variables are plotted in red, and blue has been used for the RMSSD variables. The HF variables are in black, and green has been used for LF. The LF/HF ratio is plotted in brown.

Figure 3 shows that participants with a low score on the first principal component (the patients using anti-depressants) have a tendency to have a higher HR (red) and LF/HF (brown) compared to the rest of the patients.

The recovery phase is an indicator on how the body adjusts to the normal situation after a period of mild stress. It was thought to be of interest to see if the patients on anti-depressants responded differently during recovery. This was done by creating PLS regression models that modeled recovery behavior as a function of behavior during baseline and the test period. Separate models

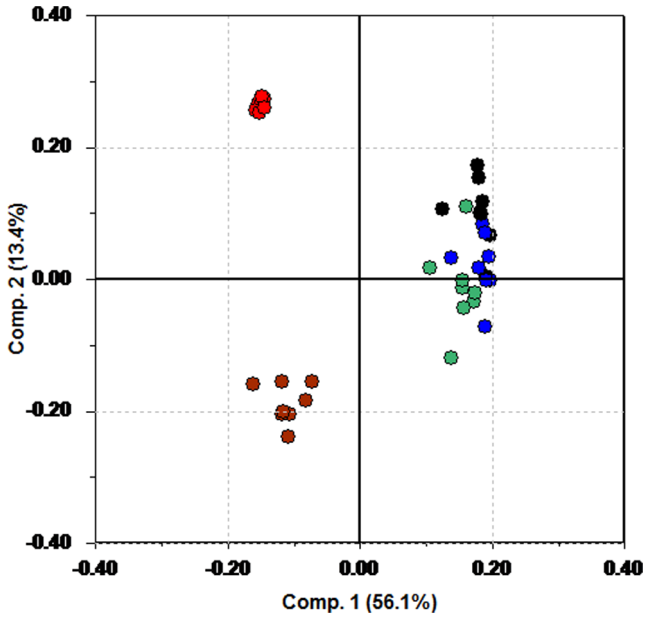


FIGURE 3 | Loading plot from PCA of the pre-test data. Red is HR, blue is RMSSD, black is HF, green is LF, and brown is used for LF/HF.

were created for HR and each of the individual HRV measures. In **Figure 4A**, a plot of modeled recovery versus actual recovery for HR is shown. Again, blue color is used to denote participants using anti-depressants. The blue line represents perfect match between modeled and actual response. **Figure 4A** shows a good correspondence between measured and estimated HR for the whole group. The HR behavior during recovery is possible to estimate for all test participants, regardless of medication use.

This situation changes when looking at some of the HRV measures. **Figure 4B** illustrates the situation for RMSSD. For several participants the model performance is poor. For five of the participants (to the right), the actual RMSSD is far higher than expected based on the model. There is also one participant (to the left) with a measured RMSSD far lower than expected according to the model. This interesting result shows that this group of patients, all using anti-depressants, undergoes a different recovery phase with regards to RMSSD, compared to the other patients. A similar behavior is experienced when studying LF, but not for HF, or LF/HF.

THE CHANGE DATA

The most important data table investigated in this work was created by subtracting the pre-test data from the post-test data. This was done to focus on each participant's change in HR and HRV during the intervention. The score plot from PCA on this data set (not shown), shows little sign of discrimination between the fish and control group. A *t*-test on the scores confirms this ($p = 0.0794$, Cohen's $d = 0.60$).

However, clear separation between the fish and control group can be achieved by controlling for medication. In **Figure 5**, two score plots from PCA on a subset of participants free from heart medication and anti-depressants are shown. Again, blue represents intervention and red control. **Figure 5A** shows the results from a PCA on this subset of participants. The ellipses in the plot are Hotelling's T^2 limits (Jackson, 1991), which can be regarded as a multivariate generalization of a *t*-test. An outlier is present in the upper right quadrant of the plot. Removal of this outlier and recalculation of the model yields the score plot in **Figure 5B**. Separation is clearly visible, and a *t*-test on the mean value of the scores of the first principal component ($p = 0.00029$, Cohen's $d = 2.72$) show that the two groups are different, and that the fish group has undergone a change in HR and HRV different to the intervention group.

A closer look at the status of subjects in the fish group is presented in **Figure 6**. This analysis was performed on the patients whose medication was constant throughout the intervention. Red is used to indicate the participants on constant medication, while blue is used for the ones who did not receive any medication. With the small sample size here, one should be careful with drawing strong conclusions. However, separation of the groups is evident from the score plot, as the patients not using medication is positioned more in the lower right part of the plot. This indicates a difference in response to the intervention. Still, the small sample size makes this observation less certain than the others presented in this work. We have therefore not carried out any additional statistical tests, but the plot generates the interesting hypothesis that the participants on medication respond differently to the fish diet.

The loading plot in **Figure 7** illuminates why there is a separation. The same color coding is used in this loading plot as in **Figure 3**. HR variables are plotted in red, and blue has been used for the RMSSD variables. The HF variables are in black, and green has been used for LF. The LF/HF ratio is plotted in brown.

In the upper left region we find some of the LF, HF, and RMSSD measures, whereas larger change in HR and LF/HF measures seem to be associated with the participants not on medication.

DISCUSSION

The use of latent variables as a data analytical tool is probably new to many readers. We do not suggest that these methods should replace traditional statistical tests, but rather complement them. Latent variables, in conjunction with statistical tests, showed a successful randomization procedure, as seen in **Figure 1**. A priori knowledge of group membership can still be used effectively by color coding the participants and variables accordingly to highlight the information contained in the variance pattern of the data. A PCA on the change data was initially unable to separate the fish group from the control group ($p = 0.0794$, Cohen's $d = 0.60$). This is due to the criterion used to calculate the principal components. The maximum variance criterion forces the principal components to pick up the major variation sources, at the expense of leaving minor, systematic changes unaccounted for. The effect of medication was strong enough to hide the effect of the intervention. Only by controlling for medicinal use a separation of the two groups became visible in the score plots ($p = 0.00029$, Cohen's $d = 2.72$). Although there of course are huge individual HR and HRV variations due to other external factors, the intervention group in general scores higher on the first principal component. This demonstrates that whether or not a participant is

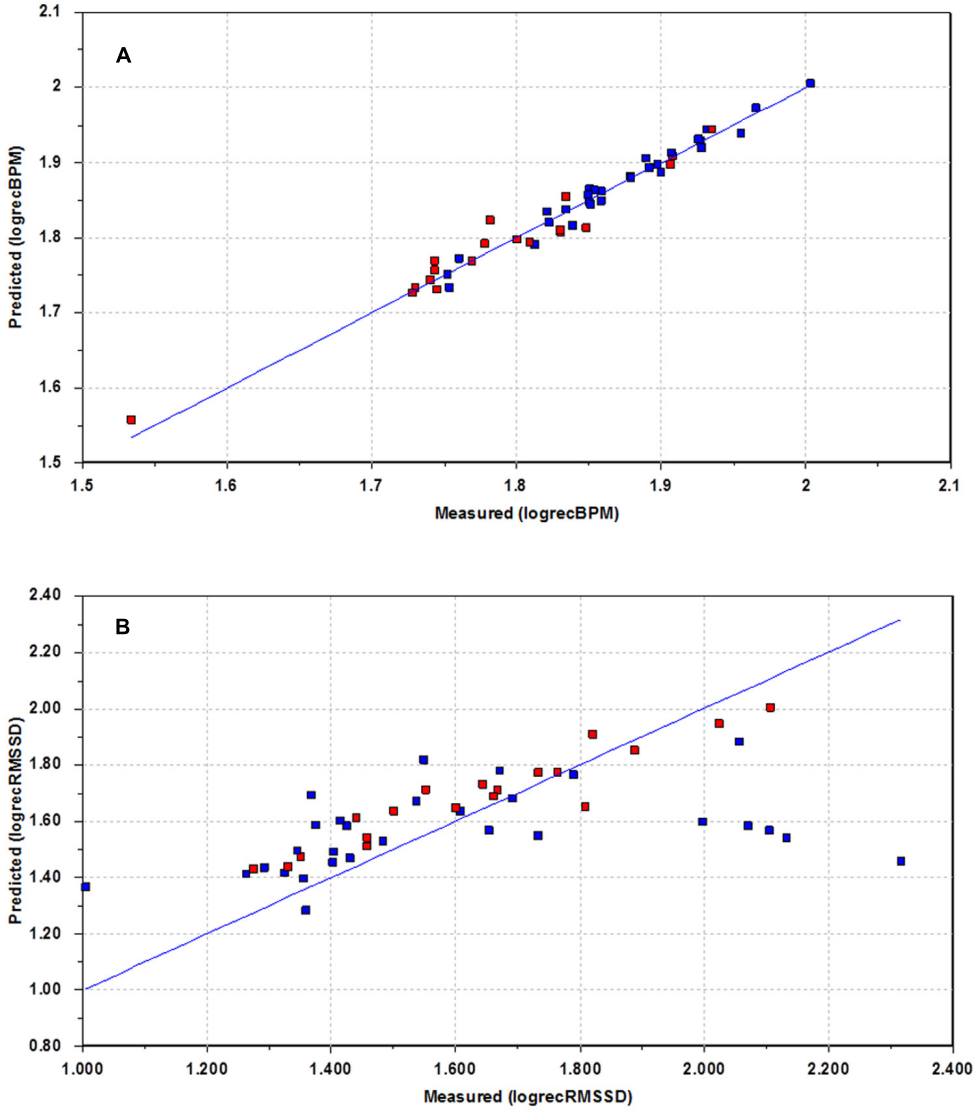


FIGURE 4 | Plots of estimated recovery versus measured recovery. Blue – uses heart medication and/or anti-depressants. Red – does not use drugs. **(A)** Recovery HR. **(B)** Recovery RMSSD.

on the fish diet is a major source of variation in the data. A similar plot (not shown here) appears if one investigates only the subset of participants, whose medication remained constant throughout the intervention period, again indicating that the introduction of fatty fish to the diet is a major source of variation once medication is controlled for.

The use of quantitative regression modeling using latent variables has been shown for parts of the recovery data. The poor performance of the model for RMSSD prediction for some of the participants indicates that the recovery phase is different for some of the participants on heavy medications. For the majority of participants the model performance is good, so an interesting question is to ask why the model fails for some participants. A closer look at the medical records reveals that the participants in question are using a variety of heart medications and anti-depressants; up to eight different drugs at the same time. Thus, participants with a higher level of medication respond differently during the recovery phase to the normal population. Five of the six participants for which the prediction fails were using statins, a cholesterol lowering drug which previously has been shown to improve HRV (Riahi et al., 2002). Four participants were using fibrates, another cholesterol lowering drug which has been reported to improve HRV (Melenovsky et al., 2003). There seems to be little consensus in the literature as to what the effects of SSRIs are on HRV (van Zyl et al., 2008; Kemp et al., 2010; Licht et al., 2010). However, a recent large independent cohort study showed that different antidepressants (e.g., tricyclic antidepressant, SSRI) were related to lower HRV (Kemp et al., 2014). The fact that the present recovery prediction model fails for the HRV data for the participants on heavy medication, again

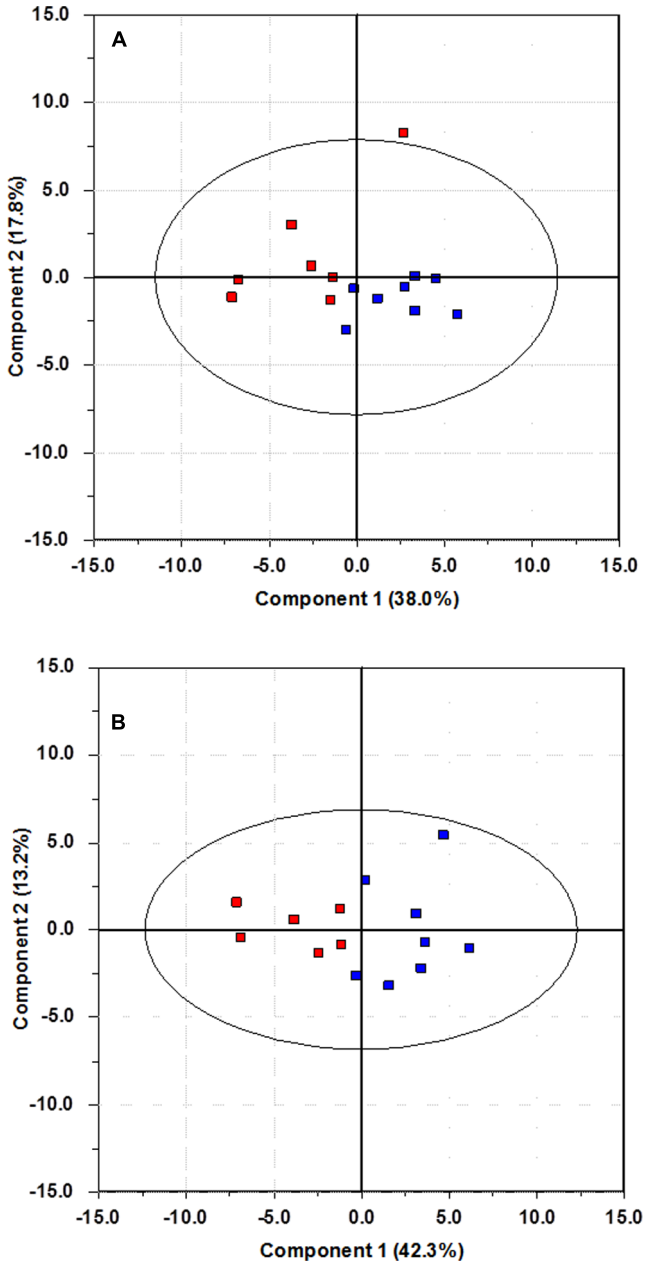


FIGURE 5 | Score plot of the first two principal components of the change data for the participants using neither heart medication nor anti-depressants. Blue color is used for the fish group, and red for the control group. **(A)** Outlier included. **(B)** Outlier excluded.

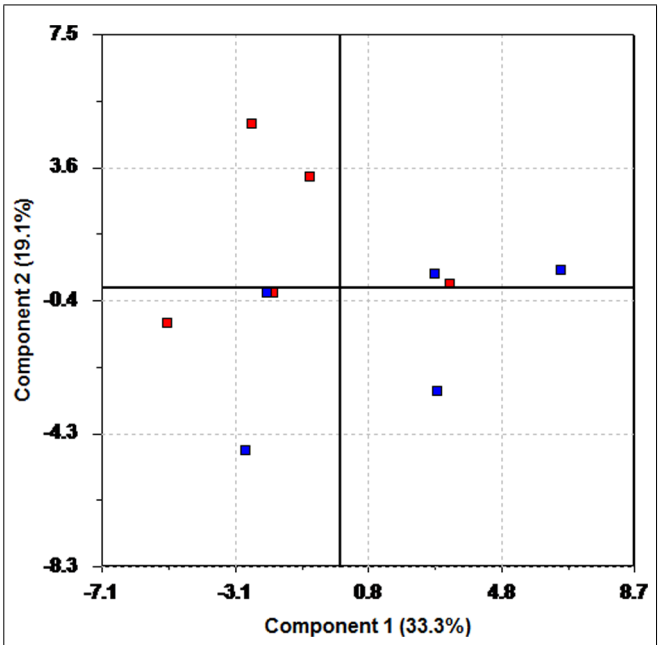


FIGURE 6 | Score plot of the first two principal components of the change data for the participants in the fish group having either a constant drug regime (blue) or receiving no medication (red).

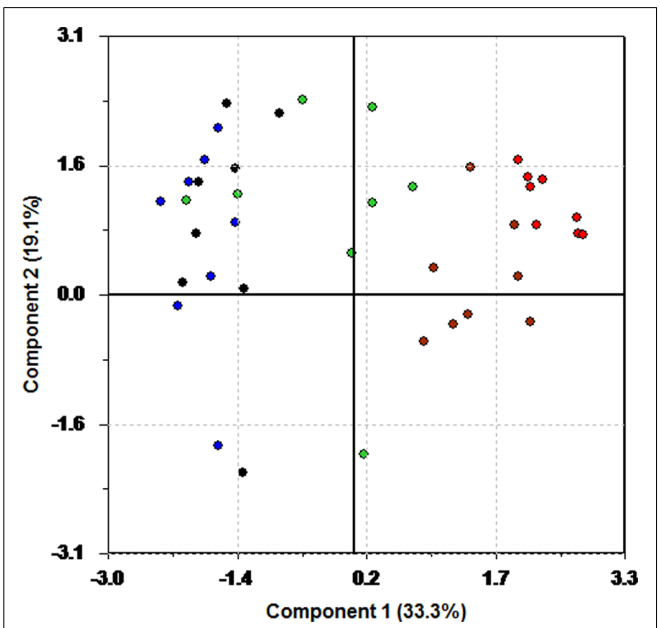


FIGURE 7 | Loading plot of the change data using only the participants in the fish group having a constant drug regime or receiving no medication. Red: HR. Blue: RMSSD. Green: LF. Black: HF. Brown: LF/HF.

demonstrates the strong masking effect medication has on the HRV.

The participants in this study were adult male forensic inpatients. This is a group for which cognitive and executive functions deficits have been demonstrated before (Galski et al., 1990). Thus, based on the very high RMSSD during recovery in these participants on a mix of medication, one can speculate whether the level of HRV during recovery may add some information about the physiological effort invested in the task before the recovery. The results fit a previously observed pattern, where increased HRV was observed after exposure to a similar experimental mild-stress procedure (Hansen et al., 2003). Another study (Hansen and Johnsen, 2013) investigating the relationship between levels of neuroticism and performance on non-executive functioning tasks (tasks based on processes driven automatically), in both non-threatening and

threatening situations, showed that participants with high neuroticism exposed to a threat had a significant increase in HRV from the task to recovery. This effect was absent when high neuroticism participants were not exposed to threats. For low neuroticism participants, this effect was absent regardless of threat level. In this latter study it was speculated whether the absence of threat in the recovery period caused the significant increase in HRV from test to recovery, since subjects with high neuroticism usually are characterized by low HRV. However, more research is needed to conclude whether information from the recovery measure can add something significant concerning mental and physical health.

Recently it was reported a strong relationship between tricyclic antidepressant and coronary heart disease (Kemp et al., 2015). Thus, as persons on heart medication and anti-depressants on average can be regarded as having poorer health (and thus lower life expectancy) than the general population, it is of interest to look deeper into the role of a fish diet for this group. The score and loading plots in **Figures 6** and **7** generate the hypothesis that the participants using heart medication and/or anti-depressants throughout the fish intervention period experienced a larger reduction in LF than the ones not using drugs. Thus, considering the debate about the autonomic origins of LF power (Thayer et al., 2010; Xhyheri et al., 2012), the fact that 2 min data epochs provide better estimates of LF (Thayer et al., 2010), and the present findings, one could speculate whether the LF represents the sympathetic activity, and that intake of fatty fish may be even more beneficial for the participants on heart medication. However, there is empirical evidence that LF power correlate highly with HF power (Thayer and Lane, 2007). Because of this and the small sample size, further investigation is necessary.

As with many diet intervention studies, the number of participants in this study is low. This becomes even more evident if trying to control for the different types of medication in use. The number of participants is not large enough to warrant a more thorough analysis of the effects of the different medicines. One may also speculate as to whether the effect of the medicines may really be due to the underlying pathology, and not the medication. There is a debate concerning this relationship and a recent study focusing on depression demonstrated that reduction in HRV was related to pathophysiological mechanisms rather than the effect of antidepressant (SSRI; see Brunoni et al., 2013). In the present study, all participants diagnosed with CVD or depression was on medication. To discern between medication and pathology is therefore not possible using this data set. Still, there are numerous reports in the literature about the effects of various medications on HR and HRV, and the effect we observe in our study thus seems reasonable. The consistency in which the participants group when controlling for medication gives credence to the findings presented here. The focus on the change data and the subsequent explorative multivariate analysis is not meant to replace traditional statistics. We have also employed *t*-tests on the latent variables to indicate whether the observed groupings are reasonable. Used in this way, this approach represents a valuable addition to the data analytical tools normally employed.

The original motivation behind this intervention experiment was to look at the effect of the nutrition on mental health (e.g., Hansen et al., 2014a,b). The *physical* health benefits of introducing fatty fish to our diet has been amply demonstrated in earlier publications. Van Horn et al. (2008) present a thorough review of this dietary aspect with regards to CVDs. For institutions having a clientele with a lower than average life expectancy, this effect should be factored in when designing health improvement plans. The question that remains to be answered is whether increased fatty fish consumption can enhance the effect of traditional medical treatment such as antidepressants and heart medication and further increase the life expectancy. It has been reported that while anti-depressants reduce the symptoms of depression, they may leave the HRV at sub-optimal levels. As HRV is a strong predictor of cardiac status, this indicates that persons suffering from depression are vulnerable to CVDs. Thus, based on the present data exploration it can be hypothesized that the beneficial effects of an increased fatty fish intake are even larger for this group. More knowledge about this will have important implications with regards to the development of health improvement interventions in psychiatric institutions.

AUTHOR CONTRIBUTIONS

All authors have contributed to the design, analysis, interpretation, or acquisition. Drafting, revision, and final approval has been conducted by all authors. All authors agree to be accountable for all aspects of the work.

ACKNOWLEDGMENTS

The present study was supported by grants from the "Program board Nutrition, University of Bergen, Norway." The authors wish to thank all participants for their cooperation. Thanks to Grethe Rosenlund at Skretting for providing the Atlantic salmon. We also wish to thank the kitchen staff for preparing all the meals and the health department for collecting blood samples at the secure forensic inpatient facility in US.

REFERENCES

Anda, R., Williamson, D., Jones, D., Macera, C., Eaker, E., Glassman, A., et al. (1993). Depressed affect, hopelessness, and the risk of ischemic heart disease in a cohort of US adults. *Epidemiology* 4, 285–294. doi: 10.1097/00001648-199307000-00003

Appelhans, B. M., and Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Rev. Gen. Psychol.* 10, 229–240. doi: 10.1037/1089-2680.10.3.229

Balogh, S., Fitzpatrick, D. F., Hendricks, S. E., and Paige, S. R. (1993). Increases in heart rate variability with successful treatment in patients with major depressive disorder. *Psychopharmacol. Bull.* 29, 201–206.

Barefoot, J. C., Helms, M. J., Mark, D. B., Blumenthal, J. A., Califf, R. M., Haney, T. L., et al. (1996). Depression and long-term mortality risk in patients with coronary artery disease. *Am. J. Cardiol.* 78, 613–617. doi: 10.1016/S0002-9149(96)00380-3

Bonaduce, D., Petretta, M., Ianniciello, A., Apicella, C., Cavallaro, V., and Marciano, F. (1997). Comparison of verapamil versus felodipine on heart rate variability after acute myocardial infarction. *Am. J. Cardiol.* 79, 564–569. doi: 10.1016/S0002-9149(96)00816-8

Brage, S., Brage, N., Franks, P. W., Ekelund, U., and Wareham, N. J. (2005). Reliability and validity of the combined heart rate and movement sensor actiheart. *Eur. J. Clin. Nutr.* 59, 561–570. doi: 10.1038/sj.ejcn.1602118

Brunoni, A. R., Kemp, A. H., Dantas, E. M., Goulart, A. C., Nunes, M. A., Boggio, P. S., et al. (2013). Heart rate variability is a trait marker of major depressive disorder: evidence from the sertraline vs. electric current therapy to treat depression clinical study. *Int. J. Neuropsychopharmacol.* 16, 1937–1949. doi: 10.1017/S1461145713000497

Carney, R. M., Rich, M. W., Tevelde, A., Saini, J., Clark, K., and Jaffe, A. S. (1987). Major depressive disorder in coronary artery disease. *Am. J. Cardiol.* 60, 1273–1275. doi: 10.1016/0002-9149(87)90607-2

Christensen, J. H., Skou, H. A., Fog, L., Hansen, V. E., Vesterlund, T., Dyerberg, J., et al. (2001a). Marine n-3 fatty acids, wine intake, and heart rate variability in patients referred for coronary angiography. *Circulation* 103, 651–657. doi: 10.1161/01.CIR.103.5.651

Christensen, J. H., Skou, H. A., Madsen, T., Tørring, I., and Schmidt, E. B. (2001b). Heart rate variability and n-3 polyunsaturated fatty acids in patients with diabetes mellitus. *J. Intern. Med.* 249, 545–552. doi: 10.1046/j.1365-2796.2001.00841.x

Cook, J. R., Bigger, J. T. Jr., Kleiger, R. E., Fleiss, J. L., Steinman, R. C., and Rolnitzky, L. M. (1991). Effect of atenolol and diltiazem on heart period variability in normal persons. *J. Am. Coll. Cardiol.* 17, 480–484. doi: 10.1016/S0735-1097(10)80119-6

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., et al. (2008). Independent association of low serum 25-hydroxyvitamin D and 1, 25-Dihydroxyvitamin D levels with all-cause and cardiovascular mortality. *Arch. Intern. Med.* 168, 1340–1349. doi: 10.1001/archinte.168.12.1340

Ebbehøj, E., Poulsen, P. L., Hansen, K. W., Knudsen, S. T., Mølgaard, H., and Mogensen, C. E. (2002). Effects on heart rate variability of metoprolol supplementary to ongoing ACE-inhibitor treatment in Type I diabetic patients with abnormal albuminuria. *Diabetologia* 45, 965–975. doi: 10.1007/s00125-002-0869-7

Galski, T., Thornton, K. E., and Shumsky, D. (1990). Brain dysfunction in sex offenders. *J. Offender Rehabil.* 16, 65–80. doi: 10.1300/J076v16n01_04

Giovannucci, E., Liu, Y., Hollis, B. W., and Rimm, E. B. (2008). 25-Hydroxyvitamin D and risk of myocardial infarction in men. *Arch. Intern. Med.* 168, 1174–1180. doi: 10.1001/archinte.168.11.1174

Gomes, M. E., Tack, C. J., Verheugt, F. W., Smits, P., and Lenders, J. W. (2010). Sympathoinhibition by atorvastatin in hypertensive patients. *Circulation* 74, 2622–2626. doi: 10.1253/circj.CJ-10-0427

Gonzalez, M. B., Snyderman, T. B., Colket, J. T., Arias, R. M., Jiang, J. W., O'Connor, C. M., et al. (1996). Depression in patients with coronary artery disease. *Depression* 4, 57–62. doi: 10.1002/(SICI)1522-7162(1996)4:2<57::AID-DEPR3>3.0.CO;2-C

Guedon-Moreau, L., Pinaud, A., Logier, R., Caron, J., Lekieffre, J., Dupuis, B., et al. (1997). Effect of ramipril on heart rate variability in digitalis-treated patients with chronic heart failure. *Cardiovasc. Drugs Ther.* 11, 531–536. doi: 10.1023/A:1007763131888

Hansen, A. L., Dahl, L., Bakke, L., Frøyland, L., and Thayer, J. F. (2010). Fish consumption and heart rate variability. Preliminary results. *J. Psychophysiol.* 24, 41–47. doi: 10.1027/0269-8803/a000005

Hansen, A. L., Dahl, L., Olson, G., Thornton, D., Graff, I. E., Frøyland, L., et al. (2014a). Fish consumption, sleep, daily functioning, and heart rate variability. *J. Clin. Sleep Med.* 10, 567–575. doi: 10.5664/jcsm.3714

Hansen, A. L., Olson, G., Dahl, L., Thornton, D., Grung, B., Graff, I. E., et al. (2014b). Reduced anxiety in forensic inpatients after a long-term intervention with Atlantic salmon. *Nutrients* 6, 5405–5418. doi: 10.3390/nu6125405

Hansen, A. L., and Johnsen, B. H. (2013). Relationship between neuroticism, threat of shock and HRV reactivity. *Int. Marit. Health* 64, 54–60.

Hansen, A. L., Johnsen, B. H., Sollers, J. J., Stenvik, K., and Thayer, J. F. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur. J. Appl. Physiol.* 93, 263–272. doi: 10.1007/s00421-004-1208-0

Hansen, A. L., Johnsen, B. H., and Thayer, J. F. (2003). Vagal influence on working memory and attention. *Int. J. Psychophysiol.* 48, 263–274. doi: 10.1016/S0167-8760(03)00073-4

Hansen, A. L., Johnsen, B. H., and Thayer, J. F. (2009). Relationship between heart rate variability and cognitive function during threat of shock. *Anxiety Stress Coping* 22, 1–12. doi: 10.1080/10615800802272251

Hibbeln, J. R., Ferguson, T. A., and Blasbalg, T. L. (2006). Omega-3 fatty acid deficiencies in neurodevelopment, aggression, and autonomic dysregulation: opportunities for intervention. *Int. Rev. Psychiatry* 18, 107–118. doi: 10.1080/09540260600582967

Huikuri, H., Raatikainen, M., Moerch-Joergensen, R., Hartikainen, J., Virtanen, V., Boland, J., et al. (2009). Prediction of fatal or near-fatal cardiac arrhythmia events in patients with depressed left ventricular function after an acute myocardial infarction. *Eur. Heart J.* 30, 689–698. doi: 10.1093/eurheartj/ehn537

Jackson, J. E. (1991). *A User's Guide To Principal Components*. New York: John Wiley & Sons, Inc. doi: 10.1002/0471725331

Joliffe, I. T. (2002). *Principal Component Analysis*. New York: Springer.

Kemp, A. H., Brunoni, A. R., Bittencourt, M. S., Nunes, M. A., Benseñor, I. M., and Lotufo, P. A. (2015). The association between antidepressant medications and coronary heart disease in Brazil: a cross-sectional analysis on the Brazilian longitudinal study of adult health (ELSA-Brazil). *Front. Public Health* 3:9. doi: 10.3389/fpubh.2015.00009

Kemp, A. H., Brunoni, A. R., Santos, I. S., Nunes, M. A., Dantas, E. M., de Figueiredo, R. C., et al. (2014). Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil cohort baseline study. *Am. J. Psychiatry* doi: 10.1176/appi.ajp.2014.13121605 [Epub ahead of print].

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., and Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol. Psychiatry* 67, 1067–1074. doi: 10.1016/j.biopsych.2009.12.012

Khaykin, Y., Dorian, P., Baker, B., Shapiro, C., Sandor, P., Mironov, D., et al. (1998). Autonomic correlates of antidepressant treatment using heart-rate variability analysis. *Can. J. Psychiatry* 43, 183–186.

Kleiger, R. E., Stein, P. K., and Bigger, J. T. (2005). Heart rate variability: measurement and clinical utility. *Ann. Noninvasive Electrocardiol.* 10, 88–101. doi: 10.1111/j.1542-474X.2005.10101.x

Lansdowne, A. T. G., and Provost, S. C. (1998). Vitamin D3 enhances mood in healthy subjects during winter. *Psychopharmacology (Berl.)* 135, 319–323. doi: 10.1007/s002130050517

Licht, C. M., de Geus, E. J., van Dyck, R., and Penninx, B. W. (2010). Longitudinal evidence for unfavorable effects of antidepressants on heart rate variability. *Biol. Psychiatry* 68, 861–868. doi: 10.1016/j.biopsych.2010.06.032

Malik, M., and Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Malliani, A., Pagani, M., Lombardi, F., and Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency-domain. *Circulation* 84, 482–492. doi: 10.1161/01.CIR.84.2.482

Manne, R. (1987). Analysis of two partial-least-squares algorithms for multivariate calibration. *Chemometr. Intell. Lab. Syst.* 2, 187–197. doi: 10.1016/0169-7439(87)80096-5

Melenovsky, M., Wichterle, D., Simek, J., Malik, J., Haas, T., Ceska, R., et al. (2003). Effect of atorvastatin and fenofibrate on autonomic tone in subjects with combined hyperlipidemia. *Am. J. Cardiol.* 92, 337–341. doi: 10.1016/S0002-9149(03)00643-X

Mølgaard, H., Mickley, H., Pless, P., Bjerregaard, P., and Møller, M. (1993). Effects of metoprolol on heart rate variability in survivors of acute myocardial infarction. *Am. J. Cardiol.* 71, 1357–1359. doi: 10.1016/0002-9149(93)90555-Q

Mozaffarian, D., Stein, P. K., Prineas, R. J., and Siscovick, D. S. (2008). Dietary fish ω-3 fatty acid consumption and heart rate variability in US adults. *Circulation* 117, 1130–1137. doi: 10.1161/CIRCULATIONAHA.107.732826

Niemelä, M. J., Airaksinen, K. E., and Huikuri, H. V. (1994). Effect of beta-blockade on heart rate variability in patients with coronary artery disease. *J. Am. Coll. Cardiol.* 23, 1370–1377. doi: 10.1016/0735-1097(94)90379-4

Nome, S., and Holsten, F. (2012). Changes in mortality after first psychiatric admission: a 20-year prospective longitudinal clinical study. *Nord. J. Psychiatry* 66, 97–106. doi: 10.3109/08039488.2011.605170

Partonen, T. (1998). Vitamin D and serotonin in winter. *Med. Hypotheses* 51, 267–268. doi: 10.1016/S0306-9877(98)90085-8

Penninx, B. W., Beekman, A. T., Honig, A., Deeg, D. J., Schoevers, R. A., van Eijk, J. T., et al. (2001). Depression and cardiac mortality: results from a community-based longitudinal study. *Arch. Gen. Psychiatry* 58, 221–227. doi: 10.1001/archpsyc.58.3.221

Perica, M. M., and Delas, I. (2011). Essential fatty acids and psychiatric disorders. *Nutr. Clin. Pract.* 26, 409–425. doi: 10.1177/0884533611411306

Pinar, E., Garcia-Alberola, A., Llamas, C., Vicente, T., Lopez-Candel, J., Rojo, J. L., et al. (1998). Effects of verapamil on indexes of heart rate variability after acute myocardial infarction. *Am. J. Cardiol.* 81, 1085–1089. doi: 10.1016/S0002-9149(98)00128-3

Porges, S. W., and Raskin, D. C. (1969). Respiratory and heart rate components of attention. *J. Exp. Psychol.* 81, 497–503. doi: 10.1037/h0027921

Pratt, L. A., Ford, D. E., Crum, R. M., Armenian, H. K., Gallo, J. J., and Eaton, W. W. (1996). Depression, psychotropic medication, and risk of myocardial infarction. Prospective data from the Baltimore ECA follow-up. *Circulation* 94, 3123–3129. doi: 10.1161/01.CIR.94.12.3123

Quintana, D. S., and Heathers, J. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Front. Psychol.* 5:805. doi: 10.3389/fpsyg.2014.00805

Riahi, S., Christensen, J. H., Toft, E., Skou, H. A., and Schmidt, E. B. (2002). HMG-CoA reductase inhibitors improve heart rate variability in patients with a previous myocardial infarction. *Pharmacol. Res.* 45, 479–483. doi: 10.1006/phrs.2002.0988

Ross, B. M., Seguin, J., and Sieswerda, L. E. (2007). Omega-3 fatty acids as treatments for mental illness: which disorder and which fatty acid? *Lipids Health Dis.* 6, 1–19. doi: 10.1186/1476-511X-6-21

Sandrone, G., Mortara, A., Torzillo, D., La Rovere, M. T., Malliani, A., and Lombardi, F. (1994). Effects of beta blockers (atenolol or metoprolol) on heart rate variability after acute myocardial infarction. *Am. J. Cardiol.* 74, 340–345. doi: 10.1016/0002-9149(94)90400-6

Shehab, A., Elnour, A. A., and Struthers, A. D. (2008). A randomised, controlled, double-blind, cross-over pilot study assessing the effects of spironolactone, losartan and their combination on heart rate variability and QT dispersion in patients with chronic heart failure. *Cardiovasc. J. Afr.* 19, 292–296.

Shimamura, A. P. (2000). The role of the prefrontal cortex in dynamic filtering. *Psychobiology* 28, 207–218. doi: 10.3758/BF03331979

Stumpf, W. E., and Privette, T. H. (1989). Light, vitamin D and psychiatry: role of 1,25- dihydroxyvitamin D3 (soltriol) in etiology and therapy of seasonal affective disorder and other mental processes. *Psychopharmacology (Berl.)* 97, 285–294. doi: 10.1007/BF00439440

Thayer, J. F., Hansen, A. L., and Johnsen, B. H. (2010). "The non-invasive assessment of autonomic influences on the heart using impedance cardiography and heart rate variability," in *Handbook of Behavioural Medicine – Methods and Applications*, ed. A. Steptoe (London: Springer), 723–740.

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biol. Psychol.* 74, 224–242. doi: 10.1016/j.biopsycho.2005.11.013

Thayer, J. F., and Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Timonen, M., Horrobin, D., Jokelainen, J., Laitinen, A. H., and Räsänen, P. (2004). Fish consumption and depression: the Northern Finland 1966 birth cohort study. *J. Affect. Disord.* 82, 447–452. doi: 10.1016/j.jad.2004.02.002

Van Horn, L., McCoin, M., Kris-Etherton, P. M., Burke, F., Carson, J. A., Champagne, C. M., et al. (2008). The evidence for dietary prevention and treatment of cardiovascular disease. *J. Am. Diet. Assoc.* 108, 287–331. doi: 10.1016/j.jada.2007.10.050

van Zyl, L. T., Hasegawa, T., and Nagata, K. (2008). Effects of antidepressant treatment on heart rate variability in major depression: a quantitative review. *Biopsychosoc. Med.* 2, 12 doi: 10.1186/1751-0759-2-12

Welzig, C. M., Shin, D. G., Park, H. J., Kim, Y. J., Saul, J. P., and Galper, J. N. (2003). Lipid lowering by pravastatin increases parasympathetic modulation of heart rate: Galpha(i2), a possible molecular marker for parasympathetic responsiveness. *Circulation* 108, 2743–2746. doi: 10.1161/01.CIR.0000103680.61390.16

Xhyheri, B., Manfrini, O., Mazzolini, M., Pizzi, C., and Bugiardini, R. (2012). Heart rate variability today. *Prog. Cardiovasc. Dis.* 55, 321–331. doi: 10.1016/j.pcad.2012.09.001

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 September 2014; accepted: 26 January 2015; published online: 17 February 2015.

Citation: Grung B, Hansen AL, Berg M, Møen-Knudseth MP, Olson G, Thornton D, Dahl L and Thayer JF (2015) Exploratory multivariate analysis of the effect of fatty fish consumption and medicinal use on heart rate and heart rate variability data. Front. Psychol. ***6****:135. doi: 10.3389/fpsyg.2015.00135*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in
PSYCHOLOGY

REVIEW ARTICLE
published: 04 March 2015
doi: 10.3389/fpsyg.2015.00147

A brief review of exercise, bipolar disorder, and mechanistic pathways

Daniel Thomson[1], Alyna Turner[2,3,4]*, Sue Lauder[3,5], Margaret E. Gigler[6], Lesley Berk[2,7], Ajeet B. Singh[2], Julie A. Pasco[2,8], Michael Berk[2,3,9,10] and Louisa Sylvia[6,11]

[1] Department of Applied Sciences, Royal Melbourne Institute of Technology University, Bundoora, VIC, Australia
[2] Innovation in Mental and Physical Health and Clinical Treatment Strategic Research Centre, School of Medicine, Deakin University, Geelong, VIC, Australia
[3] Department of Psychiatry, University of Melbourne, Parkville, VIC, Australia
[4] Centre for Translational Neuroscience and Mental Health, School of Medicine and Public Health, University of Newcastle, Callaghan, NSW, Australia
[5] Federation University Australia, Ballarat, VIC, Australia
[6] Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA
[7] Mental Health and Wellbeing Strategic Research Centre, School of Psychology, Deakin University, Geelong, VIC, Australia
[8] Department of Medicine, NorthWest Academic Centre, University of Melbourne, St Albans, VIC, Australia
[9] Florey Institute for Neuroscience and Mental Health, Parkville, VIC, Australia
[10] Orygen, The National Centre of Excellence in Youth Mental Health, Parkville, VIC, Australia
[11] Harvard Medical School, Harvard University, Boston, MA, USA

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Tim Outhred, University of Sydney, Australia
Davy Vancampfort, KU Leuven, Belgium

***Correspondence:**
Alyna Turner, Innovation in Mental and Physical Health and Clinical Treatment Strategic Research Centre, School of Medicine, Deakin University, Kitchener House, P.O. Box 281, Geelong, VIC 3220, Australia
e-mail: alyna.turner@barwonhealth.org.au

Despite evidence that exercise has been found to be effective in the treatment of depression, it is unclear whether these data can be extrapolated to bipolar disorder. Available evidence for bipolar disorder is scant, with no existing randomized controlled trials having tested the impact of exercise on depressive, manic or hypomanic symptomatology. Although exercise is often recommended in bipolar disorder, this is based on extrapolation from the unipolar literature, theory and clinical expertise and not empirical evidence. In addition, there are currently no available empirical data on program variables, with practical implications on frequency, intensity and type of exercise derived from unipolar depression studies. The aim of the current paper is to explore the relationship between exercise and bipolar disorder and potential mechanistic pathways. Given the high rate of medical co-morbidities experienced by people with bipolar disorder, it is possible that exercise is a potentially useful and important intervention with regard to general health benefits; however, further research is required to elucidate the impact of exercise on mood symptomology.

Keywords: bipolar disorder, exercise, mechanistic pathways, depression, hypomania, neurogenesis

BACKGROUND

Bipolar disorder is a chronic condition characterized by elevated (manic) and depressive episodes often associated with difficulty functioning and poor quality of life. A diagnosis of bipolar disorder is also associated with an increased risk of cardiovascular disease leading to premature mortality (Roshanaei-Moghaddam and Katon, 2009; Dome et al., 2012; Crump et al., 2013). Further, obesity and a sedentary lifestyle are risk factors for diabetes, metabolic syndrome and cardiovascular disease, all of which disproportionally affect people with bipolar disorder (Elmslie et al., 2001; Morriss and Mohammed, 2005; Alsuwaidan et al., 2009; Cairney et al., 2009; Kilbourne et al., 2009). Thus, individuals with bipolar disorder face the dual struggle of needing to focus their attention and treatment on not only their mental health but also their physical health.

Exercise may be an excellent candidate to meet this need. Exercise unequivocally improves physical heath (e.g., obesity, cardiorespiratory fitness, blood pressure, cholesterol; Cornelissen and Fagard, 2005; Church et al., 2007; Department of Health, 2011), but recent data also suggest that exercise is an effective treatment of depression and anxiety (Daley, 2008; Wipfli et al., 2008; Rethorst et al., 2009; Moylan et al., 2013; Rethorst and Trivedi, 2013). These data have prompted some to view exercise as a first line of treatment for mild to moderate depression (Carek et al., 2011). Given the promising data for depression and anxiety, exercise may also prove to be beneficial for the management of bipolar disorder. Specifically, evidence suggests that exercise is neuroprotective at least in part by increasing brain derived neurotrophic factor (BDNF; Sylvia et al., 2010). Other mechanisms will be explored, including the genetic expression and endorphin hypothesis.

The aim of this review is to understand the amount of exercise and physical activity currently engaged in by individuals with bipolar disorder. For the purpose of this review, exercise is defined as a conscious, planned decision to move and be physically active, whereas physical activity refers to any movement, including leisure activity, occupational activity, or other activities of daily living (Caspersen et al., 1985; Thompson et al., 2003). A second aim is to evaluate the research on the role of exercise in improving physical (obesity, blood pressure) and mental (symptoms, quality

of life) health outcomes in bipolar disorder. Finally, we will discuss the potential mechanisms of how exercise is suspected of improving mood and functioning in bipolar disorder.

METHODS

We conducted our search using Google Scholar, Proquest, CINAHL Complete, PubMed, and PSYCINFO, including unpublished papers in the form of dissertation abstracts using the search terms bipolar disorder and exercise and bipolar disorder and physical activity. Based on this initial search, we found 628 articles. We conducted two independent reviews of the literature by two separate authors (DT and MEG). The review period was September through November, 2014. We found over 600 articles when searching for "bipolar disorder" and "exercise" or "bipolar disorder" and "physical activity." We then limited this search by only using studies that focused on adult participants with a diagnosis of bipolar disorder; studies that included participants below the age of 18 were excluded, as were studies that were not in the English language. Studies were also excluded if they did not explicitly focus on the effects of exercise on patients with mental illness or if they did not relate directly to the review topic (i.e., a study that monitored activity levels on patients via their smartphones saw lower levels of activity, but was merely correlational; Faurholt-Jepsen et al., 2014). Thus, 13 studies are included in this review (see **Figure 1**, **Table 1**).

RESULTS

PHYSICAL ACTIVITY LEVELS AND BIPOLAR DISORDER

We found 13 empirical studies that have examined the physical activity levels of individuals with bipolar disorder. In a sample of 60 outpatient adults with bipolar disorder, Janney et al. (2014) found that 78% of the 17 h day that participants wore their actigraphs was classified as sedentary (13.5 h per day) and that no participants achieved 150 min per week of moderate/vigorous exercise as recommended by UK national guidelines (National Institute for Health and Clinical Excellence, 2006). These findings are consistent with several other reports of high rates of physical inactivity in people with bipolar disorder (Elmslie et al., 2001; Kilbourne et al., 2007; Shah et al., 2007). These data are limited as the Elmslie et al. (2001) study only included patients that were currently euthymic and Kilbourne et al. (2007) despite having a large sample ($N = 2032$), utilized only a veteran population and included individuals with schizophrenia and did not have data on bipolar subtype or mood state.

Overall, physical activity levels in bipolar disorder appear to be lower than that of the general population (Elmslie et al., 2001; Janney et al., 2014), but the data are not conclusive. For example, a national survey in Canada found no significant differences in physical activity between people with and without bipolar disorder (Cairney et al., 2009). However, this study was limited by the use of self-report measures and the assessment of leisure-time physical activity only. Moreover, methodological variations, particularly with regard to the method of assessment of physical activity, make it difficult to compare across studies. In sum, given the many factors that negatively impact physical activity in bipolar disorder, such as higher rates of smoking, obesity, and medication side effects, it is not surprising that the data suggest that they are more likely to have sedentary lifestyles (Williams et al., 2009; Dodd et al., 2010; Vancampfort et al., 2013).

ADJUNCT PHYSICAL ACTIVITY AND BIPOLAR DISORDER

Reported studies are summarized in **Table 1**. Very few studies have examined the potential therapeutic effects of physical activity on bipolar disorder. Ng, Dodd, and Berk invited inpatients to participate in a walking group and found that it was associated with improvements in the domains of depression, anxiety and stress (Ng et al., 2007). Despite several limitations of this study, including small sample size, lack of control for confounding variables, open nature, and no specific measure for mania, the study provides useful preliminary data in establishing exercise as a viable treatment option for patients with bipolar disorder.

In a study conducted by Hays et al. (2008), 26 patients diagnosed with either Bipolar I or Bipolar II Disorder walked or ran on a treadmill for 20 min at 70% of their age-predicted maximal heart rate. Findings revealed significant increases in self-reported well-being and the hormone dehydroepiandrosterone sulfate, a precursor of the adrenal steroid dehydroepiandrosterone (Wright et al., 2009). Although levels of dehydroepiandrosterone and overall well-being improved over the study duration, there was no significant correlation between the two variables (Hays et al., 2008).

Daumit et al. (2013) recruited participants ($N = 291$) who suffered from serious mental illness—including bipolar disorder ($n = 64$, 22%), as well as schizophrenia, schizoaffective disorder, and major depression—to participate in an 18-month behavioral weight-loss intervention. The treatment consisted of group exercise sessions as well as individualized weight-management sessions. The intervention group lost more weight than the control group, such that 37.8% of participants in the intervention group lost at least 5% of their initial weight, compared with 22.7% in the control group. These findings show that overweight and obese individuals with serious mental illness are capable of implementing lifestyle changes taught by an intervention, despite the daily difficulties posed by their illness.

In another community-integrated program, Van Citters et al. (2010) developed a manual for an intervention program known as "In SHAPE," a lifestyle intervention manual for patients with serious mental illness. The pilot study included participants with schizophrenia, bipolar disorder, or major depressive disorder. Participants were assigned an individual health mentor and over 9 months work together to set goals regarding healthier dietary decisions as well as other modules of wellness. Importantly, mental health functioning significantly improved among participants, as did negative symptoms. Participation in the program was associated with increased exercise, vigorous activity, and leisurely walking. Participants also demonstrated a significant reduction in waist circumference.

Sylvia et al. (2011) developed an integrated psychosocial treatment, or the Nutrition, Exercise, and Wellness Treatment (NEW Tx), specifically to help individuals with bipolar disorder engage in healthier lifestyle habits. NEW Tx consists of three modules to target changes related to eating more nutritiously and with better portion control, increase weekly exercise as well as improve other areas of wellness (i.e., sleep, smoking/substance use). In

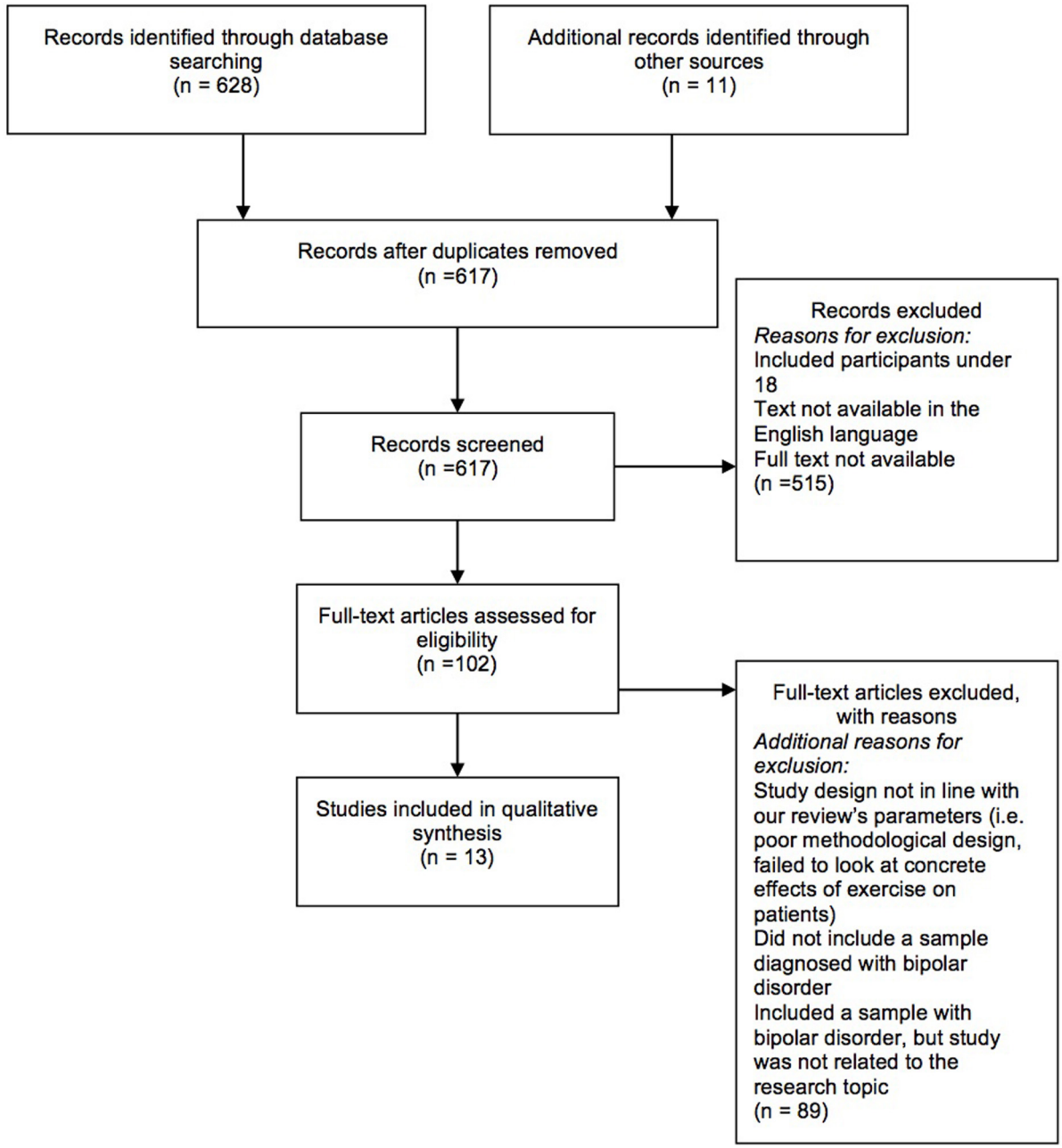


FIGURE 1 | Consort flowchart for study selection.

the NEW Tx pilot study, five participants completed the 20-week individual cognitive behavioral therapy-based treatment. Participants entering the study tended to be mildly to moderately depressed [baseline MADRS = 17.2 (5.2); baseline YMRS = 4.4 (2.0); Sylvia et al., 2013b]. Participants attended most of the NEW Tx sessions and reported high satisfaction with the treatment. Participants increased intake of vegetables and decreased their daily intake of sweets. Participants' weight, cholesterol (total, high-density lipoprotein cholesterol and low-density lipoprotein) triglycerides, and plasma glucose declined from baseline to 20 weeks follow-up. Moreover, participants experienced improvement of depressive symptoms and overall functioning as well as tripling their amount of exercise. This is one of the first studies to demonstrate the feasibility and tolerability of an intensive lifestyle intervention for bipolar disorder with promising data for its efficacy.

These lifestyle interventions hold promise in that they demonstrate that participants with serious mental illnesses can succeed in wellness programs that have been proven successful in the general population. In order to further examine the efficacy of

Table 1 | Summary of reported studies and their characteristics.

Author	Study design	Participants	Intervention	Results	Methodological quality
Elmslie et al. (2001)	Cross-sectional	89 participants with bipolar disorder, all of whom were currently in outpatient treatment psychiatric diagnoses were based on ICD-9 codes	Main outcome measures included macronutrient intakes, percentage of energy derived from food sources and physical activity levels	Mean total energy intake was higher in female patients than reference subjects. Patients also reported lower frequencies of physical activity compared to the reference subjects	Participants' data were collected from a VA's office, which may not be representative of the general population
Ng et al. (2007)	Retrospective cohort study	Admissions to inpatient unit with primary diagnosis of ICD-10 bipolar disorder ($N = 98$ admissions across 49 patients), 15 males	Participation in walking group—40 min walking intensity determined by participants up five times weekly. Comparison—non-participating patients	The two groups did not differ significantly in demographics or admission clinical global impression (CGI) and depression anxiety stress scale (DASS) measures, except for a lower DASS stress subscore for participants ($p = 0.049$) than non-participants ($p = 0.005$) and all its subscales (Depression $p = 0.048$, Anxiety $p = 0.002$, Stress $p = 0.01$)	Retrospective design, small sample size, lack of randomization or control, and indirect measure of manic symptoms
Kilbourne et al. (2007)	Cross-sectional	Patients who completed the VA's Large Health Survey of Veteran Enrollees section on health and nutrition in 1999 and who received a diagnosis of bipolar disorder (BD) ($n = 2032$), schizophrenia ($n = 1895$) or were included in a random sample of non-SMI VA patients ($n = 3065$)	Authors compared nutrition and exercise behaviors using multivariate logistic regression, controlling for patients socioeconomic status (SES) and clinical factors and adjusting for patients clustered by sites using generalized estimating equations	Patients with BD were more likely to report poor exercise habits, including infrequent walking or strength exercises compared to those with no standardised mortality index (SMI)	The nature of the data was self-report
Shah et al. (2007)	Between-groups AB	$N = 24$ (14 individuals with bipolar I disorder clinically assessed as euthymic, 10 controls), 14 males	Treadmill exercise at 10% gradient at 70% maximal predicted oxygen consumption. Duration until exhaustion	Exercise duration significantly shorter in BD group ($d = 0.47$). No significant between group differences in electrocardiograph (ECG) variables	More BD patients smoked (28.6 vs. 0% controls) and patients tended to be heavier, (189.1 ± 29.3 vs. 165.0 ± 29.5 lb, $t = 2.0$, $p = 0.06$)
Hays et al. (2008)	Within-participants AB	$N = 26$ individuals with DSM-IV bipolar I or II disorder, 13 males	Treadmill exercise for 20 min at 70% age-predicted maximal heart rate	Significant increase in dehydroepiandrosterone sulfate (DHEAS) level post-exercise and significant increase in self-reported well being post-exercise	Most of the participants were relatively asymptomatic (87%)
Cairney et al. (2009)	Cross-sectional	Data used from the 2002 Canadian Community Health Survey. physical activity (PA) levels were compared among individuals with BD ($n = 831$) to those with major depression ($n = 4713$) and those with no identifiable mood disorder ($n = 31,834$)	Using multivariate logistic regression, the independent effects of sociodemographic and clinical factors in active and inactive BD individuals stratified by relative weight status	No differences in the proportion of individuals characterized as active, moderately active or inactive among individuals with BD, major depressive disorder (MDD), or the general population	The nature of the data was self-report

Van Citters et al. (2010)	Within-group	$n = 76$, nearly three-quarters were female ($n = 54$), psychiatric diagnoses primarily included major depressive disorder ($n = 30$, 39.5%), bipolar disorder ($n = 19$, 25.0%), and schizophrenia or schizoaffective disorder ($n = 18$, 23.7%)	Participants were assigned an individual health mentor and over 9 months work together to set goals regarding healthier dietary decisions as well as other modules of wellness	Mental health functioning significantly improved among participants, as did negative symptoms. Participation in the program was associated with increased exercise, vigorous activity, and leisurely walking. Participants also demonstrated a significant reduction in waist circumference	No control group
Sylvia et al. (2011)	Within-groups	After the first group ($N = 4$) had completed the treatment, it was revised, and then a second group ($N = 6$) completed the revised treatment. Participants completed all of the study assessments and attended 82% of the sessions	Three treatment modules, Nutrition, Exercise, and Wellness (NEW Tx), were administered in twelve 60-min group sessions over 14 weeks	Both groups added over 100 min of weekly exercise to their baseline duration. Group 1 did not show any significant changes in any of the outcome measures, Group 2 showed improvements in their quality of life, depressive symptoms, and weight	Small sample size, predominantly college students and a lack of a finalized treatment manual
Wright et al. (2012)	Cross-sectional	25 individuals with BD	Semi-structured interview concerning their views on the relationship between exercise and BD. The data was then subjected to qualitative analysis using an Interpretative Phenomenological Analysis approach	Three themes emerged—regulating exercise for mood regulation, exercise as a double-edged sword, and exercise potentially bringing structure to chaos	Qualitative analyses
Daumit et al. (2013)	Between-groups	($N = 291$) who suffered from serious mental illness—including bipolar disorder ($n = 64$, 22%), as well as schizophrenia, schizoaffective disorder, and major depression	Participants took part in an 18-month behavioral weight loss intervention. The treatment consisted of group exercise sessions as well as individualized weight-management sessions	The intervention group lost more weight than the control group, such that 37.8% of participants in the intervention group lost at least 5% of their initial weight, compared with 22.7% in the control group	
Janney et al. (2014)	Between-groups	60 adults with BD were matched 1:1 to users and non-users of mental health services by gender, closest body mass index (BMI), and age	Adult outpatients treated for BD (>18 year) wore accelerometers for seven consecutive days. Each minute epoch was assigned an activity level based on the number of counts per minute	The majority of monitoring time (78%) was classified as sedentary. Light PA accounted for 21% and none achieved 150 min/week of moderate to vigorous activity (as is recommended by national guidelines)	
Sylvia et al. (2013a)	Within-group	482 individuals with BD (either BP I or II, in accordance with DSM IV) TR (aged 18–68)	Exercise frequency in BD patients was assessed in a multi-site comparative effectiveness study that examined a second generation antipsychotic (quetiapine) versus a classic mood stabilizer (lithium)	Approximately 40% of participants reported not exercising regularly. Less frequent exercise was associated with higher BMI, more depressive symptoms, and lower quality of life functioning. More frequent exercise was associated with experiencing more mania in the past year and more current manic symptoms	Cross sectional analysis and self-report. Intensity and state of exercise (e.g., compulsive or not compulsive) were not measured
Sylvia et al. (2013b)	Within-group	Five participants ages 23–64 years ($M = 44$). All participants had a primary diagnosis of BD as determined by a clinician-administered neuropsychiatric interview	Participants took part in NEW Tx, a 20-week individual cognitive behavioral therapy-based treatment comprising of three modules: Nutrition, Exercise, and Wellness (NEW)	Participants' weight, cholesterol, and triglycerides decreased over the study duration as well as number of daily calories and sugar intake. Weekly exercise duration more than tripled and depressive symptoms and overall functioning improved	Open trial, no control group. Small sample size limits ability to draw stronger conclusions

these programs, it is necessary to conduct more studies of this nature in randomized, controlled trials.

PHYSICAL ACTIVITY AND MANIA

Despite the lack of literature on exercise and physical activity in bipolar disorder, there is preliminary evidence that exercise may be a double-edged sword for patients with bipolar disorder due to it potentially polar-specific effect (Wright et al., 2012; Sylvia et al., 2013a). Wright et al. (2012) conducted a semi-structured study with 25 participants diagnosed with bipolar disorder, in which participants were interviewed on their experiences with exercise and their illness. Several themes emerged, including that of the "double-edged sword" theory, or that exercise brought structure and support for some patients while not helping others. Specifically, they found that exercise could be beneficial in helping to direct excess energy, but potentially detrimental in exacerbating manic symptoms and potentially putting patients at risk for a spiraling of manic and hypomanic symptoms. The aggravation of manic symptoms could be mediated by direct effects on mood or indirectly on excessive goal striving, which has been hypothesized to be a psychological risk pathway in the disorder (Nusslock et al., 2007; Alloy et al., 2012). Interestingly, patients described that forms of exercise with an inherent rhythm may provide a somewhat calming effect and facilitate mood regulation due to the cadenced nature of activities such as walking, running, or swimming (Wright et al., 2012). Importantly, in another study conducted by Suto et al. (2010), exercise and rest were identified as being; among the most helpful factors in managing bipolar disorder, with a specific theme on finding the right type of exercise, which could be individually dependent. Although qualitative in nature, these studies highlight that components of an exercise program, including type, intensity, frequency, and duration may be particularly important to investigate when examining the relationship between exercise and bipolar disorder.

Although it has been proposed that exercise may have a double-edged effect on people with bipolar disorder, empirical evidence is needed to support this claim. In their qualitative study, Wright et al. (2012) also suggested that while some participants experienced increased activation levels following exercise, other participants found exercise to have a calming effect on hypomania while Suto et al. (2010) recognized exercise as a popular wellness strategy for patients with bipolar disorder, with a particular theme on finding the right type of exercise (Suto et al., 2010). This is a topical debate with important implications, and future studies are suggested to examine the effects of exercise during mania and hypomania including potential addiction to exercise in this population.

Similarly, Sylvia et al. (2013a) conducted a multi-site comparative study of a second generation antipsychotic (quetiapine) versus a classic mood stabilizer (lithium) in a cohort of 482 people with bipolar disorder. Importantly, individuals in a manic, hypomanic or mixed state at study entry tended to exercise at a greater frequency than currently depressed individuals. These data further support that there may be a complex relationship between bipolar disorder and exercise, although it was unclear if their mood was driving the exercise behavior, or if there was a bidirectional relationship. The authors suggested a specific relationship between exercise frequency and mood polarity, such that depression is associated with less exercise and mania with more exercise in people with bipolar disorder. While the association of increased energy and activity with mania, and its converse with depression, may simply be an illustration of the core symptomatology of the disorder, another explanation for this polar-specific relationship could be the behavioral activation system (BAS; Meyer et al., 2007; Proudfoot et al., 2012; Wright et al., 2012). The BAS, a neurobehavioural system thought to regulate behavior in response to incentives and reward, is thought to be hyper-responsive in individuals with bipolar disorder. While depressive symptoms may emerge following a failure to achieve, or loss of goals/reward (BAS deactivation), hypomania or mania may be triggered in vulnerable individuals following a BAS activation event (an opportunity to gain a desired reward/goal; Urosevic et al., 2008). Individuals who are prone to hypomania or mania; therefore, may be more likely to pursue potentially pleasurable activities with greater vigor and enthusiasm due to the increased responsivity of this reward system (Meyer et al., 2007). Exercise could be considered a goal striving activity, explaining why some people demonstrate an addiction-like tendency to over exercise during a manic episode (Meyer et al., 2007; Wright et al., 2012).

In sum, the relationship of physical activity and mania is still unclear. For example, regular physical activity is associated with better sleep quality in individuals with bipolar disorder (Nusslock et al., 2007; Wright et al., 2012), and meta-analytical reviews have noted that exercise results in increased total sleep, increased slow wave sleep and decreased REM sleep (Kubitz et al., 1996; Youngstedt et al., 1997). Given that sleep problems are a prodromal symptom of mania, physical activity may still have some benefit just before and during a manic phase (Ng et al., 2007; Suto et al., 2010; Proudfoot et al., 2011, 2012).

POTENTIAL MECHANISMS OF PHYSICAL ACTIVITY AND BIPOLAR DISORDER

The association of physical activity and bipolar disorder might be better understood if the mechanistic pathways could be clarified (**Table 2**). This next section will examine the current theories on how exercise may impact bipolar disorder.

Neurogenesis

One likely mechanism for the benefits observed in bipolar disorder is the causal relationship of increased physical activity and neurogenesis. Exercise is likely a pleiotropic intervention that engages a wide spectrum of neurobiological systems implicated in neurogenesis and neuroplasticity, neurotransmission function, metabolism, immune-inflammatory function and cellular respiration. Data suggest that structured exercise exerts a salutary effect on these interacting networks and therefore, are capable of improving psychiatric and somatic health in bipolar disorder (Alsuwaidan et al., 2009). Several studies have highlighted the beneficial effects of exercise on brain health, with a particular focus on the relationship between voluntary exercise and increased growth factors resulting in neurogenesis, metabolism, vascular function and neurodegeneration and alleviation of depressed mood (Ernst et al., 2006; Zheng et al., 2006; Cotman et al., 2007; Marais et al., 2009; Kucyi et al., 2010; Berk et al., 2011).

Table 2 | Summary of mechanisms between exercise and bipolar disorder.

Mechanism	Process	Implications for bipolar disorder
Neurogenesis	Pleiotropic, thought to increase neuroplasticity, neurotransmission function, regulation of growth	Improved somatic and psychiatric health for patients with bipolar disorder
Epigenetics	Facilitation of differential gene expression	"Good stress" of physical exercise could increase BDNF expression to improve neurogenesis
Endorphins	Exercise releases endogenous opiates that enhance mood	Improved mood, amelioration of mood symptoms, potential double-edged sword for patients experiencing mania

Exercise is thought to ensure improved brain function by increasing synaptic plasticity, regulation of growth factors and reduction of peripheral and central risk factors (Cotman et al., 2007).

One of the best candidates for explaining the relationship of exercise with neurogenesis—to ultimately improve outcomes in bipolar disorder—is BDNF (Sylvia et al., 2010). Up-regulation of hippocampal BDNF is a well-documented result of chronic antidepressant administration as well as one of the most robust, sustained and consistently demonstrated changes as a result of exercise (Duman et al., 2008). BDNF is a member of the neurotrophin family and promotes neuronal survival and regeneration and is implicated as a biomarker of disease activity in psychiatric disorders (Frey et al., 2013; Fernandes et al., 2014). This past year, researchers further clarified the exercise-BDNF pathway. Specifically, they found that FNDC5, a recently discovered muscle protein, is elevated by endurance exercise in the hippocampus of mice and that peroxisome proliferator-activated receptors (PGC-1α) and FNDC5 regulate BDNF expression in the brain (Wrann et al., 2013). This model supposes that exercise leads to increased transcription of PGC-1α and up-regulation of Erra α (a nuclear receptor estrogen-related receptor) which is necessary to induce FNDC5 gene expression and ultimately, increase BDNF. Of note, the upregulation of BDNF through exercise shares a similar pathway to that of antidepressants which could theoretically lead to exercise triggering potential manic episodes. Supporting this theory, studies of animals have found that exercise may also impact BDNF by increasing serotonin in the frontal cortex and ventral hippocampus, or mimic the SSRI pathway (Eyers and Parker, 1997; Marais et al., 2009).

Endorphins

It is also possible that the beneficial effects of exercise on mood may be due to its association with endorphins (Steinberg and Sykes, 1985). This theory proposes that exercise is associated with release of endogenous opiates including α endorphins that improve mood and feelings of well-being. Similarly, the monoamine hypothesis suggests that exercise results in an increase release of the monoamine molecules dopamine, serotonin and norepinephrine that are typically reduced in depression (Pierce et al., 1976). Ernst et al. (2006) also found that an increase in α endorphins, BDNF, vascular endothelial growth factor (VEGF), and serotonin release may account for the relationship exercise and positive outcomes on mood and functioning.

Epigenetics

Among hypothesized pathways of exercise and bipolar disorder is epigenetics as exercise may elevate BDNF via these mechanisms (Gomez-Pinilla et al., 2011). Epigenetic mechanisms facilitate differential gene expression, which are subject to environmental influence and have been implicated in the pathophysiology of bipolar disorder (Rao et al., 2012; Banigan et al., 2013; Gamazon et al., 2013; Niculescu, 2013). These mechanisms may mediate some of the physiological impacts of exercise on body tissues (McGee and Hargreaves, 2011; Barres et al., 2012). Epigenetic gene expression alterations induced by "eustress" or "good stress" of physical exercise appear to have beneficial effects (Sanchis-Gomar et al., 2012). For example, BDNF methylation has been implicated in several psychiatric disorders, including bipolar disorder (Ikegame et al., 2013). Taken together, it seems plausible that some of the beneficial associations between exercise and outcome of bipolar disorders are mediated by epigenetic mechanisms.

Other pathways

Exercise increases mitochondrial energy generation (Boushel et al., 2014), and it is known that in depression, particularly in bipolar depression that there is decreased mitochondrial bioenergetics capacity (de Sousa et al., 2014). Similarly, in bipolar disorder there is increased inflammation and oxidative stress (Berk et al., 2011, 2013; Moylan et al., 2014), and exercise reduces both markers of systemic inflammation and oxidative stress (Jatoi, 2013). Exercise reduces cortisol, long known as elevated in depression (Rezaee et al., 2014). Lastly, other factors such as adipokines are implicated as depression biomarkers (Carvalho et al., 2014), and the effects of exercise may be mediated by adipokines such as adiponectin (Yau et al., 2014).

CONCLUSION

Despite the promise of exercise to meet the physical and mental health needs of individuals with bipolar disorder, there is a dearth of literature investigating the role of exercise for bipolar disorder. Furthermore, the current literature is riddled with limitations, such as small samples, heterogeneous treatment groups, no control groups, no distinction between types of exercise (structured exercise programs vs. lifestyle physical activity), clear definitions of the amount (duration, frequency and intensity) of exercise, as well as empirical data with regards to mood-state-dependent effects of exercise for individuals specifically with bipolar disorder. Finally, high attrition rates are often observed in research with this population, potentially leading to biased results. As a result, there is limited information to guide clinicians as to the appropriate intensity, frequency and duration of exercise for people with bipolar disorder and it is thus impossible to give bipolar-specific guidelines for exercise (Barbour et al., 2007; Wright et al., 2012).

There are promising data that exercise may be a viable and effective strategy to deal with the depressive phase of bipolar disorder, but further research is needed to determine the recommended intensity, duration and frequency of exercise programs. It is also necessary for researchers in the future to differentiate between physical activity as leisure-based pursuits, occupational and incidental activity, and more structured, planned, and voluntary exercise. In short, due to the unique problems that patients with bipolar disorder face, such as pharmacotherapy needs, often extreme fluctuations in mood symptoms, and a high comorbidity rate, it is imperative that more research be conducted in this arena so that we can better tailor adjunct lifestyle programs for them.

AUTHOR CONTRIBUTIONS

DT and MG conducted the literature searches; all authors contributed to data interpretation, manuscript preparation and final approval.

REFERENCES

Alloy, L. B., Bender, R. E., Whitehouse, W. G., Wagner, C. A., Liu, R. T., Grant, D. A., et al. (2012). High Behavioral Approach System (BAS) sensitivity, reward responsiveness, and goal-striving predict first onset of bipolar spectrum disorders: a prospective behavioral high-risk design. *J. Abnorm. Psychol.* 121, 339–351. doi: 10.1037/a0025877

Alsuwaidan, M. T., Kucyi, A., Law, C. W. Y., and Mcintyre, R. S. (2009). Exercise and bipolar disorder: a review of neurobiological mediators. *Neuromolecular Med.* 11, 328–336. doi: 10.1007/s12017-009-8079-9

Banigan, M. G., Kao, P. F., Kozubek, J. A., Winslow, A. R., Medina, J., Costa, J., et al. (2013). Differential expression of exosomal microRNAs in prefrontal cortices of schizophrenia and bipolar disorder patients. *PLoS ONE* 8:e48814. doi: 10.1371/journal.pone.0048814

Barbour, K. A., Edenfield, T. M., and Blumenthal, J. A. (2007). Exercise as a treatment for depression and other psychiatric disorders: a review. *J. Cardiopulm. Rehabil. Prev.* 27, 359–367. doi: 10.1097/01.HCR.0000300262.69645.95

Barres, R., Yan, J., Egan, B., Treebak, J. T., Rasmussen, M., Fritz, T., et al. (2012). Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab.* 15, 405–411. doi: 10.1016/j.cmet.2012.01.001

Berk, M., Kabczinski, F., Andreazza, A. C., Dean, O. M., Giorlando, F., Maes, M., et al. (2011). Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. *Neurosci. Biobehav. Rev.* 35, 804–817. doi: 10.1016/j.neubiorev.2010.10.001

Berk, M., Williams, L. J., Jacka, F. N., O'Neil, A., Pasco, J. A., Moylan, S., et al. (2013). So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med.* 11:200. doi: 10.1186/1741-7015-11-200

Boushel, R., Lundby, C., Qvortrup, K., and Sahlin, K. (2014). Mitochondrial plasticity with exercise training and extreme environments. *Exerc. Sport Sci. Rev.* 42, 169–174. doi: 10.1249/JES.0000000000000025

Cairney, J., Veldhuizen, S., Faulkner, G., Schaffer, A., and Rodriguez, M. C. (2009). Bipolar disorder and leisure-time physical activity: results from a national survey of Canadians. *Ment. Health Phys. Act.* 2, 65–70. doi: 10.1016/j.mhpa.2009.09.003

Carek, P. J., Laibstain, S. E., and Carek, S. M. (2011). Exercise for the treatment of depression and anxiety. *Int. J. Psychiatry Med.* 41, 15–28. doi: 10.2190/PM.41.1.c

Carvalho, A. F., Rocha, D. Q., McIntyre, R. S., Mesquita, L. M., Köhler, C. A., Hyphantis, T. N., et al. (2014). Adipokines as emerging depression biomarkers: a systematic review and meta-analysis. *J. Psychiatr. Res.* 59, 28–37. doi: 10.1016/j.jpsychires.2014.08.002

Caspersen, C. J., Powell, K. E., and Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep.* 100, 126–131.

Church, T. S., Earnest, C. P., Skinner, J. S., and Blair, S. N. (2007). Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. *JAMA* 297, 2081–2091. doi: 10.1001/jama.297.19.2081

Cornelissen, V. A., and Fagard, R. H. (2005). Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. *Hypertension* 46, 667–675. doi: 10.1161/01.HYP.0000184225.05629.51

Cotman, C. W., Berchtold, N. C., and Christie, L.-A. (2007). Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci.* 30, 464–472. doi: 10.1016/j.tins.2007.06.011

Crump, C., Sundquist, K., Winkleby, M. A., and Sundquist, J. (2013). Comorbidities and mortality in bipolar disorder: a Swedish national cohort study. *JAMA Psychiatry* 70, 931–939. doi: 10.1001/jamapsychiatry.2013.1394

Daley, A. (2008). Exercise and depression: a review of reviews. *J. Clin. Psychol. Med. Settings* 15, 140–147. doi: 10.1007/s10880-008-9105-z

Daumit, G. L., Dickerson, F. B., Wang, N. Y., Dalcin, A., Jerome, G. J., Anderson, C. A., et al. (2013). A behavioral weight-loss intervention in persons with serious mental illness. *N. Engl. J. Med.* 368, 1594–1602. doi: 10.1056/NEJMoa1214530

Department of Health. (2011). *At Least Five a Week: Start Active, Stay Active: A Report on Physical Activity from the Four Home Countries' Chief Medical Officers*. London: Department of Health.

de Sousa, R. T., Machado-Vieira, R., Zarate, C. A. Jr., and Manji, H. K. (2014). Targeting mitochondrially mediated plasticity to develop improved therapeutics for bipolar disorder. *Expert Opin. Ther. Targets* 18, 1131–1147. doi: 10.1517/14728222.2014.940893

Dodd, S., Brnabic, A. J. M., Berk, L., Fitzgerald, P. B., De Castella, A. R., Filia, S., et al. (2010). A prospective study of the impact of smoking on outcomes in bipolar and schizoaffective disorder. *Compr. Psychiatry* 51, 504–509. doi: 10.1016/j.comppsych.2009.12.001

Dome, P., Gonda, X., and Rihmer, Z. (2012). Effects of smoking on health outcomes in bipolar disorder with a special focus on suicidal behavior. *Neuropsychiatry* 2, 429–441. doi: 10.2217/npy.12.51

Duman, C. H., Schlesinger, L., Russell, D. S., and Duman, R. S. (2008). Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Res.* 1199, 148–158. doi: 10.1016/j.brainres.2007.12.047

Elmslie, J. L., Mann, J. I., Silverstone, J. T., Williams, S. M., and Romans, S. E. (2001). Determinants of overweight and obesity in patients with bipolar disorder. *J. Clin. Psychiatry* 62, 486–491. doi: 10.4088/JCP.v62n0614

Ernst, C., Olson, A. K., Pinel, J. P., Lam, R. W., and Christie, B. R. (2006). Antidepressant effects of exercise: evidence for an adult-neurogenesis hypothesis? *J. Psychiatry Neurosci.* 31, 84–92.

Eyers, K., and Parker, G. (1997). *Mastering Bipolar Disorder: An Insider's Guide to Managing Mood Swings and Finding Balance*. Sydney, NSW: Allen & Unwin.

Faurholt-Jepsen, M., Frost, M., Vinberg, M., Christensen, E. M., Bardram, J. E., and Kessing, L. V. (2014). Smartphone data as objective measures of bipolar disorder symptoms. *Psychiatry Res.* 217, 124–127. doi: 10.1016/j.psychres.2014.03.009

Fernandes, B. S., Berk, M., Turck, C. W., Steiner, J., and Goncalves, C. A. (2014). Decreased peripheral brain-derived neurotrophic factor levels are a biomarker of disease activity in major psychiatric disorders: a comparative meta-analysis. *Mol. Psychiatry* 19, 750–751. doi: 10.1038/mp.2013.172

Frey, B. N., Andreazza, A. C., Houenou, J., Jamain, S., Goldstein, B. I., Frye, M. A., et al. (2013). Biomarkers in bipolar disorder: a positional paper from the International Society for Bipolar Disorders Biomarkers Task Force. *Aust. N. Z. J. Psychiatry* 47, 321–332. doi: 10.1177/0004867413478217

Gamazon, E. R., Badner, J. A., Cheng, L., Zhang, C., Zhang, D., Cox, N. J., et al. (2013). Enrichment of *cis*-regulatory gene expression SNPs and methylation quantitative trait loci among bipolar disorder susceptibility variants. *Mol. Psychiatry* 18, 340–346. doi: 10.1038/mp.2011.174

Gomez-Pinilla, F., Zhuang, Y., Feng, J., Ying, Z., and Fan, G. (2011). Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. *Eur. J. Neurosci.* 33, 383–390. doi: 10.1111/j.1460-9568.2010.07508.x

Hays, A. E., Goss, F., Aaron, D., Abt, K., Friedman, E., Gallagher, M., et al. (2008). Hormonal and perceptual changes in bipolar subjects after acute aerobic exercise. *Med. Sci. Sports Exerc.* 40, S17. doi: 10.1249/01.mss.0000321518.49773.7e

Ikegame, T., Bundo, M., Murata, Y., Kasai, K., Kato, T., and Iwamoto, K. (2013). DNA methylation of the BDNF gene and its relevance to psychiatric disorders. *J. Hum. Genet.* 58, 434–438. doi: 10.1038/jhg.2013.65

Janney, C. A., Fagiolini, A., Swartz, H. A., Jakicic, J. M., Holleman, R. G., and Richardson, C. R. (2014). Are adults with bipolar disorder active? Objectively measured physical activity and sedentary behavior using accelerometry. *J. Affect. Disord.* 152–154, 498–504. doi: 10.1016/j.jad.2013.09.009

Jatoi, A. (2013). Anti-inflammatory therapy: exploring exercise, serum-derived bovine immunoglobulin/protein isolates, and ruxolitinib for the cancer-associated weight loss syndrome. *Curr. Opin. Support. Palliat. Care* 7, 339–341. doi: 10.1097/SPC.0000000000000014

Kilbourne, A. M., Morden, N. E., Austin, K., Ilgen, M., Mccarthy, J. F., Dalack, G., et al. (2009). Excess heart-disease-related mortality in a national study of patients with mental disorders: identifying modifiable risk factors. *Gen. Hosp. Psychiatry* 31, 555–563. doi: 10.1016/j.genhosppsych.2009.07.008

Kilbourne, A. M., Rofey, D. L., Mccarthy, J. F., Post, E. P., Welsh, D., and Blow, F. C. (2007). Nutrition and exercise behavior among patients with bipolar disorder. *Bipolar Disord.* 9, 443–452. doi: 10.1111/j.1399-5618.2007.00386.x

Kubitz, K. A., Landers, D. M., Petruzzello, S. J., and Han, M. (1996). The effects of acute and chronic exercise on sleep. a meta-analytic review. *Sports Med.* 21, 277–291. doi: 10.2165/00007256-199621040-00004

Kucyi, A., Alsuwaidan, M. T., Liauw, S. S., and McIntyre, R. S. (2010). Aerobic physical exercise as a possible treatment for neurocognitive dysfunction in bipolar disorder. *Postgrad. Med.* 122, 107–116. doi: 10.3810/pgm.2010.11.2228

Marais, L., Stein, D. J., and Daniels, W. M. (2009). Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. *Metab. Brain Dis.* 24, 587–597. doi: 10.1007/s11011-009-9157-2

McGee, S. L., and Hargreaves, M. (2011). Histone modifications and exercise adaptations. *J. Appl. Physiol.* 110, 258–263. doi: 10.1152/japplphysiol.00979.2010

Meyer, B., Rahman, R., and Shepherd, R. (2007). Hypomanic personality features and addictive tendencies. *Pers. Indiv. Dif.* 42, 801–810. doi: 10.1016/j.paid.2006.08.024

Morriss, R., and Mohammed, F. A. (2005). Metabolism, lifestyle and bipolar affective disorder. *J. Psychopharmacol.* 19, 94–101. doi: 10.1177/0269881105058678

Moylan, S., Berk, M., Dean, O. M., Samuni, Y., Williams, L. J., O'Neil, A., et al. (2014). Oxidative & nitrosative stress in depression: why so much stress? *Neurosci. Biobehav. Rev.* 45, 46–62. doi: 10.1016/j.neubiorev.2014.05.007

Moylan, S., Eyre, H. A., Maes, M., Baune, B. T., Jacka, F. N., and Berk, M. (2013). Exercising the worry away: how inflammation, oxidative and nitrogen stress mediates the beneficial effect of physical activity on anxiety disorder symptoms and behaviours. *Neurosci. Biobehav. Rev.* 37, 573–584. doi: 10.1016/j.neubiorev.2013.02.003

National Institute for Health, and Clinical Excellence. (2006). *Bipolar Disorder Clinical Guidelines*. London: NICE.

Ng, F., Dodd, S., and Berk, M. (2007). The effects of physical activity in the acute treatment of bipolar disorder: a pilot study. *J. Affect. Disord.* 101, 259–262. doi: 10.1016/j.jad.2006.11.014

Niculescu, A. B. (2013). Convergent functional genomics of psychiatric disorders. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 162B, 587–594. doi: 10.1002/ajmg.b.32163

Nusslock, R., Abramson, L. Y., Harmon-Jones, E., Alloy, L. B., and Hogan, M. E. (2007). A goal-striving life event and the onset of hypomanic and depressive episodes and symptoms: perspective from the behavioral approach system (BAS) dysregulation theory. *J. Abnorm. Psychol.* 116, 105–115. doi: 10.1037/0021-843X.116.1.105

Pierce, D., Kupprat, I., and Harry, D. (1976). Urinary epinephrine and norepinephrine levels in women athletes during training and competition. *Eur. J. Appl. Physiol. Occup. Physiol.* 36, 1–6. doi: 10.1007/BF00421628

Proudfoot, J., Doran, J., Manicavasagar, V., and Parker, G. (2011). The precipitants of manic/hypomanic episodes in the context of bipolar disorder: a review. *J. Affect. Disord.* 133, 381–387. doi: 10.1016/j.jad.2010.10.051

Proudfoot, J., Whitton, A., Parker, G., Doran, J., Manicavasagar, V., and Delmas, K. (2012). Triggers of mania and depression in young adults with bipolar disorder. *J. Affect. Disord.* 143, 196–202. doi: 10.1016/j.jad.2012.05.052

Rao, J. S., Keleshian, V. L., Klein, S., and Rapoport, S. I. (2012). Epigenetic modifications in frontal cortex from Alzheimer's disease and bipolar disorder patients. *Transl. Psychiatry* 2, e132. doi: 10.1038/tp.2012.55

Rethorst, C. D., and Trivedi, M. H. (2013). Evidence-based recommendations for the prescription of exercise for major depressive disorder. *J. Psychiatr. Pract.* 19, 204–212. doi: 10.1097/01.pra.0000430504.16952.3e

Rethorst, C. D., Wipfli, B. M., and Landers, D. M. (2009). The antidepressive effects of exercise: a meta-analysis of randomized trials. *Sports Med.* 39, 491–511. doi: 10.2165/00007256-200939060-00004

Rezaee, S., Kahrizi, S., and Hedayati, M. (2014). Hormonal responses of combining endurance-resistance exercise in healthy young men. *J. Sports Med. Phys. Fitness* 54, 244–251.

Roshanaei-Moghaddam, B., and Katon, W. (2009). Premature mortality from general medical illnesses among persons with bipolar disorder: a review. *Psychiatr. Serv.* 60, 147–156. doi: 10.1176/ps.2009.60.2.147

Sanchis-Gomar, F., Garcia-Gimenez, J. L., Perez-Quilis, C., Gomez-Cabrera, M. C., Pallardo, F. V., and Lippi, G. (2012). Physical exercise as an epigenetic modulator: eustress, the "positive stress" as an effector of gene expression. *J. Strength Cond. Res.* 26, 3469–3472. doi: 10.1519/JSC.0b013e31825bb594

Shah, A., Alshaher, M., Dawn, B., Siddiqui, T., Longaker, R. A., Stoddard, M. F., et al. (2007). Exercise tolerance is reduced in bipolar illness. *J. Affect. Disord.* 104, 191–195. doi: 10.1016/j.jad.2007.03.002

Steinberg, H., and Sykes, E. A. (1985). Introduction to symposium on endorphins and behavioural processes; review of literature on endorphins and exercise. *Pharmacol. Biochem. Behav.* 23, 857–862. doi: 10.1016/0091-3057(85)90083-8

Suto, M., Murray, G., Hale, S., Amari, E., and Michalak, E. E. (2010). What works for people with bipolar disorder? Tips from the experts. *J. Affect. Disord.* 124, 76–84. doi: 10.1016/j.jad.2009.11.004

Sylvia, L. G., Ametrano, R. M., and Nierenberg, A. A. (2010). Exercise treatment for bipolar disorder: potential mechanisms of action mediated through increased neurogenesis and decreased allostatic load. *Psychother. Psychosom.* 79, 87–96. doi: 10.1159/000270916

Sylvia, L. G., Friedman, E. S., Kocsis, J. H., Bernstein, E. E., Brody, B. D., Kinrys, G., et al. (2013a). Association of exercise with quality of life and mood symptoms in a comparative effectiveness study of bipolar disorder. *J. Affect. Disord.* 151, 722–727. doi: 10.1016/j.jad.2013.07.031

Sylvia, L. G., Salcedo, S., Bernstein, E. E., Baek, J. H., Nierenberg, A. A., and Deckersbach, T. (2013b). Nutrition, Exercise, and Wellness Treatment in bipolar disorder: proof of concept for a consolidated intervention. *Int. J. Bipolar Disord.* 1, 24. doi: 10.1186/2194-7511-1-24

Sylvia, L. G., Nierenberg, A. A., Stange, J. P., Peckham, A. D., and Deckersbach, T. (2011). Development of an integrated psychosocial treatment to address the medical burden associated with bipolar disorder. *J. Psychiatr. Pract.* 17, 224–232. doi: 10.1097/01.pra.0000398419.82362.32

Thompson, P. D., Buchner, D., Pina, I. L., Balady, G. J., Williams, M. A., Marcus, B. H., et al. (2003). Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* 107, 3109–3116. doi: 10.1161/01.CIR.0000075572.40158.77

Urosevic, S., Abramson, L. Y., Harmon-Jones, E., and Alloy, L. B. (2008). Dysregulation of the behavioral approach system (BAS) in bipolar spectrum disorders: review of theory and evidence. *Clin. Psychol. Rev.* 28, 1188–1205. doi: 10.1016/j.cpr.2008.04.004

Vancampfort, D., Correll, C. U., Probst, M., Sienaert, P., Wyckaert, S., De Herdt, A., et al. (2013). A review of physical activity correlates in patients with bipolar disorder. *J. Affect. Disord.* 145, 285–291. doi: 10.1016/j.jad.2012.07.020

Van Citters, A. D., Pratt, S. I., Jue, K., Williams, G., Miller, P. T., Xie, H., et al. (2010). A pilot evaluation of the in SHAPE individualized health promotion intervention for adults with mental illness. *Community Ment. Health J.* 46, 540–552. doi: 10.1007/s10597-009-9272-x

Williams, L. J., Pasco, J. A., Henry, M. J., Jacka, F. N., Dodd, S., Nicholson, G. C., et al. (2009). Lifetime psychiatric disorders and body composition: a population-based study. *J. Affect. Disord.* 118, 173–179. doi: 10.1016/j.jad.2009.02.001

Wipfli, B. M., Rethorst, C. D., and Landers, D. M. (2008). The anxiolytic effects of exercise: a meta-analysis of randomized trials and dose-response analysis. *J. Sport Exerc. Psychol.* 30, 392–410.

Wrann, C. D., White, J. P., Salogiannnis, J., Laznik-Bogoslavski, D., Wu, J., Ma, D., et al. (2013). Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. *Cell Metab.* 18, 649–659. doi: 10.1016/j.cmet.2013.09.008

Wright, K., Armstrong, T., Taylor, A., and Dean, S. (2012). "It's a double edged sword": a qualitative analysis of the experiences of exercise amongst people with Bipolar Disorder. *J. Affect. Disord.* 136, 634–642. doi: 10.1016/j.jad.2011.10.017

Wright, K. A., Everson-Hock, E. S., and Taylor, A. H. (2009). The effects of physical activity on physical and mental health among individuals with bipolar disorder: a systematic review. *Mental Health Phys. Act.* 2, 86–94. doi: 10.1016/j.mhpa.2009.09.001

Yau, S. Y., Li, A., Hoo, R. L., Ching, Y. P., Christie, B. R., Lee, T. M., et al. (2014). Physical exercise-induced hippocampal neurogenesis and antidepressant effects are mediated by the adipocyte hormone adiponectin. *Proc. Natl. Acad. Sci. U.S.A.* 111, 15810–15815. doi: 10.1073/pnas.1415219111

Youngstedt, S. D., O'Connor, P. J., and Dishman, R. K. (1997). The effects of acute exercise on sleep: a quantitative synthesis. *Sleep* 20, 203–214.

Zheng, H., Liu, Y., Li, W., Yang, B., Chen, D., Wang, X., et al. (2006). Beneficial effects of exercise and its molecular mechanisms on depression in rats. *Behav. Brain Res.* 168, 47–55. doi: 10.1016/j.bbr.2005.10.007

Conflict of Interest Statement: No funding has been received to support this study. Ajeet B. Singh is a casual speaker for Servier Australia, Pfizer Australia, and Lundbeck Australia. Julie A. Pasco has received grant/research support from the NHMRC, Perpetual, Amgen (Europe) GmBH, and BUPA Foundation and has received speaker fees from Amgen and Sanofi Aventis. Michael Berk is supported by a NHMRC Senior Principal Research Fellowship 1059660 and has received Grant/Research Support from the NIH, Cooperative Research Centre, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, MBF, NHMRC, Beyond Blue, Rotary Health, Geelong Medical Research Foundation, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Meat and Livestock Board, Organon, Novartis, Mayne Pharma, Servier and Woolworths, has been a speaker for Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck, Merck, Pfizer, Sanofi Synthelabo, Servier, Solvay and Wyeth, and served as a consultant to Astra Zeneca, Bioadvantex, Bristol Myers Squibb, Eli Lilly, Glaxo SmithKline, Janssen Cilag, Lundbeck Merck and Servier. Louisa Sylvia was a shareholder in Concordant Rater Systems and serves as a consultant for United Biosource Corporation, Clinical Trials Network and Institute, Clintara. She receives royalties from New Harbinger.

Received: 10 October 2014; accepted: 28 January 2015; published online: 04 March 2015.

Citation: Thomson D, Turner A, Lauder S, Gigler ME, Berk L, Singh AB, Pasco JA, Berk M and Sylvia L (2015) A brief review of exercise, bipolar disorder, and mechanistic pathways. Front. Psychol. ***6****:147. doi: 10.3389/fpsyg.2015.00147*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in Psychology

ORIGINAL RESEARCH
published: 25 June 2015
doi: 10.3389/fpsyg.2015.00839

Affective responses after different intensities of exercise in patients with traumatic brain injury

Patricia Rzezak[1], Luciana Caxa[1], Patricia Santolia[1], Hanna K. M. Antunes[1], Italo Suriano[2], Sérgio Tufik[1] and Marco T. de Mello[1]*

[1] Department of Psychobiology, Universidade Federal de São Paulo, São Paulo, Brazil, [2] Department of Neurosurgery, Universidade Federal de São Paulo, São Paulo, Brazil

Background: Patients with traumatic brain injury (TBI) usually have mood and anxiety symptoms secondary to their brain injury. Exercise may be a cost-effective intervention for the regulation of the affective responses of this population. However, there are no studies evaluating the effects of exercise or the optimal intensity of exercise for this clinical group.

Methods: Twelve male patients with moderate or severe TBI [mean age of 31.83 and SD of 9.53] and 12 age- and gender-matched healthy volunteers [mean age of 30.58 and SD of 9.53] participated in two sessions of exercise of high and moderate-intensity. Anxiety and mood was evaluated, and subjective assessment of experience pre- and post-exercise was assessed. A mixed between and within-subjects general linear model (GLM) analysis was conducted to compare groups [TBI, control] over condition [baseline, session 1, session 2] allowing for group by condition interaction to be determined. Planned comparisons were also conducted to test study hypotheses.

Results: Although no group by condition interaction was observed, planned comparisons indicated that baseline differences between patients and controls in anxiety (Cohens' $d = 1.80$), tension ($d = 1.31$), depression ($d = 1.18$), anger ($d = 1.08$), confusion ($d = 1.70$), psychological distress ($d = 1.28$), and physical symptoms ($d = 1.42$) disappear after one session of exercise, independently of the intensity of exercise.

Conclusion: A single-section of exercise, regardless of exercise intensity, had a positive effect on the affective responses of patients with TBI both by increasing positive valence feelings and decreasing negative ones. Exercise can be an easily accessible intervention that may alleviate depressive symptoms related to brain injury.

Keywords: traumatic brain injury, mood, anxiety, exercise

OPEN ACCESS

Edited by:
Andrew Kemp,
Universidade de São Paulo, Brazil

Reviewed by:
Andrew Kemp,
Universidade de São Paulo, Brazil
Trevor Archer,
University of Gothenburg, Sweden
Brandon L. Alderman,
Rutgers University, USA

****Correspondence:***
Patricia Rzezak,
Department of Psychobiology,
Universidade Federal de São Paulo,
Rua Abdo Ambuba,
75/31 Vila Andrade, São Paulo, Brazil
patriciarzezak@gmail.com

Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *30 July 2014*
Accepted: *03 June 2015*
Published: *25 June 2015*

Citation:
Rzezak P, Caxa L, Santolia P, Antunes HKM, Suriano I, Tufik S and de Mello MT (2015) Affective responses after different intensities of exercise in patients with traumatic brain injury. Front. Psychol. 6:839. doi: 10.3389/fpsyg.2015.00839

Introduction

Traumatic brain injury (TBI) is defined by the Brain Injury Association of America (BIAA, 2011) as "an alteration in brain function, or other evidence of brain pathology, caused by an external force." Patients with TBI have a greater risk of experiencing emotional distress and depression due to their acquired brain lesion (Rosenthal et al., 1998). In a study of 559 TBI patients followed for 1 year following trauma, 53% of the sample met the criteria for major depression (Bombardier et al., 2010). The practice of regular physical activities has

an indisputable positive effect for several health conditions (Blair, 1994; Allen, 1996; Magnusson et al., 1998; Uusitupa et al., 2000), including mental health (Stephens, 1988;Ströhle et al., 2007; Deslandes et al., 2009). Nevertheless, the World Health Organization (World Health Organization [WHO], 2010) recommendation for 150 min moderate-intensity aerobic exercise per week is a difficult target to achieve for people with disabilities (Paluska and Schwenk, 2000). Yet, the experience of improved mood states following acute bouts of physical activity in patients with TBI could serve as an immediate psychological reward for continuing exercise, and also serve public campaigns that encourage this population to adhere to a regular exercise practice. Thus, we aimed to determine the effects of a single bout of aerobic exercise and to establish the ideal exercise intensity in patients with TBI.

Studies demonstrating the positive effects of regular exercise on mental health have focused on healthy participants as well as patients with psychiatric disorders. In a large cohort of 55,000 healthy volunteers, leisure-time physical activity alone was associated with improved mental health, including reduced anxiety and depression symptoms, even after controlling for confounding factors such as age, sex, sociodemographic status, and physical illness (Stephens, 1988). A more recent, longitudinal-prospective study (Ströhle et al., 2007) of 2,548 participants from the community followed over a 4-years period demonstrated that regular exercise was associated with decreased prevalence of any mental disorder. However, there is evidence that this positive relationship is more robust in patients with a diagnosis of major depression (Conn, 2010). A program of supervised exercise in clinically depressed participants may have the same beneficial effects as psychotropic medication, with a better prognosis at a 6 months follow-up assessment (Blumenthal et al., 1999; Babyak et al., 2000). There is also evidence that mood may improve rapidly after only a single session of exercise, as compared to psychotropic medication, for which 40% of patients fail to respond, even after fourth-line therapy (Wade et al., 2014).

Researchers have shown that acute exercise can lead to an immediate improvement in positive affect (Reed, 2005; Barnes et al., 2010) and reductions in negative affect (Reed, 2005). Anxiety is reduced following bouts of exercise even in participants with normal or elevated levels of anxiety. This reduction may be seen 5–15 min after the cessation of exercise and can remain decreased for 2–4 h (Raglin, 1997).

An important question is whether the intensity of the exercise moderates the affective response of the practitioner. Research examining affective responses during exercise in non-athlete participants identified a negative relationship between exercise intensity and affect, such that as exercise intensity increased above ventilatory threshold (i.e., high-intensity exercise), people tend to feel greater psychological distress (Blanchard et al., 2001), less enjoyment (Brewer et al., 2000; Kilpatrick et al., 2003) and higher feelings of displeasure (Hall et al., 2002) as compared to moderate-intensity aerobic activities.

There have been few attempts to evaluate the effects of exercise on the mood and anxiety of patients with TBI, and there is no consensus as to whether exercise improves affective responses in this population. A study that investigated the relationship between exercise level and depressive symptoms in a community sample of individuals with TBI showed that TBI patients who exercised were less depressed than those who did not exercise (Gordon et al., 1998). Another report demonstrated that patients with TBI displayed reduced depression severity scores after a 10-weeks exercise program (Wise et al., 2012). On the other hand, two randomized control trials did not find a significant relationship between an implemented exercise program and depression scores (Bateman et al., 2001; Hoffman et al., 2010). Nevertheless, several methodological differences make it difficult to compare these studies. The first study (Gordon et al., 1998) did not implement an exercise program in an experimental study to investigate the effect of exercise on the mood of patients with TBI. The second study only investigated the effects of exercise on depression severity and did not have a comparison group (Wise et al., 2012). Finally, despite having a randomized control trial design, the other studies had different comparison groups. The first of these (Bateman et al., 2001) compared patients in a 12-weeks exercise program to patients who were allocated to a relaxation condition. The other study compared patients who exercised for 10 weeks to patients who did not receive any intervention (Hoffman et al., 2010).

The present study aimed to determine if aerobic exercise has a positive impact on the affective responses of patients with mild to moderate TBI. We also investigated whether workload is associated with a positive or a negative effect on the mood and anxiety of patients with TBI. Finally, we sought to establish if the impact of exercise on mood and anxiety is similar for patients with TBI and healthy controls. We hypothesized that patients submitted to a moderate-intensity aerobic exercise will display improvement on depression/anxiety scores, while a high-intensity exercise protocol will decrease positive feelings and evaluation of wellbeing in patients with TBI. We also hypothesized that controls would not show differences in affective responses after different exercise intensities.

Materials and Methods

The Research Ethics Committee of the Universidade Federal de São Paulo (CEP 1858/09) approved all methods and procedures in accordance with the Declaration of Helsinki. All volunteers signed a written informed consent. This protocol was also registered at ClinicalTrial.gov (# NCT01395472).

Subjects

Eligible Criteria

The study included male patients under treatment at the Neurology and Neurosurgery Department of the Universidade Federal de São Paulo, aged 18–55 years-old, with at least 5 years of formal education, with a diagnosis of mild to moderate closed head injury (TBI in which the skull and dura mater remain intact)

occurring more than 6 months prior, but after the age of 17. TBI severity was determined by the patient's neurologist according to the following criteria: Glasgow Coma Scale between 3 and 12 or loss of consciousness ranging from 20 min to 36 h and post-traumatic amnesia for more than a day. Finally, all patients were seizure free for 1 year prior to the commencement of the study. A significant other accompanied all patients.

Any patient in acute phase of recovery (e.g., mental confusion), suffering post-traumatic amnesia, with a previous history of drug or alcohol abuse, brain lesions, or neurological or psychiatric disorders was excluded from the study. Additionally, patients who could not verbally express meaningful ideas clearly as determined by a neuropsychologist (P.R.) in a comprehensive neuropsychological evaluation or had compromised motor skills that could impede exercise in a recumbent cycle-ergometer were not included. Patients who had received psychiatric or psychological treatment for mood or anxiety disorders were also not included in the study.

Participants were first selected by the patient's neurologist (I.S.) who was responsible for evaluating: the type (open or closed), the severity (mild, moderate or severe) and the phase of recovery (acute or chronic) of TBI. The same physician attested that exercise would not be dangerous for patient's health. After initial selection, patients were interviewed by a neuropsychologist (P.R.) who guaranteed that patients met all eligible criteria for participating in the study.

The control group comprised of sedentary healthy male volunteers (not exercising regularly for at least 2 months before participation in the study), aged 18–55 years old. Subjects were selected using the following criteria: must have at least 5 years of formal education; sedentary lifestyle (i.e., no habitual exercise); no clinical symptoms or indicators of cardiovascular disease; no medication that could alter cardiovascular and cognitive function; no psychotropic drug use or any pharmaceutical drug for which exercise is a contraindication, or that may negatively influence cognitive function; and no recent surgical intervention. Volunteers were recruited from the University and were either university staff or post-graduate students.

Twenty-five patients were contacted between 2009 and 2011. Of these, 21 fulfilled the inclusion and exclusion criteria. Five patients dropped out before finishing the three sessions required (cardiovascular examination, high-intensity, and moderate-intensity physical activities), three patients were not allowed to participate in the study after the rest and effort electrocardiogram, and one patient had a severe aphasia. Therefore, only 12 patients completed the three sessions required in this study protocol and their data are presented in the present study. The Control group also comprised 12 age and sex-matched volunteers.

In the evaluated sample, the TBIs were due to vehicular accidents (58.33%), falls (30%), and injured in a physical fight (8.33%). The mean age of the injury was 3.56 years (SD ± 4.13; median = 2.58).

Table 1 provides further information on participant characteristics, including cardiovascular history as wells as comparison between the patients and controls.

Procedures

Exercise

First, the participants completed an examination of cardiovascular responses to dynamic and static effort to evaluate cardiorespiratory aptitude. Those who did not show signs of harm after exercise completed two distinct exercise protocols: (1) progressive cycling to voluntary physical exhaustion (VPE; i.e., high-intensity exercise) and (2) 30 min of constant workload at a ventilatory threshold of 1 (CW; i.e., moderate-intensity exercise). As is further explained below, the physical workload of the CW session is based on the VPE session; the order of the sessions was therefore fixed.

The intervals between protocols 1 and 2 were a minimum of 1 week and a maximum of 2 weeks.

The tests were conducted in an acclimatized laboratory. In the VPE protocol, the subjects were equipped with a heart rate monitor (Polar, model FS1; Polar Kempele, Finland) and a mask (Hans Rudolph; Shawnee, KS, USA) before entering the adapted cycle-ergometer for inferior limbs (Load Angio with automatic stand, Netherlands). The warm-up was conducted for 3 min at a workload of 30 watts, and then the workloads were increased by 10 watts at 1-min intervals until VPE. Ventilation time course analysis, possible with ergospirometry test, reveals two disproportionate increases in the VO_2 defining the first (VTI) and second (VTII) ventilatory thresholds. These disproportionate increases are related to exercise induced acidosis compensation and are related mostly to respiratory frequency increase during exercise (Wasserman, 1987; Wasserman and Koike, 1992).

The volunteers were asked to maintain a pedaling speed of 50 rpm during the whole protocol. Ergospirometry (COSMED, Quark PFT – Pulmonary Function Testing – FRC & DLCO, Italy) was used to measure the cardiorespiratory variables. These respiratory variables were maximal oxygen consumption (VO_{2max}) in ventilatory thresholds I and II (VT-I and VT-II) and maximal heart rate (HRmax) and HR in VT-I and VT-II. To determine the oxygen consumption in VT-I and VT-II, the criteria described by Wasserman (1987)and Wasserman and

TABLE 1 | Demographic and cardiovascular data for traumatic brain injury [TBI] patients and controls [mean (SD)].

	TBI Patients	Controls	*t*	*p*
Age (years)	31.83 ± 9.53	30.58 ± 9.53	0.35	0.727
Height (cm)	173.75 ± 9.00	178.00 ± 7.15	−1.28	0.213
Weight (kg)	74.36 ± 16.85	80.31 ± 9.67	−1.05	0.304
BMI (kg/m^2)	24.48 ± 4.24	25.29 ± 2.28	−0.58	0.566
Ergospirometry				
VO_2 peak (ml/kg/min^{-1})	33.18 ± 14.37	40.02 ± 5.30	−1.55	0.136
HR_{max} (bpm)	157.58 ± 15.34	175.67 ± 10.59	−3.36	0.003*
HR_{max} achieved (%)	83.91 ± 9.15	92.72 ± 4.35	−3.01	0.008*
VE max	75.82 ± 18.73	133.62 ± 44.45	−4.15	0.000*
Work-load max (W)	145.83 ± 26.10	221.67 ± 38.34	−5.66	0.000*
Work-load VT-1 (W)	86.67 ± 21.46	129.17 ± 31.18	−3.89	0.000*

*cm, centimeters; kg, kilograms; m, meters; ml, milliliters; min, minutes; bpm, beats per minute; HR_{max}, maximal heart rate; W, watts; VT-1, ventilatory threshold; VE max, maximal ventilation. *$p < 0.05$.*

Koike (1992) were followed. Before every test, the equipment was calibrated by trained personnel using a known gas concentration.

The CW protocol used the same equipment previously described. The subjects performed a 30-min constant workload in VT-I after the 3-min warm-up at predetermined 30-watt workloads. The affective response evaluations are referred to by the following abbreviations: before voluntary physical exhaustion (VPE-1), after voluntary physical exhaustion (VPE-2), before a constant workload at a ventilatory threshold of 1 (CW-1) and after a constant workload at a ventilatory threshold of 1 (CW-2).

Affective Response Evaluation

The psychological evaluation was performed in a quiet and acclimatized environment that was maintained at a temperature of 22 ± 2°C with 55% humidity. Two trained neuropsychologists conducted all evaluations in a standard sequence and with a maximum duration of 20 min. To minimize circadian variations, both sessions of exercise were performed at the same time of the day. Participants completed questionnaires to assess affective responses immediately before and after each exercise protocol. Mood, anxiety and well-being questionnaires were selected based on previous published studies that investigated the impact of exercise on affective responses (Blanchard et al., 2001; Bartholomew et al., 2005; Cassilhas et al., 2010). The following questionnaires were administered:

State–Trait Anxiety Inventory (STAI, Spielberger et al., 1980)

This questionnaire evaluated trait (how the person generally feels) and state (how the person is feeling at that moment) anxiety using 20 statements each. The volunteer was asked to consider a Likert-scale of 4 points. A higher total score relates to increased anxiety severity.

Brunel Mood Scale (BRUMS, Terry et al., 2003)

This scale was adapted from The Profile of Mood States (POMS). The BRUMS is a 24-item inventory with a Likert scale of 4 points that assesses the mood dimensions of Anger, Confusion, Depression, Fatigue, Tension, and Vigor.

Visual Analoge Mood Scale (VAMS, Bond and Lader, 1974)

This scale is a self-rating instrument used to measure mood. The participant is required to mark, according to their feelings, a 100 mm line that separates adjectives with opposite meanings. Each end of the scale is supposed to reflect extreme states of that condition. The VAMS score is determined by measuring the distance in millimeters from the left end of the card to the participant's mark for each of the 16 adjectives. These items are organized into 4 subscales: 'Anxiety,' 'Physical Sedation,' 'Psychological Sedation,' and 'Other Feelings.'

Subject Exercise Experience Scale (SEES, McAuley and Courneya, 1994)

This scale is a 12-item self-report measure of the subjective experiences that are unique to the exercise domain. These items are organized into three subscales: 'Positive Well-Being,' 'Psychological Distress,' and 'Fatigue.'

Data Analysis

A parametric analysis was performed after verifying the normality of the data through a Kolmogorov–Smirnov Test. Participant characteristics of demographic and physical data were compared using Student's *t*-test. The research questions were addressed using a general linear model (GLM) to examine the main effect of Group (TBI, Controls) and Condition (baseline, VPE, CW), as well as the interaction between the two factors (Group × Condition). Baseline scores were calculated as the average across the two baseline sessions (VPE1 and CW1). Tukey's HSD *post hoc* pairwise comparisons were performed after significant effects were obtained in GLM analysis. Planned comparisons were also performed according to the hypotheses of this study, which predict that mood and anxiety differences in baseline conditions between patients and controls would disappear after exercise (Tabachnick and Fidell, 2013). Therefore, a series of Student *t*-tests were run with between-group comparisons in each of the three measure points. According to Tabachnick and Fidell (2013), it is appropriate to conduct planned comparisons in relation to hypotheses even in the absence of a significant interaction in a GLM.

As a measure of the magnitude of change in mood and anxiety measures we calculated standardized indices of effect size (Cohen's *d*: Cohen, 1977). Cohen (Cohen, 1977) defined effect sizes as "small, $d = 0.2$," "medium, $d = 0.5$," and "large, $d = 0.8$." For statistical analysis, we used the Statistical Package for the Social Sciences, version 14.0 for Windows (SPSS Inc., Chicago, IL, USA), with level of significance set at $\alpha = 0.05$.

Results

Demographic and Fitness Information

The patients and controls had similar age [$t(22) = 0.35$; $p = 0.727$], height [$t(22) = -1.28$; $p = 0.213$], weight [$t(22) = -1.05$; $p = 0.304$] and body mass index (BMI) [$t(22) = -0.58$; $p = 0.566$] and were thus considered comparable samples. In the ergospirometry test, although both patients and controls had a similar maximal oxygen volume [$t(22) = -1.55$; $p = 0.136$], the TBI patients had a lower maximal heart rate (total and percentage) [$t(22) = -3.36$; $p = 0.003$ and $t(22) = -3.01$; $p = 0.008$, respectively], maximal ventilation [$t(22) = -4.15$; $p < 0.001$], maximal workload [$t(22) = -5.66$; $p < 0.001$] and workload at VT-1 [$t(22) = -3.89$; $p < 0.001$]. Refer to **Table 1** for more information.

Basal Affective Response Profile

Compared with healthy volunteers, patients had a higher trait and state anxiety in STAI [$t(1,22) = 4.38$; $p < 0.001$; Cohen's $d = 1.80$ and $t(1,22) = 4.31$; $p < 0.001$; $d = 1.77$, respectively]; higher levels of tension [$t(1,22) = 2.85$; $p = 0.009$; $d = 1.31$], depression [$t(1,22) = 2.36$; $p = 0.028$; $d = 1.18$], anger [$t(1,22) = 2.22$; p:0.036; $d = 1.08$], confusion [$t(1,22) = 3.16$; $p = 0.005$; $d = 1.70$] and total score [$t(1,22) = 2.71$; $p = 0.013$; $d = 1.23$] of BRUMS; more psychological distress in SEES [$t(1,22) = 2.45$; $p = 0.023$; $d = 1.28$]; and more physical sedation [$t(1,22) = 3.37$; $p = 0.003$;

$d = 1.42$] in VAMS. A comparison between the two groups' basal affective responses are shown in **Tables 2** and **3**.

Comparison between Affective Responses of the Two Groups in Pre- and Post-Exercise

A GLM analysis of variance examined main effects of Group and Condition and their interaction. A significant effect of group was observed. Patients scored significantly higher on the following measures: STAI state [$F(1,22) = 7.33$; $p = 0.009$; $d = 0.64$]; BRUMS tension [$F(1,22) = 15.92$; $p < 0.001$; $d = 1.00$], depression [$F(1,22) = 8.60$; $p = 0.005$; $d = 0.81$], anger [$F(1,22) = 4.14$; $p = 0.046$; $d = 0.56$], confusion [$F(1,22) = 13.65$; $p < 0.001$; $d = 0.94$] and total score [$F(1,22) = 4.85$; $p = 0.031$; $d = 0.54$]; and VAMS physical symptoms [$F(1,22) = 9.41$; $p = 0.003$; $d = 0.75$]. A statistics trend was observed for the SEES psychological distress [$F(1,22) = 3.79$; $p = 0.056$; $d = 0.46$; **Table 2**].

A significant effect of condition was also observed for BRUMS fatigue [$F(1,22) = 9.94$; $p < 0.001$] and SEES fatigue [$F(1,22) = 14.33$; $p < 0.001$]. A statistical trend was observed for VAMS anxiety [$F(1,22) = 2.62$; $p = 0.081$; **Table 3**). *Post hoc* analysis revealed differences in basal responses and VPE [$t(1,22) = 4.34$; $p < 0.001$; $d = -1.65$] and CW [$t(1,22) = 3.07$; $p = 0.009$; $d = -1.08$] of BRUMS fatigue and in basal responses and VPE [$t(1,22) = 5.26$; $p < 0.001$; $d = -1.95$] and CW[$t(1,22) = 3.46$; $p = 0.003$; $d = -1.10$] of SEES fatigue.

General linear model analysis of the interaction between Group and Condition was not statistically significant for any of the scales used. However, planned comparisons revealed baseline differences between groups in STAI state [$t(1,22) = 4.31$; $p < 0.001$; $d = 1.77$], BRUMS depression [$t(1,22) = 2.36$; $p = 0.028$; $d = 1.18$], anger [$t(1,22) = 2.22$; $p = 0.037$; $d = 1.15$], confusion [$t(1,22) = 3.16$; $p = 0.005$; $d = 1.69$], SEES psychological distress [$t(1,22) = 2.45$; $p = 0.023$; $d = 1.28$], VAMS physical symptoms [$t(1,22) = 3.36$; $p = 0.003$; $d = 1.39$], disappeared following both VPE and CWE sessions suggesting patient improvement following exercise. These data suggest that the mood, anxiety and psychological distress symptomatology present in patients before exercise decrease to healthy levels after exercise, despite exercise intensity. On the other hand, patients and controls respond similarly in fatigue and other well-being factors after exercise. To exemplify such response style, **Figure 1** shows slopes of depression, anxiety, fatigue and well-being scores for each sample and in each measure condition.

However, BRUMS tension scores, that were higher in patients in baseline evaluation [$t(1,22) = 2.85$; $p = 0.009$; $d = 1.31$], remained higher after CWE exercise [$t(1,22) = 2.16$; $p = 0.042$; $d = 0.92$] and tended to remain higher after VPE protocol [$t(1,22) = 2.03$; $p = 0.054$; $d = 0.85$].

Discussion

The aim of this study was to evaluate the effects of exercise on the affective responses of patients with TBI and to determine whether the intensity of this exercise differentially impacts on negative and positive affect. Our most striking finding is that following exercise, patients with TBI no longer reported significant differences relative to healthy controls on affective responses. Moreover, the two groups showed similar responses after a moderate and a high-intensity exercise, demonstrating

TABLE 2 | Comparing the two modalities of exercise in the TBI patients and the healthy controls.

	TBI			Controls			Group	Condition	Interaction
	Basal	VPE Mean (SD)	CW Mean (SD)	Basal Mean (SD)	VPE Mean (SD)	CW Mean (SD)	*p*	*p*	*p*
STAI S	36.00 (7.53)	36.83 (9.66)	34.17 (7.74)	29.83 (5.06)	34.25 (5.56)	32.83 (6.28)	0.009*	0.553	0.113
BRUMS TE	2.45 (2.16)	2.50 (2.15)	2.58 (2.61)	0.67 (1.15)	0.92 (1.62)	0.75 (1.36)	<0.001*	0.936	0.958
BRUMS D	2.73 (3.82)	1.25 (2.90)	1.33 (2.02)	0.33 (0.65)	0.42 (0.79)	0.25 (0.87)	0.005*	0.382	0.337
BRUMS A	0.55 (1.04)	0.67 (2.02)	0.08 (0.29)	0.17 (0.39)	0.17 (0.58)	0.00 (0.00)	0.046*	0.231	0.388
BRUMS V	9.00 (3.63)	9.83 (3.33)	9.25 (3.55)	9.92 (2.61)	8.58 (3.00)	8.50 (3.29)	1.000	0.925	0.171
BRUMS F	1.64 (2.29)	5.25 (4.43)	4.92 (5.58)	1.83 (1.19)	6.83 (3.38)*	4.58 (2.87)	0.550	<0.001*	0.630
BRUMS C	2.36 (1.80)	2.08 (2.50)	1.75 (2.22)	0.17 (0.39)	0.75 (1.60)	0.42 (1.00)	<0.001*	0.800	0.690
BRUMS T	0.82 (10.20)	2.42 (12.15)	1.42 (13.02)	−6.75 (3.98)	0.50 (7.98)	−2.50 (6.49)	0.031*	0.328	0.404
SEES PW	15.36 (5.07)	15.27 (4.54)	16.75 (4.56)	17.08 (2.57)	14.83 (5.06)	15.00 (3.93)	0.950	0.648	0.302
SEES PD	6.64 (3.35)	8.00 (5.27)	6.50 (3.75)	4.17 (0.58)	6.75 (3.36)	5.58 (2.78)	*0.056*	0.175	0.646
SEES F	6.45 (2.81)	14.73 (6.56)	12.83 (9.88)	6.17 (2.66)	16.17 (5.70)*	11.67 (4.08)	0.841	<0.001*	0.694
VAMS A	25.62 (11.79)	23.87 (16.08)	20.30 (15.07)	14.12 (14.10)	30.23 (11.50)*	23.43 (12.14)	0.911	*0.081*	0.132
VAMS PS	30.54 (9.78)	25.33 (15.96)	21.39 (16.68)	13.11 (6.89)	17.09 (13.59)	14.41 (10.52)	0.003*	0.669	0.714
VAMS MS	16.28 (10.60)	11.33 (10.68)	16.81 (18.39)	20.50 (11.35)	13.68 (12.61)	16.52 (13.57)	0.501	0.333	0.838
VAMS O	18.09 (12.72)	16.40 (17.16)	11.59 (12.64)	7.91 (6.78)	12.56 (9.81)	10.07 (12.22)	0.144	0.581	0.698

*STAI T, Trait-State Anxiety Inventory – Trait Scale; STAI S, Trait-State Anxiety Inventory – State Scale; BRUMS, Brunel Mood Scale; BRUMS TE, Tension Scale; BRUMS D, Depression Scale; BRUMS A, Anger scale: BRUMS V, Vigor Scale; BRUMS F, Fatigue Scale; BRUMS C, Confusion Scale; BRUMS T, Total Scale; SEES, Subjective Exercise Experience Scale; SEES PW, Positive well-being Scale; SEES PD, Psychological Distress Scale; SEES F, Fatigue Scale; VAMS, Visual Analog Mood Scale; VAMS A, Anxiety Scale; VAMS PS, Physical Sedation Scale; VAMS MS, Mental Sedation Scale; VAMS O, Other Feelings Scale. *$p < 0.05$.*

TABLE 3 | *Post-hoc* comparison between groups according to physical exertion.

	Baseline		VPE		CW	
	***p*-value**	**Cohens' *d***	***p*-value**	**Cohens' *d***	***p*-value**	**Cohens' *d***
STAI S	<0.001*	1.77	0.431	0.34	0.648	0.19
BRUMS TE	0.009*	1.31	0.054	0.85	0.042*	0.92
BRUMS D	0.028*	1.18	0.347	0.45	0.101	0.75
BRUMS A	0.037*	1.15	0.418	0.38	0.328	0.55
BRUMS V	0.110	−0.69	0.344	0.39	0.597	0.22
BRUMS F	0.677	−0.17	0.336	−0.41	0.856	0.08
BRUMS C	0.005*	1.69	0.134	0.65	0.071	0.83
SEES PW	0.177	-0.60	0.829	0.09	0.325	0.41
SEES PD	0.023*	1.28	0.501	0.29	0.504	0.21
SEES F	0.427	0.34	0.579	−0.23	0.709	0.17
VAMS A	0.095	0.71	0.277	−0.46	0.581	−0.23
VAMS PS	0.003*	1.39	0.187	0.56	0.232	0.51
VAMS MS	0.340	−0.40	0.627	−0.20	0.965	0.002
VAMS O	0.084	0.75	0.508	0.28	0.767	0.12

*STAI S, Trait-state Anxiety Inventory – State Scale; BRUMS, Brunel Mood Scale; BRUMS TE, Tension Scale; BRUMS D, Depression Scale; BRUMS A, Anger Scale: BRUMS V, Vigor Scale; BRUMS F, Fatigue Scale; BRUMS C, Confusion Scale; SEES, Subjective Exercise Experience Scale; SEES PW, Positive Well-Being Scale; SEES PD, Psychological Distress Scale; SEES F, Fatigue Scale; VAMS: Visual Analog Mood Scale; VAMS A, Anxiety Scale; VAMS PS, Physical Sedation Scale; VAMS MS, Mental Sedation Scale; VAMS O, Other Feelings Scale. *$p < 0.05$.*

that patients with TBI responded similarly to different exercise protocols. The major finding here – based on planned comparisons – is that patients displayed reductions in depression and anxiety symptoms comparable to controls after both exercise intensities.

To the best of our knowledge, this is the first study to evaluate the effects of a single session of exercise on the psychological responses of patients with TBI in addition of being the first to test the hypothesis that the intensity of exercise may play a pivotal role in the beneficial effects of this type of activity on the affective responses of these patients. It is still unclear how long a person should exercise in order to benefit from its effect. In the present study we tried to establish if the positive effect on mental health, already demonstrated in healthy individuals (Stephens, 1988; Ströhle et al., 2007; Conn, 2010), could be observed following only a single session of exercise.

Three groups have previously studied the impact of a longer exercise program on the mood and anxiety symptoms of brain injury patients. While Gordon et al. (1998) demonstrated that exercise in individuals with TBI was associated with elevated mood and perceptions of better health, both Bateman et al. (2001) and Hoffman et al. (2010) did not find a significant relationship between aerobic exercise training and psychological aspects. Our findings are in agreement with the suggestion that aerobic exercise can improve affective responses in patients with TBI. Nevertheless, it is important to notice differences in our methodologies that limit the comparison between studies. First, Bateman et al. (2001) used a sample of heterogeneous brain injury patients comprising traumatic and vascular brain injury subjects. Moreover, they included patients with a recent history of brain injury, disregarding that premature exercise, close to the time of injury, can have a detrimental effect on brain functioning (Griesbach et al., 2004) and because of that is a trouble time to determine the real origin of any relationship between exercise and mental disorders. Additionally, both studies (Bateman et al., 2001; Hoffman et al., 2010) asked the subjects to self-control the intensity of their exercise by checking their heart rate and adjusting their pace to stay within a range of 60–80% of the maximum heart rate. However, it is impossible to guarantee that subjects actually work out at the desired intensity because they may intentionally exercise below the stipulated heart rate at a more comfortable pace or because they could not self-control their heart rate due to cognitive disabilities. In the present study the intensity of exercise was fixed and stipulated according to measures obtained during the ergospirometric exam. Most importantly, we recruited patients with no depression complaints and the affective response to exercise might be different for people with or without depression.

Prior research in non-TBI populations has suggested that the positive effect of exercise on mood, particularly of a single bout of exercise, was significantly greater in those with more depressive symptoms (Lane and Lovejoy, 2001). In agreement with such hypothesis, our TBI sample, which had some degree of depression and anxiety feelings, as evidenced by their baseline scores on STAI, BRUMS, and VAMS scores, showed improvement after one session of exercise, even though they did not have psychiatric disorder diagnosis. Besides, our control group composed of healthy volunteers with no complaints of mood disorders and no signs of depression in basal BRUMS or VAMS scores, did not show improvement after exercise in either positive or negative valence affective responses.

Depression-related changes have been described as one of the most common psychiatric syndromes following TBI. Studies on consecutive samples have found prevalence rates of depression ranging from 9 to 36% (Jorge et al., 2004; Bryant et al., 2010). Our findings suggest that a single session of exercise could serve as an

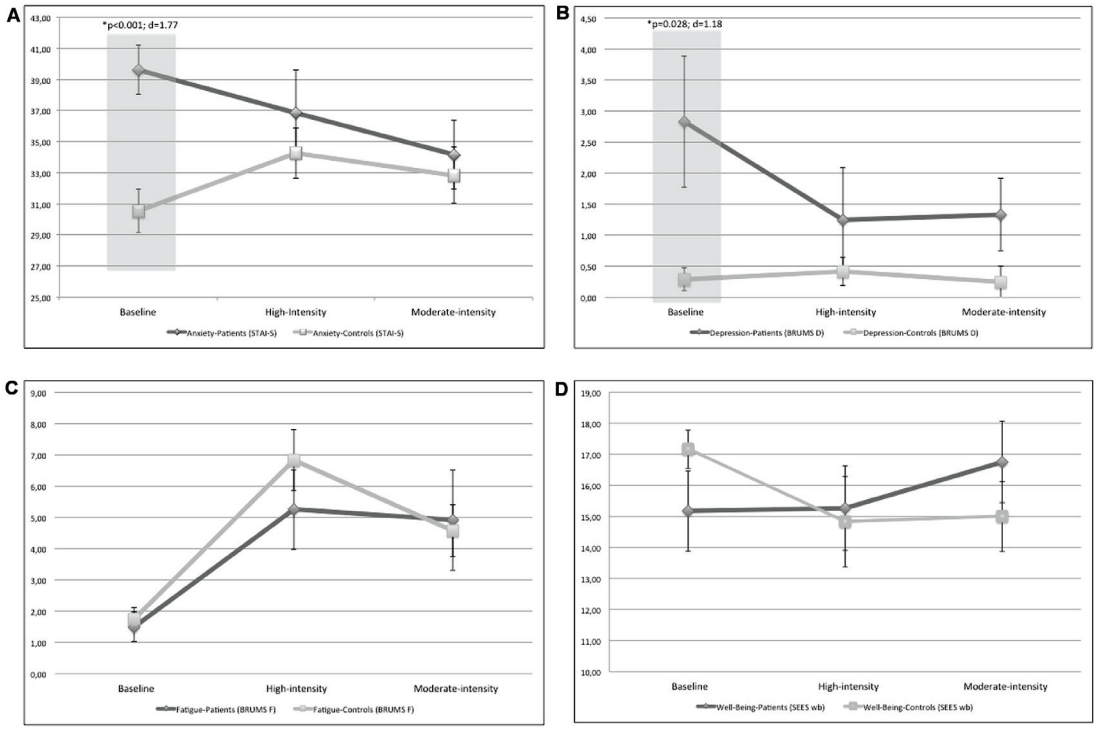


FIGURE 1 | Secondary exploratory omnibus ANOVA analysis comparing affective states of patients and controls in each of the three stages of the protocol; baseline, post high-intensity, and moderate-intensity exercises. (A,B) Showing that baseline differences in anxiety and depression symptoms disappears after a session of exercise, regardless of the intensity of such activity. **(C,D)** Showing that states more related to physical evaluation (i.e., fatigue and well-being) remains similar between groups across the three measurements.

intervention to aid daily mood regulation in patients with TBI. Future studies, following patients with TBI during a program of regular exercise are necessary to explore the long-term effect of this type of activity on mood and anxiety symptoms of this population.

One may pose the question of why exercise could be related to an improvement on mood. Some studies have demonstrated that exercise is associated with increased activity in both noradrenergic and serotonergic systems, which in turn have been associated to depression (Dunn and Dishman, 1991; Meeusen and De Meirleir, 1995; Dishman, 1997). Besides, changes in neurotrophic factors also have been noted, particularly for brain-derived neurotrophic factor (BDNF). The resultant increases in BDNF are associated with increased activity in monoaminergic systems, such as growth and regeneration of serotonergic neurons (Altar, 1999). The effect of BDNF has been likened to that of antidepressant treatment.

The view that the workload may be a moderator for the effects of exercise has been widely tested in studies that evaluated the impact of exercise on cognitive functioning. Moderate exercise is thought to improve cognitive performance in healthy adults and the elderly, whereas high-intensity exercise may instead impair brain processing (Chmura et al., 1994; McMorris et al., 1999). We have previously shown, using part of this dataset that some cognitive functions of TBI patients improved both VPE-2 and constant moderate-intensity workload exercises, although this improvement was noted on specific cognitive abilities in each case (Rzezak et al., unpublished data).

In a critical review about the acute effects of exercise on mood, Yeung (1996) demonstrated that the literature does generally support the belief that there are such effects. More than 85% of reviewed studies found at least some degree of improved mood on a wide variety of measures following exercise despite a diversity of exercise modes, durations and intensities (Cassilhas et al., 2010). Nevertheless, in non-clinical samples, a single bout of moderate-intensity exercise has been shown to reduce transiently depressive symptoms and improve moods more consistently than intense exercise (Yeung, 1996; Ekkekakis, 2003). Hence, there is still no consensus regarding the effects of high-intensity exercise on the mood and anxiety of non-clinical subjects.

In fact, one would expect that the fatigue levels after exhaustive exercise would be greater than after moderate-intensity exercise, which was not observed in the present study. One possible explanation for this observation is that exercising in the recumbent position with a heavy workload for a long period of time (30 min) could increase the discomfort level as well as the risk for cramps, which in turn would bring a decrease in positive well being and would hinder the positive valence feelings associated with moderate-intensity exercise.

For this reason, the horizontal cycle-ergometer used in this study may have been a limitation. However, because patients with TBI may have difficulties in postural control and other motor disabilities secondary to the brain injury (Gowland and Gambarotto, 1994), we believe that a vertical cycle-ergometer could impose a threat to the patient's health, and because our main purpose was to evaluate the affective responses after exercise in patients with TBI, it is reasonable that this data is relevant information. Future studies would benefit by comparing TBI patients' performance after an exercise session and other types of intervention, such as a stretching session. Another limitation of this study was the lack of a structural psychiatric interview to better describe the psychiatric status of the patients and controls. Although not as sensitive as a psychiatric evaluation, we were able to predict the presence of depressive and anxiety symptoms through the STAI, BRUMS, and VAMS scores.

Another possible weakness of this study is it small sample size. The use of rigorous selection criteria in order to have a homogeneous sample of male patients with a similar age-range, severity of TBI, in a chronic stage of recovery who have had their accident in adulthood imposed an obstacle to acquire a larger sample. Nevertheless, this was a compromise necessary for a better interpretation of our data as each one of these variables could serve as confounders for the analysis. Hence, future studies with larger samples are needed to corroborate our findings.

Although these limitations must be taken into account in the interpretation of the data, our findings are an important step in the discussion of whether exercise has a positive impact on the affective responses of patients with TBI. As this is an exploratory study, being the first to investigate this issue in this clinical population, more research is needed to corroborate the present data.

Conclusion

A single-section of exercise, regardless of exercise intensity, had a positive effect on the affective responses of patients with TBI both by increasing positive valence feelings (such as psychological well-being) and decreasing negative ones (such as depression and anxiety feelings). This is a relevant finding, as exercise can be an easily accessible intervention that could alleviate depressive symptoms related to brain injury. Moreover, the identification of the positive effects of a single-session of aerobic exercise on mood and anxiety symptoms of patients can encourage the practice of such activities in a more regular basis.

Acknowledgments

The authors would like to thank Dr. Carolina Ackel D'Elia and Luciana Ortega for their helpful comments. This work was supported by grants from the Associação Fundo de Incentivo à Pesquisa (AFIP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), FAP-UNIFESP, CEPE, CEMSA and Fundação de Amparo à Pesquisa do Estado de São Paulo (CEPID no. 98/14303-3 to ST). Marco Túlio de Mello and Sérgio Tufik are recipients of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) fellowship. Patricia Rzezak receives financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP no. 2012/13065-0). None of the authors disclose professional relationships with companies or manufacturers who would benefit from the results of the present study.

References

Allen, J. (1996). Coronary risk factor modification in women after coronary artery bypass surgery. *Nurs. Res.* 45, 260–265. doi: 10.1097/00006199-199609000-00002

Altar, C. A. (1999). Neurotrophins and depression. *Trends Pharmacol. Sci.* 20, 59–61. doi: 10.1016/S0165-6147(99)01309-7

Babyak, M., Blumenthal, J. A., Herman, S., Khatri, P., Doraiswamy, M., Moore, K., et al. (2000). Exercise treatment for major depression: maintenance of therapeutic benefit at 10 months. *Psychosom. Med.* 62, 633–638. doi: 10.1097/00006842-200009000-00006

Barnes, R. T., Coombes, S. A., Armstrong, N. B., Higgins, T. J., and Janelle, C. M. (2010). Evaluating attentional and affective changes following an acute e. *J. Sports Sci.* 28, 1065–1076. doi: 10.1080/02640414.2010.489196

Bartholomew, J. B., Morrison, D., and Ciccolo, J. R. (2005). Effects of acute exercise on mood and well-being in patients with major depressive disorder. *Med. Sci. Sports Exerc.* 37, 2032–2037. doi: 10.1249/01.mss.0000178101.78322.dd

Bateman, A., Culpan, F. J., Pickering, A. D., Powell, J. H., Scott, O. M., and Greenwood, R. J. (2001). The effect of aerobic training on rehabilitation outcomes after recent severe brain injury: a randomized controlled evaluation. *Arch. Phys. Med. Rehabil.* 82, 174–182. doi: 10.1053/apmr.2001.19744

Blair, S. N. (1994). "Physical activity, fitness and coronary heart disease," in *Physical Activity, Fitness and Health: International Proceedings and Consensus Statement*, eds C. Bouchard, R. J. Shephard, T. and Stephens (Champaign, IL: Human Kinetics), 579–590.

Blanchard, C. M., Rodgers, W. M., Spence, J. C., and Courneya, K. S. (2001). Feeling state responses to acute exercise of high and low intensity. *J. Sci. Med. Sport* 4, 30–38. doi: 10.1016/S1440-2440(01)80005-0

Blumenthal, J. A., Babyak, M. A., Moore, K. A., Craighead, W. E., Herman, S., Khatri, P., et al. (1999). Effects of exercise training on older patients with major depression. *Arch. Intern. Med.* 159, 2349–2356. doi: 10.1001/archinte.159.19.2349

Bombardier, C., Fann, J., Temkin, N., Esselman, P., Barber, J., and Dikmen, S. (2010). Rates of major depressive disorder and clinical outcomes following traumatic brain injury. *JAMA* 303, 1938–1945. doi: 10.1001/jama.2010.599

Bond, A., and Lader, M. (1974). The use of analogue scales in rating subjective feelings. *British J. Psychol.* 47, 211–218. doi: 10.1111/j.2044-8341.1974.tb02285.x

Brain Injury Association of America [BIAA]. (2014). *Brain Injury Association of America. BIAA Adopts New TBI Definition.* Available at: http://www.biausa.org/announcements/biaa-adopts-new-tbi-definition. (Accessed September 24, 2014).

Brewer, B. W., Manos, T. M., McDevitt, A. V., Cornelius, A. E., and Van Raalte, J. L. (2000). The effect of adding lower intensity work on perceived aversiveness of exercise. *J. Sport Exerc. Psychol.* 22, 119–130.

Bryant, R. A., O'Donnell, M. L., Creamer, M., McFarlane, A. C., Clark, C. R., and Silove, D. (2010). The psychiatric sequelae of traumatic injury. *Am. J. Psychiatry* 167, 312–320. doi: 10.1176/appi.ajp.2009.09050617

Cassilhas, R. C., Antunes, H. K., Tufik, S., and de Mello, M. T. (2010). Mood, anxiety, and serum IGF-1 in elderly men given 24 weeks of high resistance exercise. *Percept. Mot. Skills* 110, 265–276. doi: 10.2466/pms.110.1.265-276

Chmura, J., Nazar, K., and Kaciuba-Uściłko, H. (1994). Choice reaction time during graded exercise in relation to blood lactate and plasma catecholamine thresholds. *Int. J. Sports Med.* 15, 172–176. doi: 10.1055/s-2007-1021042

Cohen, J. (1977). *Statistical Power Analysis for Behavioral Sciences*, 2nd Edn, New York: Academic Press.

Conn, V. S. (2010). Depressive symptom outcomes of physical activity interventions: meta-analysis findings. *Ann. Behav. Med.* 39, 128–138. doi: 10.1007/s12160-010-9172-x

Deslandes, A., Moraes, H., Ferreira, C., Veiga, H., Silveira, H., Mouta, R., et al. (2009). Exercise and mental health: many reasons to move. *Neuropsychobiology* 59, 191–198. doi: 10.1159/000223730

Dishman, R. K. (1997). Brain monoamines, exercise, and behavioral stress: animal models. *Med. Sci. Sports Exerc.* 29, 63–74. doi: 10.1097/00005768-199701000-00010

Dunn, A. L., and Dishman, R. K. (1991). Exercise and the neurobiology of depression. *Exerc. Sport Sci. Rev.* 19, 41–98. doi: 10.1249/00003677-199101000-00002

Ekkekakis, P. (2003). Pleasure and displeasure from the body: perspectives from exercise. *Cogn. Emot.* 17, 213–239. doi: 10.1080/02699930302292

Gordon, W. A., Sliwinski, M., Echo, J., McLoughlin, M., Sheerer, M. S., and Meili, T. E. (1998). The benefits of exercise in individuals with traumatic brain injury: a retrospective study. *J. Head Trauma Rehabil.* 13, 58–67. doi: 10.1097/00001199-199808000-00006

Gowland, C., and Gambarotto, C. A. (1994). "Assessment and treatment of physical impairments leading to disability after brain injury," in *Brain Injury Rehabilitation: Clinical Considerations*, eds M. A. Finlayson and S. H. Garner (Philadelphia: Williams & Wilkins), 102–123.

Griesbach, G. S., Hovda, D. A., Molteni, R., Wu, A., and Gomez-Pinilla, F. (2004). Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor up regulation and recovery of function. *Neuroscience* 125, 129–139. doi: 10.1016/j.neuroscience.2004.01.030

Hall, E. E., Ekkekakis, P., and Petruzzello, S. J. (2002) The affective beneficence of vigorous exercise revisited. *British J. Health Psychol.* 7, 47–66. doi: 10.1348/135910702169358

Hoffman, J. M., Bell, K. R., Powell, J. M., Behr, J., Dunn, E. C., Dikmen, S., et al. (2010). A randomized controlled trial of exercise to improve mood after traumatic brain injury. *PM R* 2, 911–919. doi: 10.1016/j.pmrj.2010.06.008

Jorge, R. E., Robinson, R. G., Moser, D., Tateno, A., Crespo-Facorro, B., and Arndt, S. (2004). Major depression following traumatic brain injury. *Arch. Gen. Psychiatry* 61, 42–50. doi: 10.1001/archpsyc.61.1.42

Kilpatrick, M., Hebert, E., Bartholomew, J., Hollander, D., and Stromberg, D. (2003). Effect of exertional trend during cycle ergometry on postexercise affect. *Res. Q. Exerc. Sport* 74, 353–359. doi: 10.1080/02701367.2003.10609103

Lane, A., and Lovejoy, D. (2001). The effects of exercise on mood change: the moderating effect of depressed mood. *J. Sports Med. Phys. Fitness* 41, 539–545.

Magnusson, C., Baron, J., Persson, I., Wolk, A., Bergström, R., Trichopoulos, D., et al. (1998). Body size in different periods of life and breast cancer risk in post-menopausal women. *Int. J. Cancer* 76, 29–34. doi: 10.1002/(SICI)1097-0215(19980330)76:1<29::AID-IJC6>3.0.CO;2-#

McAuley, E., and Courneya, K. (1994). The subjective exercise experiences scale (SEES): development and preliminary validation. *J. Sport Exerc. Psychol.* 16, 163–177.

McMorris, T., Myers, S., MacGillivary, W. W., Sexsmith, J. R., Fallowfield, J., Graydon, J., et al. (1999). Exercise, plasma catecholamine concentrations and decision-making performance of soccer players on a soccer-specific test. *J. Sports Sci.* 17, 667–676. doi: 10.1080/026404199365687

Meeusen, R., and De Meirleir, K. (1995). Exercise and brain neurotransmission. *Sports Med.* 20, 160–188. doi: 10.2165/00007256-199520030-00004

Paluska, S. A., and Schwenk, T. L. (2000). Physical activity and mental health: current concepts. *Sports Med.* 29, 167–180. doi: 10.2165/00007256-200029030-00003

Raglin, J. S. (1997). "Anxiolytic effects of physical activity," in *Physical Activity and Mental Health*, ed. W. P. Morgan (Washington, DC: Taylor & Francis), 107–126.

Reed, J., (2005). "Acute physical activity and self-reported affect: a review," in *Causes, Role and Influence of Mood States*, ed. A. V. Clark (Chicago, IL: Nova Science Publishers, Inc.), 91–113.

Rosenthal, M., Christensen, B., and Ross, T. (1998). Depression following traumatic brain injury. *Arch. Phys. Med. Rehabili.* 79, 90–103. doi: 10.1016/S0003-9993(98)90215-5

Spielberger, C. D., Vagg, P. R., Barker, L. R., Donham, G. W., and Westberry, L. G. (1980). "The factor structure of the State-Trait Anxiety Inventory," in *Stress and Anxiety,* eds G. Sarason and C. D. Spielberger (Washington: Hemisphere), 95–109.

Stephens, T. (1988). Physical activity and mental health in the United States and Canada: evidence from four popular surveys. *Prev. Med.* 17, 35–47. doi: 10.1016/0091-7435(88)90070-9

Ströhle, A., Höfler, M., Pfister, H., Müller, A. G., Hoyer, J., Wittchen, H. U., et al. (2007). Physical activity and prevalence and incidence of mental disorders in adolescents and young adults. *Psychol. Med.* 37, 1657–1666. doi: 10.1017/S003329170700089X

Tabachnick, B. G., and Fidell, L. S. (2013). *Using Multivariate Statistics*, 6th Edn. Boston: Pearson.

Terry, P. C., Lane, A. M., and Fogarty, G. J. (2003). Construct validity of the POMS-A for use with adults. *Psychol. Sports Exerc.* 4, 125–139. doi: 10.1016/S1469-0292(01)00035-8

Uusitupa, M., Louheranta, A., Lindström, J., Valle, T., Sundvall, J., Eriksson, J., et al. (2000). The Finnish diabetes prevention study. *British J. Nutr.* 83, S137–S142. doi: 10.1017/s0007114500001070

Wade, R. L., Kindermann, S. L., Hou, Q., and Thase, M. E. (2014). Comparative assessment of adherence measures and resource use in SSRI/SNRI-treated patients with depression using second-generation antipsychotics or L-methylfolate as adjunctive therapy. *J. Manag. Care Pharm.* 20, 76–85.

Wasserman, K. (1987). Determinants and detection of anaerobic threshold and consequences of exercise above it. *Circulation* 76, 29–39.

Wasserman, K., and Koike, A. (1992). Is the anaerobic threshold truly anaerobic? *Chest* 101, 211–218. doi: 10.1378/chest.101.5_Supplement.211S

Wise, E. K., Hoffman, J. M., Powell, J. M., Bombardier, C. H., and Bell, K. R. (2012). Benefits of exercise maintenance after traumatic brain injury. *Arch. Phys. Med. Rehabil.* 93, 1319–1323. doi: 10.1016/j.apmr.2012.05.009

World Health Organization [WHO]. (2010). *Global Recommendations on Physical Activity for Health*. WHO Library. Available at: http://whqlibdoc.who.int/publications/2010/9789241599979_eng.pdf (acessed June 15, 2015).

Yeung, R. R. (1996). The acute effects of exercise on mood state. *J. Psychosom. Res.* 40, 123–141. doi: 10.1016/0022-3999(95)00554-4

Conflict of Interest Statement: The Associate Editor, Dr Andrew Kemp, declares that despite being affiliated with the same institution as the authors, the review process was handled objectively. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
PSYCHOLOGY

ORIGINAL RESEARCH ARTICLE
published: 02 February 2015
doi: 10.3389/fpsyg.2015.00015

Mindfulness training promotes upward spirals of positive affect and cognition: multilevel and autoregressive latent trajectory modeling analyses

***Eric L. Garland*[1,2] *, *Nicole Geschwind*[3], *Frenk Peeters*[3] *and Marieke Wichers*[4]**

[1] College of Social Work, University of Utah, Salt Lake City, UT, USA
[2] Integrative Medicine – Supportive Oncology, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA
[3] Maastricht University, Maastricht, Netherlands
[4] University of Groningen, Groningen, Netherlands

Edited by:
Andrew Kemp, Universidade de São Paulo, Brazil

Reviewed by:
Sierra A. Bainter, University of North Carolina at Chapel Hill, USA
Amanda Jane Shallcross, New York University Langone Medical Center, USA

***Correspondence:**
Eric L. Garland, Integrative Medicine – Supportive Oncology, Huntsman Cancer Institute, University of Utah, 395 South 1500 East, Salt Lake City, UT 84112, USA
e-mail: eric.garland@socwk.utah.edu

Recent theory suggests that positive psychological processes integral to health may be energized through the self-reinforcing dynamics of an upward spiral to counter emotion dysregulation. The present study examined positive emotion–cognition interactions among individuals in partial remission from depression who had been randomly assigned to treatment with mindfulness-based cognitive therapy (MBCT; $n = 64$) or a waitlist control condition ($n = 66$). We hypothesized that MBCT stimulates upward spirals by increasing positive affect and positive cognition. Experience sampling assessed changes in affect and cognition during 6 days before and after treatment, which were analyzed with a series of multilevel and autoregressive latent trajectory models. Findings suggest that MBCT was associated with significant increases in trait positive affect and momentary positive cognition, which were preserved through autoregressive and cross-lagged effects driven by global emotional tone. Findings suggest that daily positive affect and cognition are maintained by an upward spiral that might be promoted by mindfulness training.

Keywords: mindfulness training, positive emotion, broaden-and-build, emotion regulation, latent growth curve analysis

INTRODUCTION

The dynamic nature of human experience emerges from the continual demand to adapt to changing and challenging life circumstances; it is out of this process of adaptation that emotions arise (Lazarus, 1991). In turn, emotions are thought to flow from appraisal of the meaning of one's internal state and current environmental context. When life circumstances are appraised to be benign, beneficial, or rewarding, positive affect results (Lazarus and Folkman, 1984). In complementary fashion, when individuals experience positive emotion, they are more likely to notice the pleasant, beautiful, or rewarding aspects of their lives (Tamir and Robinson, 2007), and are more likely to adopt a positive attitude to self or others when making evaluative judgments (Clore and Palmer, 2009). Thus, positive cognitive processes and positive affect appear to be tightly linked. In previous theoretical work, we (Garland et al., 2010) have proposed that positive psychological processes energize and maintain one another through the self-reinforcing dynamics of a positive feedback loop, which we and others (Fredrickson and Joiner, 2002; Burns et al., 2008; Kok and Fredrickson, 2011) have termed an *upward spiral*. Yet, at present, few empirical studies have elucidated the dynamics of positive emotion–cognition interactions.

In contrast, substantially more is known about how negative affect influences cognition, and in turn, how negatively biased cognitive processes amplify and further entrench negative affect (Mathews and MacLeod, 2005). Such negatively valenced emotion–cognition interactions are particularly evident in depression. Depressed individuals preferentially attend to negative stimuli (e.g., sad faces; Gotlib et al., 2004), ruminate on negative beliefs about the self, world, and future (Nolen-Hoeksema, 2000), resolve ambiguous stimuli negatively (Mogg et al., 2006), and make negative appraisals of the valence of their own thoughts (Freeston et al., 1995) – i.e., *negative thought appraisals* (Purdon et al., 2005). These negative cognitive processes may explain why depressed individuals have fewer positive thoughts than non-depressed people (Ingram et al., 1990). Thus biased information processing in depression decreases the pleasantness of thoughts and produces feelings of dysphoria, processes which may be underpinned by alterations to functional and structural neural architecture that increase the influence of subcortical brain regions while impairing top–down, prefrontal cognitive control (Disner et al., 2011). As a possible result of functional imbalances in cortical–subcortical networks, depressed individuals are unable to sustain activations in brain regions that subserve the experience of reward (e.g., nucleus accumbens), thus accounting in part for their lower levels of positive affect (Heller et al., 2009). Indeed, a number of studies demonstrate that depressed individuals experience less daily positive affect (Peeters et al., 2006). Deprived of the ability to sustain and increase positive affect over time, depressed persons may remain mired in a downward spiral of negativity that perpetuates emotion dysregulation and prolongs depression (Garland et al., 2010; Wichers et al., 2010).

As such, positive affect may be central to countering the dysfunctional cognition–emotion interactions in depression. According to Fredrickson's (1998, 2004) broaden-and-build

theory, positive emotions undo the psychophysiological consequences of negative emotions (Fredrickson and Levenson, 1998), and expand the scope of cognition to allow individuals to access a wider-than-usual range of percepts, associations, ideas, and action urges (Fredrickson and Branigan, 2005). In addition to broadening the scope of cognition, positive affect may also increase the frequency and intensity of positively valenced cognitions (i.e., *positive cognitions*, see Macleod and Moore, 2000), by biasing attention (Wadlinger and Isaacowitz, 2010) and memory recall (Roberts-Wolfe et al., 2012) toward positive information, promoting positive interpretations of ambiguous situations (Mathews, 2012), and assigning positive value to objects or thoughts during cognitive appraisal (Clore and Huntsinger, 2007). Through these mechanisms, positive emotions foster positive cognitions that promote novel and exploratory sequences of behavior (Cohn et al., 2009), which creates more opportunities to encounter rewarding life experiences – the "fuel" for increased positive appraisals and emotions in the future. In these ways, upward spirals of positive emotion and cognition may exert a countervailing force of the dysphoric and anhedonic states characteristic of depression.

Plausibly, interventions and experiences that engender positive affective and cognitive states may amplify upward spirals above and beyond preexisting trait-like deficits in positive affect and cognition. One form of mental training that may stimulate upward spirals of positive emotion is mindfulness meditation. The practice of mindfulness meditation involves repeated placement of attention onto an object (such the sensory experience of breathing, walking, or other common activities) while accepting and letting go of distracting thoughts and emotions. This practice is thought to engender a specific metacognitive state: a non-reactive, non-evaluative monitoring of moment-by-moment cognition, emotion, perception, and sensation without fixation on thoughts of past and future (Garland, 2007; Lutz et al., 2008). Ultimately, recurrent practice of engaging the state of mindfulness is thought to develop dispositional mindfulness, the propensity to experience and express mindful attitudes in everyday life (Baer et al., 2006).

As investigators increasingly incorporate measures of positive emotion into their research protocols, more studies have identified effects of mindfulness training on positive affective processes. Community samples undergoing mindfulness training have evidenced significant increases in positive affect (Nyklíček and Kuijpers, 2008; Orzech et al., 2009). Mindfulness training has also been shown to influence positive affect among clinical samples, such as patients with comorbid recurrent depression and rheumatoid arthritis (Zautra et al., 2008). Notably, a RCT of adults with residual depressive symptoms found that mindfulness-based cognitive therapy (MBCT; Segal et al., 2002) increased positive affect and the sense of reward from savoring pleasant daily life activities (Geschwind et al., 2011). Mindfulness training was also found to enhance reward responsiveness in a RCT of opioid misusing chronic pain patients as evidenced by increased cardiac-autonomic (Garland et al., 2014a) and electrocortical responses (Garland et al., 2014b) to positive affective stimuli.

Similarly, mindfulness training may influence positive cognitive processes. For instance, mindfulness training was associated with significantly improved memory for positive information among a community sample (Roberts-Wolfe et al., 2012). Beyond its effects on memory, mindfulness training may also promote positive reappraisal (Garland et al., 2009), the capacity to reappraise negative or stressful life events as benign, beneficial, or meaningful (Lazarus and Folkman, 1984). Indeed, increases in dispositional mindfulness over the course of a mindfulness training program were reciprocally linked with increases in positive reappraisal, and that the stress-reductive effects of increases in dispositional mindfulness were partially mediated by increases in positive reappraisal (Garland et al., 2011). Another study found that relative to individuals in two control conditions, those who had been treated with MBCT evidenced significantly greater positive reappraisal ability during an experimental sad mood induction (Troy et al., 2012). And, a recent RCT found that a mindfulness-oriented intervention led to significantly greater increases in positive reappraisal than a support group control (Garland et al., 2014c).

While these studies are promising, little is known about how mindfulness training might modulate interactions between positive affect and cognition over time. Recently, Garland and Fredrickson (2013) proposed a novel theoretical model to explicate how mindfulness might stimulate upward spirals of positive affect and cognition. In brief, the practice of mindfulness is hypothesized to evoke a metacognitive state of broadened awareness with downstream effects on affect and cognition. Insofar as positive emotions may broaden cognition (Fredrickson and Branigan, 2005), the broadened scope of attention induced by mindfulness practice may in turn induce positive emotions (Garland et al., 2010). The consequent positive affective state may then bias and tune attention (Friedman and Förster, 2010) toward an expanded set of contextual information from which positive appraisals of external and internal stimuli (mental contents: e.g., thoughts) can be generated. When the consequent positive cognitions and positively valenced emotional states become the new target of mindful awareness, this amplifies positive emotion and increases attentional tuning toward mood-congruent contextual features that support further positive appraisals and a deepening of positive emotion. Thus, when paid mindful attention, momentary experiences of positive affect and cognition may interact synergistically in an autoregressive and cross-lagged fashion and thereby energize the daily maintenance and gradual development of well-being and resilience. Through the prospective cyclical relation of the upward spiral dynamic, time-specific elevations in positive affect stimulated by mindfulness practice (above and beyond an individual's average level of positive affect) are presumed to predict subsequent time-specific elevations in positive cognitions (above and beyond an individual's average level of positive cognitions), and vice versa. In this sense, mindfulness provides the perturbation or shock that reverberates through the cognitive-emotional system, resulting in momentary psychological elevations that feed back into the system to tip one's dispositional affective style (Davidson, 2004) toward a more durable positivity.

In view of this theoretical model, the purpose of the present study is to elucidate the temporal dynamics of positive emotion–cognition interactions among a sample of individuals in partial remission from depression who had been randomly assigned to treatment with MBCT or a waitlist control condition. Data from

this RCT were previously analyzed by Geschwind et al. (2011) to test the effects of MBCT on reward experience. The experience sampling method (ESM) was used to measure dynamic changes in affect and cognition to test a number of hypotheses that were distinct from those tested in this earlier investigation. Our primary aim was to test the hypothesis that MBCT will significantly increase momentary positive cognitions relative to a waitlist control group. Although positive cognition might be evidenced by positive attentional bias, positive memory bias, positive interpretation bias, etc., in this study, positive cognition was operationalized as appraising one's current thoughts as positively valenced – i.e., positive thought appraisal (c.f., Purdon and Clark, 2001). Our secondary aim was to explore how MBCT might influence the temporal dynamics of positive affect and cognition in this sample and provide a partial test of the upward spiral model. In that regard, we had several hypotheses, which, if taken together and supported by the data, comprise evidence for the upward spiral model: (a) elevations in positive affect on a given day will predict elevations in positive affect on the following day, above and beyond any trait-like propensity toward positive affect (*autoregressive component of positive affect*); (b) elevations in positive cognition on a given day will predict elevations in positive cognition on the following day, above and beyond any trait-like propensity toward positive cognition (*autoregressive component of positive cognition*); (c) elevations in positive affect on a given day will predict elevations in positive cognition on the following day, and vice versa (*cross-lagged relations between positive affect and cognition*); (d) MBCT will stimulate an upward spiral dynamic in these emotion–cognition interactions by strengthening the cross-lagged relationship between positive affect and cognition and providing the initial elevation in these variables that will then be carried forward in an autoregressive fashion.

MATERIALS AND METHODS

PARTICIPANT CHARACTERISTICS

Adults diagnosed with at least one major depressive episode who had residual depressive symptoms were recruited from outpatient mental health treatment facilities in Maastricht (the Netherlands) and through flyers posted in public spaces. The presence of residual depressive symptoms was operationalized as scoring seven or higher on the 17-item Hamilton Depression Rating Scale (HDRS; Hamilton, 1960) at the time of screening. Potential participants were excluded if they fulfilled criteria for a current depressive episode, schizophrenia, or psychotic episodes in the past year, and if they had recent (past 4 weeks) or upcoming changes in ongoing psychological or pharmacological treatment. The average ages of participants randomly assigned to the MBCT and waitlist control conditions were 44.6 (SD = 9.7) and 43.2 (SD = 9.5), respectively. The majority of participants were female (MBCT: 79%; control group: 73%). A substantial number of participants had had three or more previous depressive episodes (MBCT: 44%; control group: 45%). Other relevant sociodemographic and clinical characteristics are reported in Geschwind et al. (2011).

SAMPLING PROCEDURES

Potential participants participated in an initial phone screen to establish inclusion and exclusion criteria. During a second screening, participants were administered the Structured Clinical Interview for *DSM IV*–Axis I (First et al., 2002) and the 17-item HDRS by trained psychologists. Eligible participants were trained in using the ESM procedure, and then completed a pre-treatment assessment consisting of 6 days of experience sampling in their own environment (see Experience Sampling Method). After the baseline assessment, participants were randomized to either 8 weeks of MBCT or 8 weeks of a waitlist control condition (allocation ratio 1:1). In light of previous studies that suggest a greater benefit for those with three or more previous episodes (Teasdale et al., 2000; Ma and Teasdale, 2004), randomization to treatment condition was stratified according to number of depressive episodes (two or less vs. three or more). An independent investigator not involved in the project generated the random allocation sequence via computer in blocks of five. After completion of all baseline assessments, the researcher allocated participants to their treatment condition based on their randomization code in a sealed envelope that was opened in order of sequence. No masking of treatment condition took place. Following MBCT or the waiting period, participants again took part in 6 days of experience sampling. For their completion of the research, participants were compensated with gift vouchers worth 50 Euros. After the post-intervention assessment, individuals in the waitlist condition had the opportunity to participate in MBCT. Study procedures were approved by the Medical Ethics Committee of Maastricht University Medical Centre. The study flow diagram is depicted in **Figure 1**.

MBCT INTERVENTION

Mindfulness-based cognitive therapy training sessions closely followed the protocol outlined in Segal et al. (2002). MBCT sessions consisted of 8 weekly, 2.5 h and were run in groups of 10–15 participants (thus occasionally larger than the usual 10–12 participants per group). The time frame of assessments for control participants was matched to those of MBCT participants. MBCT sessions included instructor-led mindfulness meditation, experiential exercises, and group discussion. Participants received CDs with guided mindfulness exercises and were assigned 30–60 min of homework practice a day. MBCT was delivered by experienced trainers in a center that regularly offered mindfulness training. All trainers were supervised by an experienced health care professional who had been trained in MBCT by its co-developers, Teasdale and Williams. The mean number of MBCT sessions attended was 7.2 (SD = 1.5).

EXPERIENCE SAMPLING METHOD

Experience sampling method is a momentary assessment method that assesses participants in their daily living environment. As such, this method provides repeated, in-the moment assessments of psychological experience in a prospective and ecologically valid manner (Csikszentmihalyi and Larson, 1987; Peeters et al., 2003). ESM offers several advantages over retrospective questionnaires and interviews, including increased ecological validity, minimized retrospective bias, and improved reliability (Csikszentmihalyi and Larson, 1987). In the current study, ESM was conducted via self-assessment forms collated in a booklet for each day whose completion was prompted by a wristwatch programmed to emit a

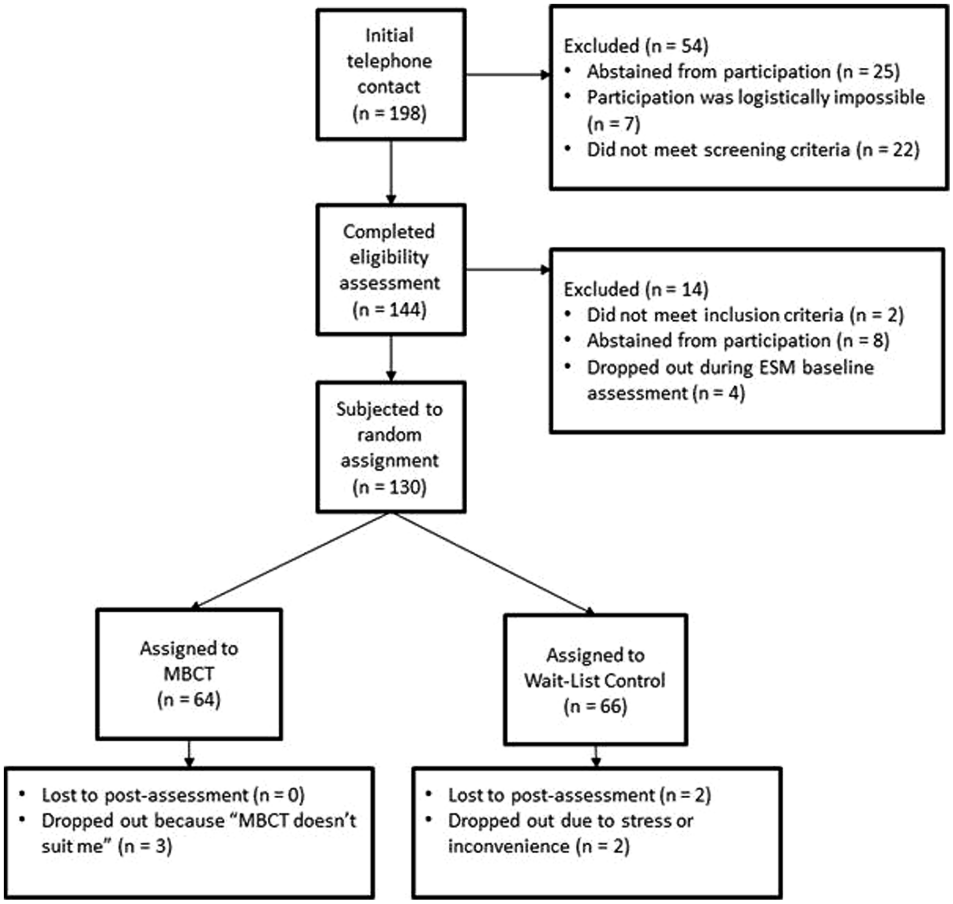


FIGURE 1 | Study flow diagram.

signal ("beep") at an unpredictable moment in each of ten 90-min time blocks between 7:30 a.m. and 10:30 p.m. ESM was conducted for six consecutive days for each study period (pre-intervention and post-intervention), resulting in a maximum of 60 beeps per study period. All self-assessments were rated on 7-point Likert scales. Trained research assistants explained the ESM procedure to the participants during an initial briefing session, and a practice form was completed to confirm that participants understood the 7-point Likert scale. To minimize memory distortion, participants were instructed to complete their ESM reports immediately after each beep, and to record the time at which they completed the form. All reports not filled in within 15 min after the actual beep were excluded from this analysis, due to decreased reliability and validity for reports completed after this interval (Delespaul, 1995). Data from a circumscribed subset of the complete ESM measurement package are reported in the present study, as detailed below.

Measurement of positive affect

At each beep, several ESM mood adjectives were assessed on 7-point Likert scales ranging from 1 (*not at all*) to 7 (*very*). Consistent with previous work (Myin-Germeys et al., 2001; Wichers et al., 2010), principal component factor analysis with oblique rotation was used to generate a factor representing PA. The mood adjectives "happy," "satisfied," "strong," "enthusiastic," "curious," "animated," and "inspired" loaded on the PA factor ($\alpha = 0.89$). One mood item ("I feel relaxed") was not included in the PA factor due to low factor loadings (<0.6). Mean levels of PA were then computed per participant for each beep moment.

Measurement of positive cognition

At each beep, participants were also asked the open-ended question "What am I thinking right now?" Subjects were encouraged to write down the first thought on their mind (this qualitative data will not be reported in the present manuscript). They were explicitly discouraged to write down chains of thoughts or multiple thoughts. Subsequently, to assess for the presence of positive cognition (i.e., positive thought appraisal), participants were asked to rate to what extent they agreed with the phrase "This is a pleasant thought" on a 7-point Likert scale ranging from 1 (*not at all*) to 7 (*very*) at each beep. If the thought was wholly unpleasant, they would rate that thought as a 0 on the pleasantness scale. Purdon and Clark (2001) and Purdon et al. (2005) assessed positive vs. negative valence of cognition employing a similar strategy of asking participants to rate the pleasantness of their thoughts.

DATA ANALYTIC PLAN

Our primary study hypothesis that MBCT would increase momentary positive cognition was tested with a multilevel linear regression model (Singer and Willett, 2003). For this analysis, ESM data were analyzed within a hierarchical structure. Multiple observations (Level 1) are clustered within participants (Level 2). Multilevel linear regression using the linear mixed modeling command in SPSS 20.0 (IBM Statistics, 2012) examined the effects of MBCT on change in momentary positive cognition. The two-way interaction between time (baseline vs. post-assessment) and treatment group (control vs. MBCT) was the parameter of interest. For this analysis, we allowed intercepts to vary randomly. Because we assumed the presence of autoregressive effects from one momentary cognitive state to the next, a first order autoregressive term (that is, AR[1]) was included in the repeated measures model.

Secondary study hypotheses were tested with a series of autoregressive latent trajectory (ALT) growth models (Bollen and Curran, 2004) following the analytic methodology employed by Rodebaugh et al. (2002). Bollen and Curran's (2004) ALT modeling strategy allows us to simultaneously estimate the time-specific autoregressive and cross-lagged effects of daily positive affect and cognition on succeeding daily levels, while accounting for stable, trait-like trajectories over time. In ALT, parameters representing trait-like stability and state-like change are estimated within a single general model. Thus, the ALT framework provides a robust way to statistically model emotion–cognition interactions of interest.

Our ALT analytic strategy was as follows. First, we averaged momentary ratings of positive affect and cognition into a mean daily level for each day in the study assessment periods. Because we were interested in the effects of MBCT on positive affect and cognition, in the final ALT model we focused on days in the post-assessment period following the treatment or waitlist control conditions, controlling for average daily levels of positive affect and cognition in pre-assessment period. Next, a succession of nested univariate ALT models were fitted separately for the repeated positive cognition and the repeated positive affect measures as a means of assessing the temporal dynamics of stability and change within each construct. Each univariate model contained a latent *intercept factor* and latent *slope factor*. The intercept factor was defined such that the factor loadings for all repeated measures were fixed to 1, save for the first assessment of positive cognition or positive affect for each respective study period, which was allowed to covary with the underlying factor. This type of parameterization, called a "predetermined model," prevents parameter bias from being introduced by not accounting for prior, unassessed levels of the construct (Bollen and Curran, 2004). The intercept factor can be interpreted to index the trait-like aspects of positive cognition and positive affect. Because the trajectory in each construct might vary randomly between individuals over time, we estimated a *linear slope factor* with factor loadings set to 1, 2, 3, 4, and 5 to account for this change.

Next, we estimated autoregressive parameters between daily measures in the presence of the latent growth factors described above. The measure of the average level of positive affect and positive cognition on a given day was regressed upon the measure of that construct on the prior day. We constrained the autoregressive parameters to equality when such constraints did not produce significant decrements in model fit.

Then, the final univariate ALT models were combined into a single multivariate ALT model to examine how positive affect and cognition interact with one another along the upward spiral over the post-assessment period. To account for the "upward spiral" hypothesis (Garland et al., 2010) whereby positive affective and cognitive processes in one point in time may preserve and energize positive affective and cognitive processes in the next, this multivariate model estimated the same autoregressive parameters from the univariate models while testing cross-lagged pathways between the previous day's level of positive affect on the following day's level of positive cognition, and vice versa. The multivariate model was then regressed on treatment condition (MBCT vs. waitlist control) and pre-treatment average daily level of positive affect and cognition to evaluate group differences in the pattern of stability and change over time in these measures. Lastly, to test whether MBCT leads to a stronger coupling between positivity on one day and positivity on subsequent days, a two-group multivariate model was then fitted, with parameters estimated separately for the MBCT and waitlist control groups and formally tested for equivalence.

Amos Version 19.0 (Arbuckle, 2010) was used to estimate all ALT models. Of the 130 participants randomly assigned to MBCT ($n = 64$) or the waitlist control condition ($n = 66$), 121 to 129 of these individuals had complete data for any given measure. To handle missing data, the models under investigation employed maximum likelihood estimation procedures, which use all available information from partially missing cases in analyses. Data were missing at random (MAR); the pattern of missingness on positive affect and positive cognition ESM responses varied randomly between individuals, and missingness could not be predicted by the present values on these variables. Only $\leq$5% of cases were missing data on the analyzed variables. Model fit was evaluated based on the relative chi-square ratio (chi-square/degrees of freedom), using an established rule of thumb of a ratio of less than 3 as indicating acceptable fit (Carmines and McIver, 1981; Marsh and Hocevar, 1985). Model fit was also evaluated using the incremental fit index (IFI; Bollen, 1990), the comparative fit index (CFI; Bentler, 1990), and the root mean squared error of approximation with 90% confidence intervals (RMSEA; Browne and Cudeck, 1993). In addition to the chi-square ratio, adequate model fit was indicated by IFI and CFI values exceeding 0.90 and RMSEA values between 0.08 and 0.10. Because studies have demonstrated that RMSEA values can be significantly inflated in modest sample sizes ($\sim$100 cases; Bollen and Ting, 2000), we expected RMSEA to be overestimated due to sample size. As such, less emphasis is placed on this fit statistic as an accurate index of model fit.

RESULTS

UNIVARIATE STATISTICS

Table 1 displays mean, standard deviation, skewness, kurtosis, and correlation for the 6 days of mean positive affect and cognition in the post-assessment period. Descriptive statistics indicate that, on the whole, participants had a moderate degree of positive affect and cognition each day. Generally, correlations

Table 1 | Correlations and univariate statistics of 6 days of positive affect and positive cognition following MBCT or a waitlist control condition.

	Positive affect						Positive cognition					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Positive affect day 1												
Positive affect day 2	0.65***											
Positive affect day 3	0.58***	0.65***										
Positive affect day 4	0.54***	0.57***	0.71***									
Positive affect day 5	0.61***	0.61***	0.70***	0.77***								
Positive affect day 6	0.61***	0.60***	0.62***	0.56***	0.66***							
Positive cognition day 1	0.70***	0.36***	0.33***	0.27**	0.38***	0.41***						
Positive cognition day 2	0.37***	0.74***	0.45***	0.45***	0.38***	0.40***	0.35***					
Positive cognition day 3	0.31**	0.37***	0.74***	0.49***	0.42***	0.44***	0.28**	0.42***				
Positive cognition day 4	0.30**	0.34***	0.45***	0.77***	0.50***	0.31***	0.33***	0.44***	0.47***			
Positive cognition day 5	0.45***	0.49***	0.41***	0.56***	0.79***	0.50***	0.42***	0.44***	0.31***	0.52***		
Positive cognition day 6	0.32***	0.30**	0.30**	0.26**	0.34***	0.72***	0.44***	0.41***	0.43***	0.30**	0.41***	
Mean	3.45	3.29	3.25	3.36	3.33	3.27	3.37	3.25	3.28	3.39	3.38	3.31
Standard deviation	1.07	1.08	1.13	1.18	1.17	1.07	0.90	0.92	0.98	1.01	0.99	1.02
Kurtosis	–0.21	–0.10	–0.06	–0.33	–0.76	–0.35	0.24	0.13	0.57	–0.34	–0.22	0.46
Skew	–0.01	0.07	0.13	–0.01	–0.04	–0.10	–0.31	–0.28	–0.26	0.09	–0.08	–0.40

*Statistics are presented in aggregate for participants in both the MBCT and waitlist control groups. **p < 0.01; ***p < 0.001.*

ranged from the small to large range (e.g., 0.3 to 0.8) with most correlations medium in strength. Correlations of mean positive affect across days tended to be high, suggesting stability in participants' affect, whereas positive cognition ratings were considerably less stable. Same day ratings of positive affect and cognition had the strongest associations. Importantly, all univariate measures of kurtosis and skewness were below 1.0; hence, the use of normal theory maximum likelihood estimation was warranted.

EFFECTS OF MBCT ON MOMENTARY POSITIVE COGNITION AND AFFECT

Consistent with our primary hypothesis, multilevel linear regression revealed that compared to the control condition, MBCT was associated with significant increases in momentary positive cognition, $b = 0.23$, SE = 0.07, 95% CI (0.09, 0.36), $p = 0.001$ (see **Table 2**). MBCT participants experienced a significant mean increase in positive cognition from pre- ($M = 3.96$, SD = 0.56) to post-assessment ($M = 4.21$, SD = 0.63), whereas control participants levels of pre- ($M = 3.92$, SD = 0.61) and post-assessment positive cognition ($M = 3.94$, SD = 0.62) were not significantly different. The AR correlation was also significant ($p < 0.001$). Similarly, as reported in the parent study from which the present data is derived (Geschwind et al., 2011), multilevel linear regression revealed that MBCT participants experienced a significant mean increase in positive affect from pre- to post-assessment, whereas control participants did not experience increased positive affect (Geschwind et al., 2011).

Table 2 | Multilevel regression analysis of the effects of MBCT on momentary experiences of positive cognition.

	Fixed effects		
	***B* (SE)**	***t***	***p***
Time (pre vs. post)	0.25 (0.05)	4.97	<0.001
Condition (MBCT vs. WL)	0.28 (0.10)	2.71	0.008
Condition X Time	0.23 (0.07)	3.21	0.001
	Random effects		
	***B* (SE)**	***z***	***p***
Intercept	0.25 (0.04)	6.87	<0.001

POST-ASSESSMENT POSITIVE AFFECT

The post-assessment univariate ALT model for daily positive cognition with an intercept factor and autoregressive structure exhibited excellent fit with the observed data: $\chi^2 = 4.98$, $df = 8$, $p = 0.76$; CFI = 1.00; IFI = 1.00; RMSEA = 0.00 (0.00, 0.07). Before interpreting parameter estimates, we considered several alternative models. First, we estimated an additional latent slope factor. We assessed for this change because it is possible that any potential effects of treatment on positive affect might strengthen or wane systematically over time. Factor loadings from this slope factor to average daily level of positive affect were fixed to values of 1, 2, 3, 4, 5 on the repeated measures for Days 2 through 6. Adding this latent positive affect slope factor did not improve model fit, nor was the mean of this latent slope factor statistically significant, suggesting that after accounting for the intercept factor and the autoregressive parameters, there was not a systematic trend

in changes in positive affect over the 6 days following treatment. Equality constraints were then imposed upon the autoregressive parameters, which did lead to a marginally significant decrement in model fit, χ^2 *change* = 9.59, *df* = 4, $p = 0.05$. Yet, the constrained model continued to fit the data well: $\chi^2 = 14.57$, *df* = 12, $p = 0.27$; CFI = 0.99; IFI = 0.99; RMSEA = 0.04 (CI = 0.00, 0.10). The significant variance of the latent intercept factor ($p < 0.001$) indicated that individuals did randomly vary in trait-like level of positive affect over the post-assessment period. All but one of the autoregressive parameters (i.e., between Day 5 and Day 6) were positive and significant in the unconstrained model, and in the constrained model, all the autoregressive effects were positive and significant. These findings indicate that elevations (or decreases) in positive affect on a given day predict elevations (or decreases) in positive affect on the following day.

POST-ASSESSMENT POSITIVE COGNITION

Similar to the modeling strategy described above, a set of nested models were estimated for positive cognition in the week following treatment. We estimated a post-assessment univariate ALT model for positive cognition with an intercept factor and autoregressive structure; this model fit the data well: $\chi^2 = 11.68$, *df* = 8, $p = 0.17$; CFI = 0.98; IFI = 0.98; RMSEA = 0.06 (0.00, 0.13). As in the positive affect ALT models, prior to interpreting the parameter estimates, we considered several alternative models. First, we estimated an additional latent slope factor. We assessed for this change because it is possible that any potential effects of treatment on positive cognition might strengthen or wane systematically over time. Factor loadings from this latent slope factor to average daily level of positive cognition were fixed to values of 1, 2, 3, 4, 5 on the repeated measures for Days 2 through 6. As before, adding this latent positive cognition slope factor did not improve model fit, nor was the mean of this latent slope factor statistically significant, suggesting that there was no systematic trend in fluctuations in positive cognition over the post-assessment study period, after accounting for the intercept factor and the autoregressive parameters. Finally, equality constraints were imposed upon the autoregressive parameters, which did not lead to a significant decrement in model fit, χ^2 *change* = 4.45, *df* = 4, $p = 0.35$. This model fit the data well: $\chi^2 = 16.13$, *df* = 12, $p = 0.19$; CFI = 0.98; IFI = 0.98; RMSEA = 0.05 (CI = 0.00, 0.11). The significant variance of the latent intercept factor ($p < 0.001$) indicated that individuals did randomly vary in trait-like level of positive cognition over the post-assessment period. However, contrary to our hypothesis, the autoregressive parameters were non-significant, indicating that the propensity toward experiencing positive cognition on the previous day did not influence the tendency to experience positive cognition on the following day. Thus, the observed covariance and mean structure in daily measures of positive cognition was explained sufficiently by a single, underlying, trait-like latent factor whose influence did not systematically vary over time during the post-assessment period.

INTERRELATION BETWEEN POSITIVE AFFECT AND COGNITION FOLLOWING TREATMENT

A single multivariate ALT model was constructed from the final univariate models of positive affect and cognition. Following Rodebaugh et al. (2002), because we had hypothesized an autoregressive effect in both processes *a priori,* we retained the autoregressive parameters in the multivariate model despite the fact that they were non-significant in the univariate ALT model for positive cognition. In our initial model, we estimated covariances among the two latent intercept factors and the Day 1 measures of positive affect and cognition. This multivariate model had adequate fit: $\chi^2 = 109.19$, *df* = 45, $p < 0.001$; CFI = 0.95; IFI = 0.95; RMSEA = 0.10 (CI = 0.08, 0.13). Next, we added cross-lagged effects to the model such that Day 2 positive cognition was regressed on Day 1 positive affect, and Day 2 positive affect was regressed on Day 1 positive cognition, etc. This cross-legged pattern was estimated across all 6 days. In addition, correlations were modeled between Day 1 measures of positive affect and positive cognition. Adding these lagged effects to the ALT model resulted in significantly improved model fit, χ^2 *change* = 22.55, *df* = 10, $p = 0.013$. Next, we imposed equality constraints between the autoregressive parameters for positive affect and positive cognition separately, as well as on the cross-lagged effects. None of these equality constraints diminished model fit. Thus, the final model contained constraints on both the autoregressive and cross-lagged parameters. This model fit the observed data well: $\chi^2 = 97.55$, *df* = 51, $p < 0.001$; CFI = 0.96; IFI = 0.96; RMSEA = 0.08 (CI = 0.06, 0.11).

Inspection of the final multivariate ALT model revealed a significant autoregressive effect between successive daily measures of positive affect, such that elevations (or decreases) in positive affect on a given day was partially a function of elevations (or decreases) in positive affect on the previous day. Although no such autoregressive effect was observed for positive cognition, the presence of significant cross-lagged effects indicated that elevations (or decreases) in positive affect on a given day influenced elevations (or decreases) in positive cognition on the following day. Furthermore, on each day, there were significant residual covariances for daily positive affect and positive cognition (*r*'s ranging from 0.75 to 0.82). Taken together, the multivariate ALT model results indicated that daily positive affect was driven by a stable, trait-like component and level of positive affect from the preceding day, whereas positive cognition was driven by a stable trait-like component as well as the previous day's level of positive affect.

THE INFLUENCE OF MBCT

Going beyond the unconditional multivariate ALT model described above, we sought to evaluate the influence of mindfulness training on the pattern of stability and change in positive affect and cognition by regressing the final multivariate model on a single, dichotomous item representing treatment condition (MBCT vs. waitlist control) and the average daily level of positive affect and cognition prior during the pre-assessment period. The unconstrained model fit the data fairly well: $\chi^2 = 185.53$, *df* = 69, $p < 0.001$; CFI = 0.92; IFI = 0.92; RMSEA = 0.11 (CI = 0.09, 0.13). We identified a significant effect of treatment condition on the latent intercept factor of positive affect ($p < 0.05$) such that MBCT participants had significantly higher trait-like positive affect in the post-assessment period than participants in the waitlist control condition. However, controlling for effects of treatment

on the latent intercept of positive affect, the effect of treatment condition on the latent intercept of positive cognition was non-significant ($p = 0.11$). In addition, average pre-assessment levels of positive affect and cognition were significantly associated with the latent intercept of post-assessment positive affect ($p < 0.001$) and cognition ($p < 0.001$), respectively.

Next, we imposed equality constraints between the autoregressive parameters for positive affect and positive cognition separately, as well as on the cross-lagged effects. None of these equality constraints significantly diminished model fit. Thus, the final model contained constraints on both the autoregressive and cross-lagged parameters. This model fit the observed data adequately: $\chi^2 = 197.08$, $df = 85$, $p < 0.001$; CFI = 0.92; IFI = 0.92; RMSEA = 0.10 (CI = 0.08, 0.12). Restraining the model did not lead to a significant decrement in model fit, χ^2 *change* = 11.62, $df = 16$, $p = 0.77$. In the constrained model, treatment condition remained a significant predictor of the latent intercept of positive affect ($p = 0.03$). As in the final unconditioned multivariate ALT model, we identified significant autoregressive effects on state-like positive affect, significant cross-lagged effects between positive affect and the following day's level of positive affect and cognition, and significant within-day covariances between positive affect and cognition. Significant parameter estimates for this final model are presented in **Figure 2**. These findings suggest that participants who experienced higher levels of positive affect and cognition following an 8-weeks course of MBCT were more likely to continue to experience positive emotions and thoughts energized by the momentum of a cycle of self-reinforcing positive affect.

Lastly, we fitted a series of two-group multivariate models, with autoregressive and cross-lagged parameters estimated separately for the MBCT and waitlist control groups, controlling for the average daily level of positive affect and cognition prior during the pre-assessment period. We first allowed autoregressive and cross-lagged parameters to vary freely. Next, we imposed equality constraints between the autoregressive parameters for positive affect and positive cognition separately, as well as on the cross-lagged effects. None of these equality constraints significantly

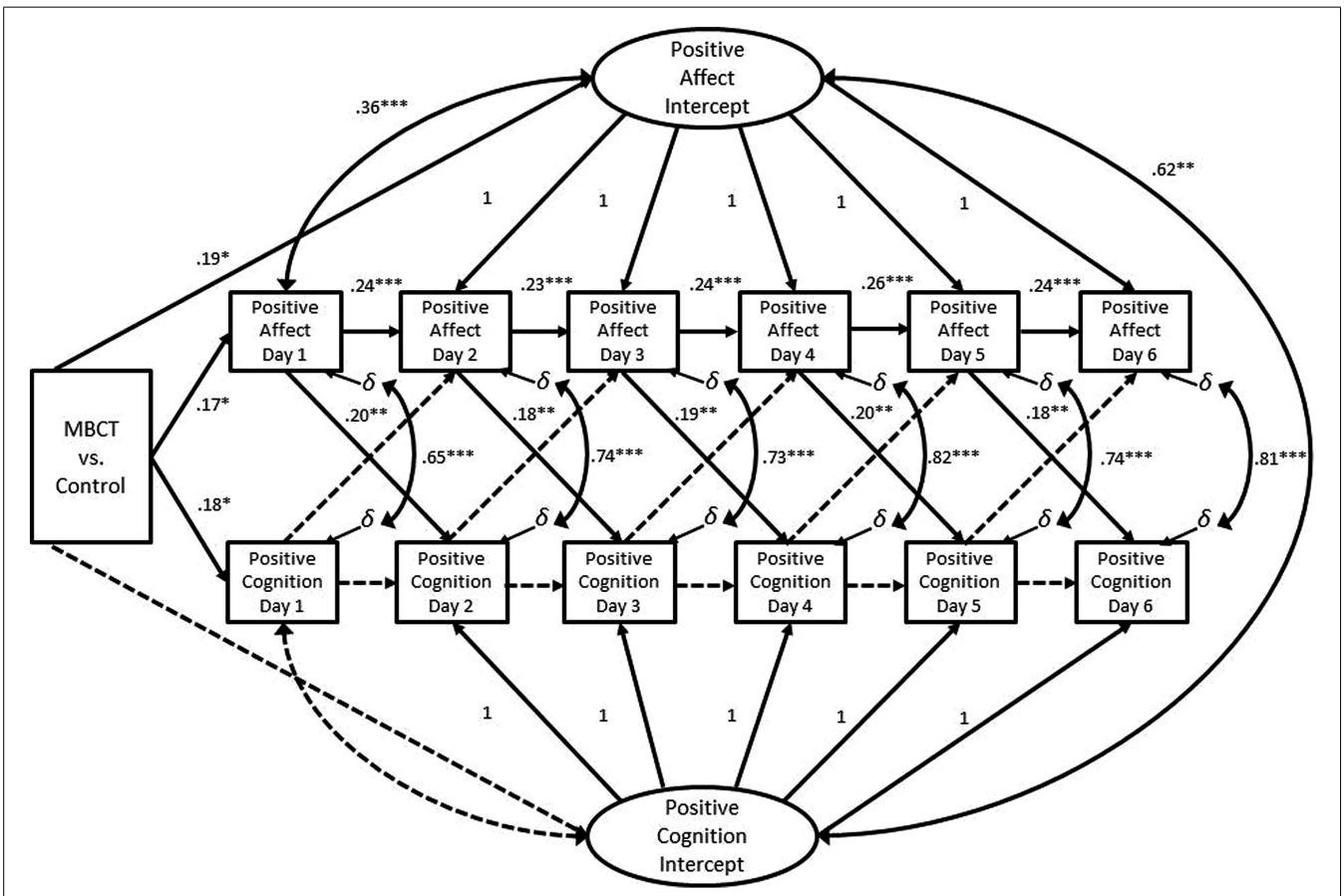


FIGURE 2 | Final autoregressive latent trajectory model of the influence of mindfulness training on upward spirals of positive emotion and cognition. This model contains post-assessment data from both the MBCT and control group; the influence of treatment condition on daily positive affect and cognition is modeled with a dichotomous predictor variable. Note – only statistically significant factor loadings and parameter estimates are shown. Although not depicted for the sake of clarity, latent positive affect and cognition factors were regressed onto average daily level of positive affect and cognition during the 6-day pre-assessment period. Similarly, error terms and non-significant parameter estimates are not shown. Non-significant paths are represented by dashed arrows with no parameter estimates. Correlations are represented by double-headed arrows. Factor loadings are marked with the value of 1; all other parameter estimates are standardized regression weights. $^*p \leq 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

diminished model fit. In the final model, autoregressive and cross-lagged parameters were constrained. This final model the data well: $\chi^2 = 256.40$, $df = 144$, $p < 0.001$; CFI = 0.92; IFI = 0.92; RMSEA = 0.07 (CI = 0.06, 0.09). Significant autoregressive effects of positive affect were observed for both the MBCT group ($b = 0.26$, SE = 0.09, $p = 0.004$) and the waitlist control group ($b = 0.22$, SE = 0.08, $p = 0.009$), but there were no significant autoregressive effects for positive cognition in either group. Significant cross-lagged effects of positive affect on the following day's level positive cognition were observed for the MBCT group ($b = 0.17$, SE = 0.08, $p = 0.04$), but there were no significant cross-lagged effects in the waitlist control group ($b = 0.11$, SE = 0.07, $p = 0.10$). Significant residual covariances were observed for same day positive affect and positive cognition for both groups. The latent intercept factors of positive affect and positive cognition significantly covaried in MBCT group ($b = 0.22$, SE = 0.08, $p = 0.004$) but not in the waitlist control group ($b = 0.07$, SE = 0.04, $p = 0.10$). In sum, with regard to model equivalence, models for the MBCT and waitlist control groups differed on: (a) cross-lagged effects of positive affect on positive cognition; and (b) covariance of latent positive affect and latent positive cognition intercept factors.

DISCUSSION

The current study revealed the temporal dynamics and interactivity of positive affect and positive cognition following treatment of partially remitted depression with MBCT. Study findings supported some of our key hypotheses: MBCT enhanced moment-by-moment positive cognition from pre- to post-treatment, and elevated daily levels of positive affect predicted higher levels of positive affect and positive cognition experienced on following days. Moreover, MBCT appeared to strengthen the cross-lagged relationship between current positive affect and positive cognition on the following day. However, contrary to our hypotheses, current positive cognition was not a significant predictor of later positive cognition or positive affect on succeeding days. Taken together, results demonstrate that at least over a 6 days period, daily positive affect and positive cognition are energized by prior experiences of positive affect – providing partial support for our hypothetical upward spiral model. Mindfulness training may play an important role in this emotionally driven upward spiral by virtue of its apparent ability to stimulate positive affect and cognition among persons with deficits in positive affectivity.

In ALT models, both positive affect and cognition were found to exhibit trait-like properties, suggesting that a significant portion of the variance in each factor as it is expressed from day to day is attributable to an underlying tendency toward experiencing positive emotions and pleasant thoughts, respectively. Yet, study findings also suggest the presence of state-like, time-specific effects whereby positive affect and cognition interact and maintain one another over time. Thus, results showed that a classical state-trait model (without a latent slope or growth structure) was sufficient to represent positive affect and cognition. Study findings suggest that positive affect is the prime mover in the upward spiral, whereby prior levels of positive affect govern subsequent experiences of positive affect and cognition. This notion is congruent with central tenets of the broaden-and-build theory, which suggest that, far from being epiphenomena, positive emotions exert consequential effects on cognition that over time build durable psychosocial resources. A number of mechanisms may account for the effects of positive affect on cognition, including: the broadening of the scope of attention (Fredrickson and Branigan, 2005; Rowe et al., 2007); the tuning of attentional selection toward affect-congruent stimuli (Friedman and Förster, 2010); the activation of state-dependent memories (Blaney, 1986); the promotion of positive evaluations of others (Forgas and Bower, 1987); the induction of positive interpretational biases (Mathews, 2012); or the increased dopaminergic activity in projections from the ventral striatum to the prefrontal and anterior cingulate cortices (Ashby et al., 1999; Mitchell and Phillips, 2007) which may serve as the neural substrate of the aforementioned processes. Through these and other putative mechanisms, affective state may color or even control the valence and quality of cognition (Clore and Palmer, 2009). The significant cross-lagged effects of daily positive affect on subsequent affect and cognition, as well as the high correlations between positive cognition and same day positive affect, suggest that emotion and thought are tightly coupled in the form of a resonant loop (Isen et al., 1978) that may preserve itself in an autoregressive fashion over time.

Although participants assigned to both the MBCT and waitlist control conditions exhibited significant autoregressive effects on positive affect, positive affect and cognition were more tightly coupled among MBCT participants following mindfulness training, as evidenced by the presence of significant cross-lagged effects within the MBCT group that were absent in the control group. This data provides partial support for our hypothesis that mindfulness training may spark an upward spiral of positive psychological processes. Findings indicate that for those individuals who respond to MBCT by experiencing enhanced positive affect, these enhancements tend to maintain themselves through a self-reinforcing cycle that is largely impelled by emotion. Thus, effects from the initial post-assessment boost in positive affect and cognition produced by mindfulness training may reverberate through subsequent iterations of the emotion–cognition cycle, thereby preserving and energizing a partial upward spiral dynamic as time progresses. Yet, to be clear, the presence of a full upward spiral could not be established, as no cross-lagged effects from positive cognition to positive affect were observed.

While mindfulness training may enhance positive affect, it may have consequential effects on negative affect as well. Indeed, in this same study sample, MBCT was found to significantly reduce momentary experiences of negative affect, and these decreases, along with increases in momentary positive affect, statistically mediated the therapeutic effect of MBCT on depressive symptoms (Batink et al., 2013). These findings converge with a meta-analysis of controlled trials which found mindfulness-based interventions to significantly reduce negative affective states (Goyal et al., 2014). At the same time, mindful emotion regulation is thought to involve increased sensory awareness of emotional experience, as evidenced by neuroimaging studies indicating a reduction in midline prefrontal reactivity in support of enhanced limbic (e.g., insular) circuitry activation (Farb et al., 2012). Thus, far from suppressing or avoiding negative emotional states, mindfulness

is associated with increased negative emotion differentiation that predicts consequent reductions in negative emotional lability (Hill and Updegraff, 2012). These other affective features of mindfulness are also important, in that negative emotions can confer insights that are crucial for longer-term psychological health and development (Parrott, 2014). Integrating findings on mindfulness and negative affect with data from the current study, it is possible that rather than merely eliminating negative emotions, mindfulness training may engender therapeutic effects by increasing the ratio of positive-to-negative emotions (Fredrickson, 2013). Although the exact mathematical parameters of this ratio remain unknown, it seems that when negative emotions are balanced by contextually appropriate positive emotions, this affective balance may decouple negative affective states from maladaptive psychological outcomes.

Beyond previously established effects of mindfulness on positive affect (Geschwind et al., 2011), to our knowledge current study findings are the first in the literature to indicate that treatment with MBCT is associated with significant increases in momentary positive cognitions (i.e., positive thought appraisal). Mindfulness is typically held to be a non-discursive, non-conceptual psychological process that is putatively antithetical to cognitive appraisal (Chambers et al., 2009). In contrast to this assumption, results of the present investigation indicate that partially remitted depressed participants of a MBCT course experienced significantly more positive momentary appraisals of their thoughts following mindfulness training. These findings provide partial support for Garland et al. (2009, 2011) hypothesis that mindfulness training promotes positive (re)appraisals by allowing for decentering from current negative situational appraisals into a non-conceptual, broadened metacognitive state from which an enlarged scope of previously unattended contextual information can be accessed for the generation of new, positive appraisals of self and world. Clearly, mindfulness and positive cognition are not identical psychological processes; to the contrary, mindfulness practice is commonly held to evoke states where sensory experience is monitored without any cognitive elaboration or evaluation (Hölzel et al., 2011). Yet, we argue that positive affect and cognition may be adaptive byproducts of mindfulness practice. According to our theoretical model (Garland and Fredrickson, 2013), the positive affect induced by mindfulness practice may provide a signal which tunes the attentional system to detect stimuli that are congruent with the induced emotional state, resulting in greater awareness of the pleasant, beautiful, or rewarding aspects of one's own mental experience or life circumstances. While mindfulness may temporarily suspend evaluative language during decentering from negative automatic thoughts, individuals will inevitably re-engage their socially constructed autobiographical narratives to make meaning out of their lives (Olivares, 2010). As the result of positive emotional tuning of the information processing system afforded by mindfulness practice, when individuals return to the semantic-narrative mode from the state of mindfulness, the new appraisals and thoughts emerging from conscious reflection on life circumstances or spontaneous insight may tend to have a positive valence. Through these processes, mindfulness training may promote positive reappraisal and facilitate traditional cognitive restructuring techniques in CBT.

The facilitative effects of mindfulness meditation training on positive emotion–cognition interactions in persons with a history of depression may have consequential effects on physical health. Indeed, meditation-induced upward spirals of positive emotion have been found to promote vagally driven increases in heart rate variability that are mediated by increased perceptions of social connectivity (Kok et al., 2013). Similarly, mindfulness training has been shown to enhance savoring of natural reward as evidenced by cue-elicited increases in heart rate variability and decreased heart rate (Garland et al., 2014b). Thus, inducing positive emotions through meditative practices seems to enhance positive appraisals of socioenvironmental stimulus contexts, a cognitive process that then exerts downstream salutary effects on cardiac-autonomic function. Such effects may indeed be consequential for health; in that regard, depression severity has been inversely associated with attenuated heart rate variability (Kemp et al., 2010), and patients exhibiting major depressive disorder with melancholia have attenuated heart rate variability relative to healthy controls which may have adverse effects on mortality and morbidity (Kemp et al., 2014). More studies are needed to elucidate the impact of meditation-induced upward spirals on vagal function, affective tone, and longevity itself.

Strengths of the present study include: the use of ESM to measure changes in positive psychological processes; the use of multiple measurement points to assess the process of stability and change; and the use of ALT modeling, a statistical technique that confers numerous advantages with regard to its ability to estimate causal models and flexibly estimate inter- and intra-individual variation over time. In addition, the study was strengthened by its randomized controlled design, which minimized potential internal threats to validity.

The study also had a number of limitations. First, because no active control condition was employed, we are unable to determine if the effects of MBCT on positive affect and cognition were specifically due to mindfulness training, or due to non-specific therapeutic factors (e.g., attention by a caring professional, social support, group dynamic, expectation of benefit, etc.). It is possible that other treatments (e.g., CBT) might have similar facilitative effects on positive psychological processes following treatment. In addition, we lacked objective treatment fidelity measures to determine to what extent instructors adhered to the MBCT protocol. The ESM procedure was also limited in that participants self-reported their completion time for ESM reports; thus, our ability to conclusively determine whether a given experience sampling report was valid or invalid (i.e., late) was somewhat reduced; however, earlier research demonstrates that self-reported completion times are reliably accurate (Jacobs et al., 2005). Also, ESM positive affect scores were derived from principal components analysis; however, some scholars assert that a latent variable factor analytic approach produces more valid data (Preacher and MacCallum, 2003). Further, our sample was comprised of persons with a history of major depressive disorder; thus, study conclusions may not generalize to non-depressed samples. Lastly, the upward spiral model includes attentional mechanisms which were not measured in the present study. Future studies employing an active control group, as well as a broader array of self-report measures, behavioral tasks

(e.g., a dot probe task comparing attentional responses to pleasant vs. neutral stimuli), and psychophysiological methods [e.g., analysis of heart rate variability or the late positive potential (LPP) component of the electroencephalogram] would allow for a more robust exploration of how MBCT might impact cognition–emotion interactions and a more complete test of the upward spiral model.

In conclusion, the present study suggests that positive affect and positive cognition are driven by upward spiral processes that might potentially be stimulated through mindfulness training. As such, this work adds to the growing body of research indicating that positive psychological processes have direct clinical relevance for the treatment of persons suffering from emotion dysregulation (Garland et al., 2010). Future studies are needed to elucidate how self-reinforcing cycles of positive psychological experience may be sparked by metacognitive processes, enhanced cognitive flexibility, and the selective tuning of attentional and interpretational systems to apprehend the positively valenced features of the natural and human environment.

ACKNOWLEDGMENT

Eric L. Garland was supported by grant number DA037005 from the National Institutes of Health during the preparation of this manuscript.

REFERENCES

Arbuckle, J. L. (2010). *Amos (Version 19.0) [Computer Program]*. Chicago, IL: SPSS.

Ashby, F. G., Isen, A. M., and Turken, A. U. (1999). A neuropsychological theory of positive affect and its influence on cognition. *Psychol. Rev.* 106, 529–550. doi: 10.1037/0033-295X.106.3.529

Baer, R. A., Smith, G. T., Hopkins, J., Krietemeyer, J., and Toney, L. (2006). Using self-report assessment methods to explore facets of mindfulness. *Assessment* 13, 27–45. doi: 10.1177/1073191105283504

Batink, T., Peeters, F., Geschwind, N., van Os, J., and Wichers, M. (2013). How does MBCT for depression work? Studying cognitive and affective mediation pathways. *PLoS ONE* 8:e72778. doi: 10.1371/journal.pone.0072778

Bentler, P. M. (1990). Comparative fit indexes in structural models. *Psychol. Bull.* 107, 238–246. doi: 10.1037/0033-2909.107.2.238

Blaney, P. H. (1986). Affect and memory: a review. *Psychol. Bull.* 99, 229–246. doi: 10.1037/0033-2909.99.2.229

Bollen, K. A. (1990). Overall fit in covariance structure models: two types of sample size effects. *Psychol. Bull.* 107, 256–259. doi: 10.1037/0033-2909.107.2.256

Bollen, K. A., and Curran, P. J. (2004). Autoregressive latent trajectory (ALT) models a synthesis of two traditions. *Sociol. Methods Res.* 32, 336–383. doi: 10.1177/0049124103260222

Bollen, K. A., and Ting, K. F. (2000). A tetrad test for causal indicators. *Psychol. Methods* 5, 3–22. doi: 10.1037/1082-989X.5.1.3

Browne, M. W., and Cudeck, R. (1993). "Alternative ways of assessing model fit," in *Testing Structural Equation Models*, eds K. A. Bollen and J. S. Long (Newbury Park, CA: SAGE Focus Editions), 154, 136–162.

Burns, A. B., Brown, J. S., Sachs-Ericsson, N., Plant, E. A., Curtis, J. T., Fredrickson, B. L., et al. (2008). Upward spirals of positive emotion and coping: replication, extension, and initial exploration of neurochemical substrates. *Pers. Individ. Dif.* 44, 360–370. doi: 10.1016/j.paid.2007.08.015

Carmines, E. G., and McIver, J. P. (1981). "Analyzing models with unobserved variables: analysis of covariance structures," in *Social measurement: Current Issues*, eds G. W. Bohrnstedt and E. F. Borgatta (Beverly Hills, CA: Sage Publications, Inc.), 65–115.

Chambers, R., Gullone, E., and Allen, N. B. (2009). Mindful emotion regulation: an integrative review. *Clin. Psychol. Rev.* 29, 560–572. doi: 10.1016/j.cpr.2009.06.005

Clore, G. L., and Huntsinger, J. R. (2007). How emotions inform judgment and regulate thought. *Trends Cogn. Sci. (Regul. Ed.)* 11, 393–399. doi: 10.1016/j.tics.2007.08.005

Clore, G. L., and Palmer, J. (2009). Affective guidance of intelligent agents: how emotion controls cognition. *Cogn. Syst. Res.* 10, 21–30. doi: 10.1016/j.cogsys.2008.03.002

Cohn, M. A., Fredrickson, B. L., Brown, S. L., Mikels, J. A., and Conway, A. M. (2009). Happiness unpacked: positive emotions increase life satisfaction by building resilience. *Emotion* 9, 361–368. doi: 10.1037/a0015952

Csikszentmihalyi, M., and Larson, R. (1987). Validity and reliability of the experience sampling method. *J. Nerv. Ment. Dis.* 175, 526–536. doi: 10.1097/00005053-198709000-00004

Davidson, R. J. (2004). Well-being and affective style: neural substrates and biobehavioural correlates. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 359, 1395–1411. doi: 10.1098/rstb.2004.1510

Delespaul, P. A. E. G. (1995). *Assessing Schizophrenia in Daily Life: The Experience Sampling Method*. Maastricht: Maastricht University Press.

Disner, S. G., Beevers, C. G., Haigh, E. A. P., and Beck, A. T. (2011). Neural mechanisms of the cognitive model of depression. *Nat. Rev. Neurosci.* 12, 467–477. doi: 10.1038/nrn3027

Farb, N. A. S., Anderson, A. K., and Segal, Z. (2012). The mindful brain and emotion regulation in mood disorders. *Can. J. Psychiatry* 57, 70–77.

First, M. B., Spitzer, R. L., Gibbon, M., and Williams, J. B. W. (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition (SCID-I/NP)*. New York: Biometrics Research, New York State Psychiatric Institute.

Forgas, J. P., and Bower, G. H. (1987). Mood effects on person-perception judgments. *J. Pers. Soc. Psychol.* 53, 53–60. doi: 10.1037/0022-3514.53.1.53

Fredrickson, B. L. (1998). What good are positive emotions? *Rev. Gen. Psychol.* 2, 300–319. doi: 10.1037/1089-2680.2.3.300

Fredrickson, B. L. (2004). The broaden-and-build theory of positive emotions. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 359, 1367–1378. doi: 10.1098/rstb.2004.1512

Fredrickson, B. L. (2013). Updated thinking about positivity ratios. *Am. Psychol.* 68, 814–822. doi: 10.1037/a0033584

Fredrickson, B. L., and Branigan, C. (2005). Positive emotions broaden the scope of attention and thought-action repertoires. *Cogn. Emot.* 19, 313–332. doi: 10.1080/02699930441000238

Fredrickson, B. L., and Joiner, T. (2002). Positive emotions trigger upward spirals toward emotional well-being. *Psychol. Sci.* 13, 172–175. doi: 10.1111/1467-9280.00431

Fredrickson, B. L., and Levenson, R. W. (1998). Positive emotions speed recovery from the cardiovascular sequelae of negative emotions. *Cogn. Emot.* 12, 191–220. doi: 10.1080/026999398379718

Freeston, M. H., Ladoceur, R., Provencher, M., and Blais, F. (1995). Strategies used with intrusive thoughts: context, appraisal, mood, and efficacy. *J. Anxiety Disord.* 9, 201–215. doi: 10.1016/0887-6185(95)00002-6

Friedman, R. S., and Förster, J. (2010). Implicit affective cues and attentional tuning: an integrative review. *Psychol. Bull.* 136, 875–893. doi: 10.1037/a0020495

Garland, E. L. (2007). The meaning of mindfulness: a second-order cybernetics of stress, metacognition, and coping. *Complement. Health Pract. Rev.* 12, 15–30.

Garland, E. L., and Fredrickson, B. A. (2013). "Mindfulness broadens awareness and builds meaning at the attention-emotion interface," in *Linking Acceptance and Commitment Therapy and Positive Psychology: A Practitioner's Guide to a Unifying Framework*, eds T. B. Kashdan and J. Ciarrochi (Oakland, CA: New Harbinger Publications, Inc.), 30–67.

Garland, E. L., Fredrickson, B. L., Kring, A. M., Johnson, D. P., Meyer, P. S., and Penn, D. L. (2010). Upward spirals of positive emotions counter downward spirals of negativity: insights from the broaden-and-build theory and affective neuroscience on the treatment of emotion dysfunctions and deficits in psychopathology. *Clin. Psychol. Rev.* 30, 849–864. doi: 10.1016/j.cpr.2010.03.002

Garland, E. L., Froeliger, B., and Howard, M. O. (2014a). Effects of mindfulness-oriented recovery enhancement on reward responsiveness and opioid cue-reactivity. *Psychopharmacology (Berl.)* 231, 3229–3238. doi: 10.1007/s00213-014-3504-7

Garland, E. L., Froeliger, B., and Howard, M. O. (2014b). Neurophysiological evidence for remediation of reward processing deficits in chronic pain and opioid misuse following treatment with mindfulness-oriented recovery enhancement: exploratory ERP findings from a pilot RCT. *J. Behav. Med.* doi: 10.1007/s10865-014-9607-0 [Epub ahead of print].

Garland, E. L., Manusov, E. G., Froeliger, B., Kelly, A., Williams, J., and Howard, M. O. (2014c). Mindfulness-oriented recovery enhancement for chronic pain and prescription opioid misuse: results from an early stage randomized controlled trial. *J. Consult. Clin. Psychol.* 82, 448–459. doi: 10.1037/a0035798

Garland, E. L., Gaylord, S. A., and Fredrickson, B. L. (2011). Positive reappraisal coping mediates the stress-reductive effect of mindfulness: an upward spiral process. *Mindfulness* 2, 59–67. doi: 10.1007/s12671-011-0043-8

Garland, E. L., Gaylord, S. A., and Park, J. (2009). The role of mindfulness in positive reappraisal. *Explore (NY)* 5, 37–44. doi: 10.1016/j.explore.2008.10.001

Geschwind, N., Peeters, F., Drukker, M., van Os, J., and Wichers, M. (2011). Mindfulness training increases momentary positive emotions and reward experience in adults vulnerable to depression: a randomized controlled trial. *J. Consult. Clin. Psychol.* 79, 618–628. doi: 10.1037/a0024595

Gotlib, I. H., Krasnoperova, E., Yue, D. N., and Joormann, J. (2004). Attentional biases for negative interpersonal stimuli in clinical depression. *J. Abnorm. Psychol.* 113, 127–135. doi: 10.1037/0021-843X.113.1.121

Goyal, M., Singh, S., Sibinga, E. M., Gould, N. F., Rowland-Seymour, A., Sharma, R., et al. (2014). Meditation programs for psychological stress and well-being: a systematic review and meta-analysis. *JAMA Intern. Med.* 174, 357–68. doi: 10.1001/jamainternmed.2013.13018

Hamilton, M. (1960). A rating scale for depression. *J. Neurol. Neurosurg. Psychiatry* 23, 56–62. doi: 10.1136/jnnp.23.1.56

Heller, A. S., Johnstone, T., Shackman, A. J., Light, S. N., Peterson, M. J., Kolden, G. G., et al. (2009). Reduced capacity to sustain positive emotion in major depression reflects diminished maintenance of fronto-striatal brain activation. *Proc. Natl. Acad. Sci. U.S.A.* 106, 22445–22450. doi: 10.1073/pnas.0910651106

Hill, C. L., and Updegraff, J. A. (2012). Mindfulness and its relationship to emotional regulation. *Emotion* 12, 81–90. doi: 10.1037/a0026355

Hölzel, B. K., Lazar, S. W., Gard, T., Schuman-Olivier, Z., Vago, D. R., and Ott, U. (2011). How does mindfulness meditation work? Proposing mechanisms of action from a conceptual and neural perspective. *Perspect. Psychol. Sci.* 6, 537–559. doi: 10.1177/1745691611419671

Ingram, R. E., Slater, M. A., Atkinson, J. H., and Scott, W. (1990). Positive automatic cognition in major affective disorder. *Psychol. Assess. J. Consult. Clin. Psychol.* 2, 209–211. doi: 10.1037/1040-3590.2.2.209

Isen, A. M., Shalker, T. E., Clark, M., and Karp, L. (1978). Affect, accessibility of material in memory, and behavior: a cognitive loop? *J. Pers. Soc. Psychol.* 36, 1–12. doi: 10.1037/0022-3514.36.1.1

Jacobs, N., Nicolson, N. A., Derom, C., Delespaul, P., van Os, J., and Myin-Germeys, I. (2005). Electronic monitoring of salivary cortisol sampling compliance in daily life. *Life Sci.* 76, 2431–2443. doi: 10.1016/j.lfs.2004.10.045

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., and Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol. Psychiatry* 67, 1067–1074. doi: 10.1016/j.biopsych.2009.12.012

Kemp, A. H., Quintana, D. S., Quinn, C. R., Hopkinson, P., and Harris, A. W. F. (2014). Major depressive disorder with melancholia displays robust alterations in resting state heart rate and its variability: implications for future morbidity and mortality. *Front. Psychol.* 5:1387. doi: 10.3389/fpsyg.2014.01387

Kok, B. E., Coffey, K. A., Cohn, M. A., Catalino, L. L., Vacharkulksemsuk, T., Algoe, S. B., et al. (2013). How positive emotions build physical health: perceived positive social connections account for the upward spiral between positive emotions and vagal tone. *Psychol. Sci.* 24, 1123–1132. doi: 10.1177/0956797612470827

Kok, B. E., and Fredrickson, B. L. (2011). Upward spirals of the heart: autonomic flexibility; as indexed by vagal tone, reciprocally and prospectively predicts positive emotions and social connectedness. *Biol. Psychol.* 85, 432–436. doi: 10.1016/j.biopsycho.2010.09.005

Lazarus, R. (1991). *Emotion and Adaptation.* New York: Oxford University Press.

Lazarus, R., and Folkman, S. (1984). *Stress, Appraisal, and Coping.* New York: Springer.

Lutz, A., Slagter, H. A., Dunne, J. D., and Davidson, R. J. (2008). Attention regulation and monitoring in meditation. *Trends Cogn. Sci. (Regul. Ed.)* 12, 163–169. doi: 10.1016/j.tics.2008.01.005

Ma, S. H., and Teasdale, J. D. (2004). Mindfulness-based cognitive therapy for depression: replication and exploration of differential relapse prevention effects. *J. Consult. Clin. Psychol.* 72, 31–40. doi: 10.1037/0022-006X.72.1.31

Macleod, A. K., and Moore, R. (2000). Positive thinking revisited: positive cognitions, well-being and mental health. *Clin. Psychol. Psychother.* 7, 1–10. doi: 10.1002/(SICI)1099-0879(200002)7:1<1::AID-CPP228>3.0.CO;2-S

Marsh, H. W., and Hocevar, D. (1985). Application of confirmatory factor analysis to the study of self-concept: first-and higher order factor models and their invariance across groups. *Psychol. Bull.* 97, 562–582. doi: 10.1037/0033-2909.97.3.562

Mathews, A. (2012). Effects of modifying the interpretation of emotional ambiguity. *J. Cogn. Psychol.* 24, 92–105. doi: 10.1080/20445911.2011.584527

Mathews, A., and MacLeod, C. (2005). Cognitive vulnerability to emotional disorders. *Annu. Rev. Clin. Psychol.* 1, 167–195. doi: 10.1146/annurev.clinpsy.1.102803.143916

Mitchell, R. L., and Phillips, L. H. (2007). The psychological, neurochemical and functional neuroanatomical mediators of the effects of positive and negative mood on executive functions. *Neuropsychologia* 45, 617–629. doi: 10.1016/j.neuropsychologia.2006.06.030

Mogg, K., Bradbury, K. E., and Bradley, B. P. (2006). Interpretation of ambiguous information in clinical depression. *Behav. Res. Ther.* 44, 1411–1419. doi: 10.1016/j.brat.2005.10.008

Myin-Germeys, I., van Os, J., Schwartz, J. E., Stone, A. A., and Delespaul, P. A. (2001). Emotional reactivity to daily life stress in psychosis. *Arch. Gen. Psychiatry* 58, 1137–1144. doi: 10.1001/archpsyc.58.12.1137

Nolen-Hoeksema, S. (2000). The role of rumination in depressive disorders and mixed anxiety/depressive symptoms. *J. Abnorm. Psychol.* 109, 504–511. doi: 10.1037/0021-843X.109.3.504

Nyklíček, I., and Kuijpers, K. F. (2008). Effects of mindfulness-based stress reduction intervention on psychological well-being and quality of life: is increased mindfulness indeed the mechanism? *Ann. Behav. Med.* 35, 331–340. doi: 10.1007/s12160-008-9030-2

Olivares, O. J. (2010). Meaning making, uncertainty reduction, and the functions of autobiographical memory: a relational framework. *Rev. Gen. Psychol.* 14, 204–211. doi: 10.1037/a0020469

Orzech, K. M., Shapiro, S. L., Brown, K. W., and McKay, M. (2009). Intensive mindfulness training-related changes in cognitive and emotional experience. *J. Posit. Psychol.* 4, 212–222. doi: 10.1080/17439760902819394

Parrott, W. G. (2014). *The Positive Side of Negative Emotions.* New York: The Guilford Press.

Peeters, F., Berkhof, J., Delespaul, P., Rottenberg, J., and Nicolson, N. A. (2006). Diurnal mood variation in major depressive disorder. *Emotion* 6, 383–391. doi: 10.1037/1528-3542.6.3.383

Peeters, F., Nicolson, N. A., Berkhof, J., Delespaul, P., and deVries, M. (2003). Effects of daily events on mood states in major depressive disorder. *J. Abnorm. Psychol.* 112, 203–211. doi: 10.1037/0021-843X.112.2.203

Preacher, K. J., and MacCallum, R. C. (2003). Repairing Tom Swift's electric factor analysis machine. *Unders. Stat.* 2, 13–43. doi: 10.1207/S15328031US0201_02

Purdon, C., and Clark, D. A. (2001). Suppression of obsession-like thoughts in nonclinical individuals: impact on thought frequency, appraisal and mood state. *Behav. Res. Ther.* 39, 1163–1181. doi: 10.1016/S0005-7967(00)00092-9

Purdon, C., Rowa, K., and Antony, M. M. (2005). Thought suppression and its effects on thought frequency, appraisal and mood state in individuals with obsessive compulsive disorder. *Behav. Res. Ther.* 43, 93–108. doi: 10.1016/j.brat.2003.11.007

Roberts-Wolfe, D., Sacchet, M., Hastings, E., Roth, H., and Britton, W. (2012). Mindfulness training alters emotional memory recall compared to active controls: support for an emotional information processing model of mindfulness. *Front. Hum. Neurosci.* 6:15. doi: 10.3389/fnhum.2012.00015

Rodebaugh, T. L., Curran, P. J., and Chambless, D. L. (2002). Expectancy of panic in the maintenance of daily anxiety in panic disorder with agoraphobia: a longitudinal test of competing models. *Behav. Ther.* 33, 315–336. doi: 10.1016/S0005-7894(02)80031-4

Rowe, G., Hirsh, J. B., and Anderson, A. K. (2007). Positive affect increases the breadth of attentional selection. *Proc. Natl. Acad. Sci. U.S.A.* 104, 383–388. doi: 10.1073/pnas.0605198104

Segal, Z., Williams, J. M., and Teasdale, J. D. (2002). *Mindfulness-Based Cognitive Therapy for Depression.* New York: The Guilford Press.

Singer, J., and Willett, J. (2003). *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence.* Oxford: Oxford University Press.

Tamir, M., and Robinson, M. D. (2007). The happy spotlight: positive mood and selective attention to rewarding information. *Pers. Soc. Psychol. Bull.* 33, 1124–1136. doi: 10.1177/0146167207301030

Teasdale, J. D., Segal, Z. V., Williams, J. M. G., Ridgeway, V. A., Soulsby, J. M., and Lau, M. A. (2000). Prevention of relapse/recurrence in major depression by mindfulness-based cognitive therapy. *J. Consult. Clin. Psychol.* 68, 615–623. doi: 10.1037/0022-006X.68.4.615

Troy, A., Shallcross, A., Davis, T., and Mauss, I. (2012). History of mindfulness-based cognitive therapy is associated with increased cognitive reappraisal ability. *Mindfulness* 4, 212–223. doi: 10.1007/s12671-012-0114-5

Wadlinger, H. A., and Isaacowitz, D. M. (2010). Fixing our focus: training attention to regulate emotion. *Pers. Soc. Psychol. Rev.* 15, 75–102. doi: 10.1177/1088868310365565

Wichers, M., Peeters, F., Geschwind, N., Jacobs, N., Simons, C. J. P., Derom, C., et al. (2010). Unveiling patterns of affective responses in daily life may improve outcome prediction in depression: a momentary assessment study. *J. Affect. Disord.* 124, 191–195. doi: 10.1016/j.jad.2009.11.010

Zautra, A. J., Davis, M. C., Reich, J. W., Nicassio, P., Tennen, H., Finan, P., et al. (2008). Comparison of cognitive behavior and mindfulness meditation interventions on adaptation to rheumatoid arthritis for patients with and without history of recurrent depression. *J. Clin. Consult. Psychol.* 76, 408–421. doi: 10.1037/0022-006X.76.3.408

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 December 2014; accepted: 06 January 2015; published online: 02 February 2015.

Citation: Garland EL, Geschwind N, Peeters F and Wichers M (2015) Mindfulness training promotes upward spirals of positive affect and cognition: multilevel and autoregressive latent trajectory modeling analyses. Front. Psychol. ***6****:15. doi: 10.3389/fpsyg.2015.00015*

This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology.

frontiers in Psychology

HYPOTHESIS AND THEORY
published: 01 May 2017
doi: 10.3389/fpsyg.2017.00635

Modernizing Relationship Therapy through Social Thermoregulation Theory: Evidence, Hypotheses, and Explorations

Hans IJzerman[1], Emma C. E. Heine[2], Saskia K. Nagel[3] and Tila M. Pronk[4]*

[1] Department of Psychology, University of Virginia, Charlottesville, VA, USA, [2] Department of Clinical Psychology, Vrije Universiteit Amsterdam, Amsterdam, Netherlands, [3] Department of Philosophy, University of Twente, Enschede, Netherlands, [4] Department of Social and Organisational Psychology, Tilburg University, Tilburg, Netherlands

In the present article the authors propose to modernize relationship therapy by integrating novel sensor and actuator technologies that can help optimize people's thermoregulation, especially as they pertain to social contexts. Specifically, they propose to integrate *Social Thermoregulation Theory* (IJzerman et al., 2015a; IJzerman and Hogerzeil, 2017) into Emotionally Focused Therapy by first doing exploratory research during couples' therapy, followed by Randomized Clinical Trials (RCTs). The authors thus suggest crafting a *Social Thermoregulation Therapy* (STT) as enhancement to existing relationship therapies. The authors outline what is known and not known in terms of social thermoregulatory mechanisms, what kind of data collection and analyses are necessary to better understand social thermoregulatory mechanisms to craft interventions, and stress the need to conduct RCTs prior to implementation. They further warn against too hastily applying these theoretical perspectives. The article concludes by outlining why STT is the way forward in improving relationship functioning.

Keywords: social thermoregulation, attachment, relationship therapy, emotion regulation, wearables, sensor technology, actuators

OPEN ACCESS

Edited by:
Andrew Kemp,
Swansea University, UK

Reviewed by:
Justine Megan Gatt,
University of New South Wales, Australia
Wataru Sato,
Kyoto University, Japan

****Correspondence:***
Hans IJzerman
h.ijzerman@gmail.com

Specialty section:
This article was submitted to Emotion Science, a section of the journal Frontiers in Psychology

Received: *11 July 2016*
Accepted: *10 April 2017*
Published: *01 May 2017*

Citation:
IJzerman H, Heine ECE, Nagel SK and Pronk TM (2017) Modernizing Relationship Therapy through Social Thermoregulation Theory: Evidence, Hypotheses, and Explorations. Front. Psychol. 8:635. doi: 10.3389/fpsyg.2017.00635

INTRODUCTION

One of the strongest predictors of one's physical health, mental health, and happiness is the quality of one's close relationships. Having high quality relationships predicts factors that we understand as *life chances,* including a longer life, greater creativity, and higher self-esteem (House et al., 1988; Argyle, 1992; Holt-Lunstad et al., 2010). However, to date, our understanding of why high quality social relationships lead to a more fulfilled and healthy life is relatively limited. The present paper serves to provide further direction to understanding some prominent underlying mechanisms through *social thermoregulation theory.* In addition, we will outline how near-future interventions can be crafted by doing research with novel technologies during relationship therapy.

Thus far, the evidence linking relationships and life chances focused at "higher order" levels: marital couples that regulate each other's emotions successfully have fewer marital problems, have better health, and are more satisfied with their relationship than couples who do not successfully co-regulate (Gottman and Levenson, 1992). But our position is broader: first, disturbances in health closely relate to dysregulated body temperature (Benzinger, 1969). Second, temperature regulation has been a major driving force for sociality in homeothermic (= warm-blooded) animals

(Ebensperger, 2001). For humans, the aggregate evidence is similarly in favor of an evolved reliance of social warmth on physical warmth (IJzerman et al., 2015a). Finally, the literature is in favor of the idea of *co-regulation,* a lower level dynamic that can help down-regulate emotional states socially (Butler and Randall, 2013).

The present article brings together these three concepts and asks the question *if* thermoregulation is crucial for physiological co-regulation in close relationships, and, consequently, proceeds to ask *whether* therapists can help improve physiological co-regulation in couples. Altogether, we propose to rely on novel technologies that can aid in developing *Social Thermoregulation Therapy* (STT) to help optimize people's social lives.

In this article, we first provide what we see as one of the main functions of relationships: relationships help distribute the burdens of the environment and help to *co-regulate.* Then, we provide the available evidence on social thermoregulation theory, integrate co-regulation with social thermoregulation theory, after which we discuss potential interventions to improve *co-thermoregulation.* Most prominently, we point to modern sensor and actuator technology as tools to help develop and then implement STT. We clarify what we know and don't know, followed by some of the risks we perceive in moving forward with such novel therapies. We anticipate that this approach will dramatically reduce the gap between researchers (theory) and therapists (application). Our position paper is much needed, as advances in this field will likely be so rapid that consequential mistakes in crafting novel relationship therapies are not unimaginable and potentially disastrous.

THE GENERAL PREMISE: RELATIONSHIPS ARE FOR CO-REGULATION

In a seminal 1992 article, Gottman and Levenson (1992) found that *co-regulation* is crucial for a relationship's success. They found that positive exchanges (e.g., responses through humor or positive problem descriptions rather than a negative, defensive response) toward a marriage partner were predictive of lower chance on divorce later, better health, and greater finger amplitude (indicative of autonomic activation). In the early days of this research, co-regulation was mostly understood through the regulation of emotions at higher, more conscious forms of attending to the other's emotion (e.g., through humor or positive problem descriptions). With more advanced equipment, researchers have also started to pay greater attention to lower level dynamics that used to be much harder to capture. As but one example, Coan et al. (2006) found that simply holding the partner's hand while under distress decreased stress-related activation in the brain while under threat of electric shock.

These insights on lower level dynamics led Butler and Randall (2013) to redefine co-regulation as the "bidirectional linkage of oscillating emotional channels (subjective experience, expressive behavior, and autonomic physiology) between partners (a linkage that) contributes to emotional and physiological stability for both partners in a close relationship" (p. 203), which thus incorporates lower level (autonomic) regulation with more conscious forms. Butler and Randall's (2013) perspective supplements the early views imparted by Gottman and Levenson (1992) with a type of social emotion regulation that is less "in the head" and more distributed and dynamic, relying on an "affective attunement" between close partners (e.g., romantic partners or caregiver and infant).

The general aim of such affective attunement is to achieve an allostatic balance in the relationship through distributing risks of environmental threats, leading to an offloading of energetic demands created by such threats (e.g., Beckes and Coan, 2011; Fitzsimons et al., 2015). The field of behavioral ecology has illustrated this idea of load sharing with conspecifics. Ostriches, for example, increase the rate of eating when they are in the presence of other ostriches, which can look out for predators (Bertram, 1980; Krebs and Davies, 1993). Homeothermic animals, like rodents, huddle up to other animals when cold to offload the energetic demands of warming up (Ebensperger, 2001). Thus, beyond distributing threat, one of the constant and very demanding threats to allostatic balance is the near-constant change in environmental temperature. For most animals their ilk help downregulate the environmental challenge that fluctuations of temperature pose on them.

WHY SOCIAL THERMOREGULATION IS VITAL FOR CO-REGULATION: THE AVAILABLE EVIDENCE

Despite modern conveniences like heaters or cloths, temperature regulation remains a considerable challenge for humans. From that perspective, Social Thermoregulation Theory complements basic approaches to co-regulation, detailing how "social warmth" (i.e., trustworthiness and social predictability) relies on more ancient needs of physical warmth. Strong evidence for the relationship between social interaction and thermoregulation can be found in studies across homoeothermic animals. In rodents, social thermoregulation has been shown to be one of the most important motivating forces behind group living, especially when temperatures drop (Ebensperger, 2001). As but one example, the Octodon Degus (a Chilean rodent) used 40% less energy and achieved a higher surface temperature when housed with three or five others (versus alone; Nuñez-Villegas et al., 2014). Studies of vervet monkeys show somewhat more complex mechanisms, with larger social networks buffering their core temperatures from the cold (McFarland et al., 2015), while even grooming a dead vervet monkey's pelt insulates against temperature variations (McFarland et al., 2015).

For humans, the aggregate evidence is similarly in favor of the evolved reliance of social on physical warmth. Psychological research has consistently shown that temperature fluctuations (either outside or lab temperature) is causally tied to social behaviors ranging from renting romance movies (Hong and

Sun, 2012) to house-purchasing decisions (Van Acker et al., 2016) to basic effects on perception, language use, and memory (IJzerman and Semin, 2009; Schilder et al., 2014; Messer et al., 2017). The effect also works the other way around: if people feel the environment to be socially unpredictable, they perceive temperatures as lower, whereas the reverse is true if people feel psychologically safe (Zhong and Leonardelli, 2008; IJzerman and Semin, 2010; IJzerman et al., 2015b, 2016; Ebersole et al., 2016). The link between psychological safety and thermoregulation extends to consumer behavior: brands that are regarded as more trustworthy induce perceptions of higher temperature, while the degree to which one is affected by temperature determines what one would pay for the brand (IJzerman et al., 2015b). This led IJzerman et al. (2015b) to conclude that temperature perceptions are a sort of social "weather report," or a temperature prediction system on the basis of which people know whether to rely on their social context (or not)[1].

Although it *seems* unlikely that social thermoregulation is still heavily involved in adult social interactions, one has to note that the evolutionary window of availability of modern conveniences (like heaters and clothes) to regulate temperature has likely been too brief to make a noticeable difference in the reliance of social on physical warmth. As a result, the need for physical warmth likely has formed as a model, or template, through which humans come to understand and interpret their social interactions.

Accordingly, interaction with others outside people's direct relationships should similarly rely on "temperature estimates." And indeed, in humans (unlike penguins) social thermoregulation is not just about huddling, but instead about attaching to different people in different contexts. Perhaps the most compelling evidence on attaching in a variety of contexts from recent work on social integration and climatic variation. IJzerman et al. (2017b) found in a relatively large sample in 12 different countries that the lower people's core temperatures, the more they engage in *complex social integration* (i.e., engage in contact with different people in different social contexts); they also found that this integration buffers their core against distance from the equator (as a proxy for colder climates). In short, the available evidence is strongly in favor of the idea that people's social networks – even the more complex ones – protect them from the cold, and that humans adapt their social behaviors and cognitions to temperature changes.[2]

HOW SOCIAL THERMOREGULATION SUPPORTS CO-REGULATION: EVIDENCE AND SPECULATIONS

We have reviewed evidence that temperature affects our social behavior and cognitions in myriad ways, while we have also reviewed evidence that shows that complex social networks still protect us against the cold. But at present, it is still unclear exactly *how* humans help regulate each other's temperature through more complex dynamics, if at all. Although there is now considerable evidence that social thermoregulation is (causally) tied to social cognitions and behaviors, the literature regarding co-thermoregulatory patterns is scarce. At best, we can extract some elementary effects and speculate about further mechanisms. Despite the limited evidence, we feel comfortable providing some first direction given the current state of diverse, but converging literatures.

For example, emotions like anxiety and sadness have come to be associated with lowered peripheral temperature (Ziegler and Cash, 1938; Ekman et al., 1983; McFarland, 1985; Ekman, 1993; Nummenmaa et al., 2014). Relatedly, adults' peripheral temperatures drop when they feel socially excluded (IJzerman et al., 2012).[3] Peripheral temperature changes also extend to early social interactions: when a mother leaves the room in the Strange Situation, the infant's skin temperature drops. Skin temperature only returns to baseline once the mother returns (and not so when a stranger enters the room; Mizukami et al., 1990). Further, people respond to close others' sadness (either partners or infants) with an increase in peripheral temperature (Vuorenkoski et al., 1969; IJzerman et al., 2015a). That these effects may be co-regulatory in nature could be inferred from studies that show that physical warmth downregulate the need for social contact after a lack of social warmth (IJzerman et al., 2012; Zhang and Risen, 2014).[4,5]

Why is the regulation of body temperature so important to our social regulation systems? Human infants – like

[1]The field of social thermoregulation in humans is its infancy. Nevertheless, a considerable amount of evidence has been gathered on the relationship between temperature regulation and social behavior. This does not mean that this field is without its criticism (and rightfully so). Given the discussion on power in psychological science, it may then also not come as a surprise that also in the field of thermoregulation some effects failed to replicate (Vess, 2012; LeBel and Campbell, 2013). Yet, other effects did replicate (IJzerman and Semin, 2009; Schilder et al., 2014; Inagaki et al., 2015; Ebersole et al., 2016; IJzerman et al., 2016). Further, original studies with larger *N*s do exist, with some studies with participant samples between 100 and 500 (e.g., IJzerman et al., 2015b; Van Acker et al., 2016), and some outliers even with samples around 30,000 (Hong and Sun, 2012) and above 6 million (Zwebner et al., 2013). We think that the criticisms should likely be directed at better specifying the models relevant for social thermoregulation theory, for which we see this paper as an important step in the right direction.

[2]Note that the more dynamic view of social thermoregulation diverges substantially from what one may understand as *conceptual embodiment,* a view advocated by for example Lakoff and Johnson (1999). They propose that warmth becomes paired with affection at an early age, and that such peripherally related constructs form a mental representation of relationships. Our view instead relies on more dynamic, and innate, co-regulation systems for which the infant searches from birth on, and that it *may* form an internal mental representation of its social network, scaffolded onto such early innate predispositions to search for warmth (cf. Bowlby, 1969; Mandler, 1992).

[3]We would like to note that when we discuss peripheral temperatures here, we mostly talk about the extremities. Little is known about temperature changes throughout the body in response to social situations, but temperature changes in the extremities are for example likely to differ from temperature changes in the face. Indeed, social exclusion has been found to lead to decreases in the extremities (IJzerman et al., 2012) but increases in the face (Paolini et al., 2016).

[4]Furthermore, the evidence on physiological patterns converging with social thermoregulation (like oxytocin and serotonin) converge with these ideas on co-thermoregulation (e.g., Beckes et al., 2015; Raison et al., 2015).

[5]We have not discussed the differences between core and peripheral temperature. Core temperatures are relatively stable, although they are influenced by time of day, distance from the equator, sex, and the quality of one's social network. Peripheral temperature is much more prone to change throughout the day. For example, peripheral temperatures drop when environmental temperatures drop and even drop about 0.7° after being socially excluded. This is so because the periphery serves as a defense mechanism from changes to the core.

many other altricial species – are not able to regulate their temperature independently and need to rely on the caregiver to thermoregulate. Early attachment processes of the human child are thus focused on its need to keep warm, likely forming the basis for an evolved model, or, rather, template, for mental (attachment-like) models concerning the relationship between physical and social warmth. Experimental evidence supports the temperature-template-attachment view: attachment has been found to moderate people's responses to temperature cues in a variety of reports (see, e.g., IJzerman et al., 2013). Furthermore, Vergara et al. (2017, unpublished) found that individual differences in need for social thermoregulation and preference for temperature predict not only individual differences in attachment but also stress and health, providing further support for thermoregulation as essential feature of our attachment system.[6]

Thermoregulation – across animals – is crucial for survival. The available evidence in humans also points to a robust link with social behavior and cognition, one that seems to be crucial for attachment. We therefore strongly suspect that thermoregulation becomes integrated into higher-order prediction systems and that this "temperature prediction system" supports us in navigating our social environment. Trustworthiness of brands for example do not only increase temperature perceptions, they also drive purchasing decisions (IJzerman et al., 2015b), while temperature fluctuations also influence people's conformity to the majority appeal (Huang et al., 2014) or their decisions to engage in social interactions (Hong and Sun, 2012; Van Acker et al., 2016).

And there are some indications that responses to others' emotions manifest through peripheral temperature changes. This is why, in line with previous work (IJzerman et al., 2015b), we have reason to believe that the "weather report" we have used as a metaphor relies on peripheral temperature to provide people with information on the basis of which they adapt to social situations. "Spending" this on others should thus only happen if we expect to be "paid back" in the future. Wagemans and IJzerman (2015, unpublished) for example found that peripheral temperature increases, but *only* if the relationship is communal. Szymkow et al. (2013) and IJzerman et al. (2015b) find that people estimate temperature higher, but *only* if the target is trustworthy (and *only* if lab temperatures are lower; Ebersole et al., 2016; IJzerman et al., 2016). Finally, people's need to thermoregulate is higher, but again *only* if they perceive others as trustworthy (i.e., are securely attached; Vergara et al., 2017, unpublished).

In other words, there is considerable variation in the degree to which we (literally) warm up to others. There is also variation in the degree to which we perceive benefits from others in relation to thermoregulation and consequently the degree to which people "spend" their thermoregulation on others. This "spending" should be contingent not only on one's past experiences, but also on the quality of the relationship. With novel technological inventions it becomes possible to study these dynamics in a methodologically sound fashion, cost efficiently, and in *real time*.

[6]We stress that the relationship between social thermoregulation and health to date has only been found in correlational studies, and no prospective studies have been conducted.

SOCIAL THERMOREGULATION'S PHYSIOLOGICAL MECHANISMS: TOWARD PREDICTING DYNAMIC PATTERNS

The key to understanding temperature prediction systems – and how they help us adapt to social contexts – is the *economy of action* (Proffitt, 2006; Schnall et al., 2010; Beckes and Coan, 2011; Coan and Sbarra, 2015). The premise is simple: organisms need to take in more energy than they exert, and overspending the energy expenditure budget is a threat to allostatic balance. In other animals, the metabolic costs of thermoregulation are decreased when regulated socially (Gilbert et al., 2006). We believe that social emotion regulation is (partly) rooted in the need to maintain temperature homeostasis and that helping to regulate another's sadness will *cost* to support if our own periphery rises in peripheral temperature. We will thus *only* offer emotion regulation if we suspect the other to "pay back" in the future (and we ask, is the relationship with the other is communal?).

In other words, the 'economy' of relationships can be understood by calculating who in the social network "pays" for survival and – in more modern days and relationships – who "pays" by facilitating day-to-day emotional functioning. Human relationships are therefore a bit like being modern-type penguins, but then in the sense that people's "modern" emotional systems are reliant on much older (penguin) systems. We think that this modern emotional system could rely on a "temperature monetizing system" that helps us regulate and bargain toward temperature homeostasis (Satinoff, 1978, 1982; Anderson, 2010). At present, there is virtually no research detailing how thermoregulation and metabolism relate to social emotion regulation, but there is some support for the fact that attachment-like processes rely on metabolic regulation. For example, people who are more avoidant in their relationship orientation have higher levels of fasting glucose, indicating a higher reliance on their own metabolic resources (Coan and Sbarra, 2015; Ein-Dor et al., 2015; see also Henriksen et al., 2014; IJzerman et al., 2015a).

Relationships and Co-thermoregulation

One of the goals of a relationship is thus to maintain a form of "temperature homeostasis"; keeping track of the health of the relationship through temperature helps us maintain an optimized social network. Despite the considerable evidence linking thermoregulation to social behaviors and cognitions, the underlying dynamics we need to understand to effectively integrate social thermoregulation theory into therapy are still highly speculative. We know that our need for social warmth relies on our need for physical warmth; we also know that the lack

of high quality relationships is metabolically costly; we further know that high quality relationships protect us from the cold; and we also know that both experiencing emotions ourselves and seeing emotions in others are associated with peripheral temperature changes in ourselves. Based on these different, but converging literatures, we thus strongly suspect that people co-thermoregulate close others by warming one's skin or hugging the other when sad, and that both acts are metabolically costly. Yet, whether this is true, and how they exactly interrelate is not at all clear.[7]

We further strongly suspect that co-thermoregulation can be responsive or unresponsive, based on how reliable the partners perceive the relationship to be (communality), or how reliable they themselves perceive the world in general to be (attachment style). With "responsive co-thermoregulation" – a new term we would like to introduce for relationship research and therapy – we mean the (non-conscious) regulation of each other's temperature toward homeostasis in dyads. The degree to which one participates is thus contingent upon *perceived social predictability* (i.e., a combination between attachment and communality of the relationship). Unresponsive co-thermoregulation would thus imply *not* hugging the partner when he or she is sad, and *not* increasing peripheral temperature when the other is in need. What constitutes responsive and unresponsive co-thermoregulation is still in need of very specific classification.

Acknowledging that relationships are complex and that multiple factors contribute to successful regulation, further caution is warranted in applying this perspective on co-thermoregulation in therapy. That is, the perceived social predictability can be accurate or inaccurate as in some situations being non-responsive to one's partner's emotions might be *functional*. When one's partner has a very bad temper or can be abusive, avoiding one's partner's anger – as opposed to engaging – can be considered more beneficial. Thus, part of classifying responsive versus unresponsive co-thermoregulation is the understanding of the social context in which co-thermoregulation occurs.

FROM SPECULATION TO APPLICATION: THE ROLE OF TECHNOLOGICAL ADVANCEMENTS

We have acknowledged that the dynamics of co-thermoregulation are yet unclear. Specifically, it is unclear how strong, in which situation, and for which types of emotion one's peripheral temperature should in- or decrease. At the same time, the available evidence supports the idea that understanding co-thermoregulation is vital to achieve optimal social functioning. Thermoregulation has further been implicated in (mental) health, such as depression (Pechlivanova et al., 2010), insomnia (Bach et al., 2002; Van den Heuvel et al., 2004; Pechlivanova et al., 2010), anxiety (Parry, 2007), and many others. Furthermore, physiological processes related to thermoregulation (like catabolization of *Brown Adipose Tissue*) have become linked to tumor growth (Shellock et al., 1986; Lee et al., 2010) or late onset obesity (Himms-Hagen, 1979, 1989, 1990; Van Marken Lichtenbelt et al., 2009). In other words, proper (social) thermoregulation seems vital for having optimal health.

Relationships, health, and thermoregulation are strongly interdependent, and understanding and applying thermo-regulation may well-present one of the most important advances in modern (relationship) therapy. The reason why integrating thermoregulation into modern therapy has become feasible is because of advances in a field called "eHealth" (short for electronic health), a field that has become trendy in clinical research, mostly due to obvious benefits in saving costs, time, and the lower threshold to receive therapy. The most common applications of eHealth have been to seek a reduction of costs, by for example moving part of the therapy process online (and thus decreasing the amount of hours invested in providing therapy or assessments). For STT, eHealth can also quickly help us decrease costs of research by advancing our understanding through measurements. Could it be that – because of all the intimate links between relationships, thermoregulation, and health, that STT can quickly and fundamentally transform and optimize the type of care we can receive, thereby optimize the quality of our social networks?

Application of Co-thermoregulation into Current Therapies

The application of STT into eHealth is likely most efficacious by adding it to an existing intervention known as *Emotionally Focused Therapy* (EFT). EFT is a short-term relationship therapy focusing on (co-regulatory) patterns in interaction (Johnson, 1999; Johnson et al., 1999; Greenberg, 2004). Various potential patterns of interaction in relationships are described and targeted through this type of therapy, one that is rooted in attachment theory (Bowlby, 1969). One example that shows these dynamics and its roots in attachment theory is the pattern that details how quality and emotionally unresponsive interactions often leading to stonewalling or emotionally "attacking" each other in the relationship (like Gottman and Levenson's, 1992, classification a *non-regulated couple*).

Some have claimed EFT to be the most researched and most effective couple's therapy (Johnson et al., 1999; Wiebe and Johnson, 2016), with 10 sessions of EFT improving dyadic adjustment of the relationship, and others regard it as a form of exposure therapy, exposing the couples to experiences that are emotionally taxing within the relationship (Greenman and Johnson, 2013). A few sessions of EFT have also been found to elicit greater emotional dependency on the partner (allowing to "distribute" the risk), as handholding after EFT reduced the stress experienced after electric shock through

[7]We have not even scratched the surface of the interrelationship between peripheral temperature changes and facial expression of emotions. We think it is likely that peripheral temperature changes are connected to facial expressions and other manifestations of emotions, which are thus dynamically connected to co-regulate emotions. Furthermore, we also have not even considered the link between individual differences in empathy or perspective taking. We suspect such relationships to exist, but how these links should be understood is beyond the scope of this article.

Coan et al.'s (2006) handholding paradigm (Johnson et al., 2013). Johnson et al.'s (1999) ideas are reminiscent of Gottman and Levenson's (1992) idea of the "regulated couple" where a positive marital exchange, as a "bidirectional linkage of oscillating patterns. . .(between partners)" contributes to the marriage's success. In more recent research, this view was supplemented with lower level interactions, not only by being vulnerable and offloading stress to others (Beckes and Coan, 2011; Butler and Randall, 2013), but now also by our proposal to offloading temperature regulation to the environment through what we have called the "temperature monetizing system."

At present, we know that people in high quality relationships increase in peripheral temperature when the other is stressed (Vuorenkoski et al., 1969). The central proposal of STT would be to *adjust* (i.e., re-associate) peripheral temperatures in a relationship to specific social situations but *only* if one's perception of social predictability within the relationship is misaligned. One could thus liken STT to better known neurofeedback paradigms (e.g., Lubar et al., 1995). Altering one's peripheral temperature without attention to context will certainly not reliably alter the relationship dynamic. Integrating STT into EFT in other words is complex. Not only is it hard for therapists to assess the level of co-regulation in real life, at present it is still unclear when temperature in- or decreases (and how strongly) occur in communal relationships to different emotions by the partner, and it is thus unclear *when* co-thermoregulation is responsive and when not. Furthermore, *some* types of emotions are probably reliant on co-regulation (e.g., a "cooling" state like sadness) whereas this may not be true for other emotions (e.g., a "heating" state like anger).[8] In addition, it is unclear how frequently one should manipulate peripheral temperature to support the relationship more permanently. What is clear is that STT has the potential to transform EFT by seamlessly tracking couples' physiology in daily life.

Finally, STT is *not* a replacement for therapy related to higher order cognition, but should complement existing therapies (like Cognitive Behavioral Therapy and EFT) by addressing lower level dynamics. This is also why not all couples may benefit from aiding the relationship for the sake of staying together. Some clients might be at the end of a relationship and be better off having the therapist mediate their separation than putting time and effort in trying to save the relationship. The challenges seem various and daunting. But we suppose most of these issues are resolvable. We will now outline the steps to create the most efficacious STT by doing research during therapists' EFT sessions with couples.

GETTING FROM HERE TO THERE: RESEARCH THERAPISTS CAN DO

With the advent of novel technologies, the gap between research and therapy decreases dramatically. For that reason, we describe how thermoregulatory dynamics can be researched during EFT sessions. We hope that interventions can be created based on this research. Between therapy sessions, therapists and researchers can monitor couples' temperature, location, and proximity continuously for longer periods of time from a distance while the couples live their regular lives. Whereas most eHealth focuses on becoming more efficient in therapy, once the mechanisms are clearly defined, such *real time* monitoring can have considerable (practical) transformative consequences as compared to normal EFT, because therapists can start tapping into lower level dynamics. Again, exactly how this could be achieved needs to be researched in between therapy sessions. One of the advantages is that once protocols for STT are developed, the therapist will not simply have to recreate difficult and emotional interactions, but can instead track his or her clients in their daily lives.

The tools to start such a research endeavor between EFT sessions with tracking are within reach: smartphones and smartwatches have become available with accurate temperature sensors that can measure continuously and store data online or on a distant server (Park and Chen, 2007; Dufau et al., 2011; Aram et al., 2012; Lee et al., 2012; Song et al., 2012). Continuous measurement will allow researchers – in collaboration with therapists – to make very fine-grained observations of couples' co-thermoregulatory responses. Pairing these co-thermoregulatory mechanisms to relationship outcomes (e.g., marital dissolution, relationship satisfaction) will help us classify clients' thermoregulatory responses as responsive or unresponsive.

Having sensor technology thus resolves a number of problems that researchers in psychology typically encounter, like lack of measurements. While psychologists typically focus on confirmatory studies, little sensible hypotheses can be formulated regarding the topic of co-thermoregulation. To create social thermoregulatory interventions, we thus advocate focusing on *descriptions* of relationships first, *without* predefined models. The idea is to measure couples in their daily lives; doing so across different situations across different relationships then allows for specifying which co-thermoregulatory patterns define high quality relationships. This means that (1) we quickly come to understand *whether* co-thermoregulation predicts relationship success, (2) which types of emotions rely on co-thermoregulation (and in which types of situations) and (3) which are the most ideal patterns to oscillate, for which types of individuals. Such approaches will thus allow us to quickly gain ground, create more accurate models, and from there design (confirmatory) randomized control trials. Furthermore, when mechanisms are understood based on exploratory research and Randomized Clinical Trials (RCTs) first and protocols developed second, such

[8]This is likely true because the regulation of body temperature can be understood asymmetrically: decreases in temperature are regulated socially, while this is not true of increases in temperature. This is highly contingent on the asymmetry of thermoregulatory systems: increases in core temperature are immediately dangerous for body and brain, whereas this is not true for decrease in temperature (thermosensitive neurons that detect increases in temperature are also more prominent in the hypothalamus, while thermoreceptors detecting coldness are more prominent in the skin; Hensel, 1974; Guyton, 1991). The idea of this asymmetry is also confirmed in the experimental literature: priming of trustworthiness (versus agency) in relationships leads to temperature increases under colder ambient conditions, but not under warmer ambient conditions (Ebersole et al., 2016; IJzerman et al., 2016). In addition, co-thermoregulation is certainly not the *only* aspect of co-regulation. Specifically, research across (human and non-human) animals also shows the importance of risk distribution (Ebensperger, 2001; Coan et al., 2006).

FIGURE 1 | Maxim's "Thermochron iButton DS1291H."

FIGURE 2 | BlueMaestro TempoDisc (left panel) and BlueMaestro TempoDisc in a Fitbug wristband (right panel) as used in our research.

an approach will allow therapists to become more client-centered, as the increased amount of measurements will afford a greater focus to study clients at the intra-individual level (Whitsett and Shoda, 2014; LeBel et al., 2016).

Measurement

In our own research, we have focused on using devices that are as non-intrusive as possible. Thermoregulation researchers consider the gold standard in measuring peripheral temperature Maxim's "Thermochron iButton DS1291H," which has a mean accuracy of −0.09°C and a precision of 0.05°C (Van Marken Lichtenbelt et al., 2006; Pouw et al., 2012; see also **Figure 1**). The advantage of the iButton is that it is wireless and can be easily attached to one's body. The downside of the iButton is that it is impractical in daily life as it is attached to the skin at the finger or arm.

In our more recent research, we therefore chose to move to a different sensor, the BlueMaestro Tempodisc (see **Figure 2**), which also has a precision of 0.05°C (but which is still in need of independent verification). The advantage of the BlueMaestro Tempodisc is that it can be inserted in a FitBug wristband and can be easily worn in daily life. Additionally, the BlueMaestro Tempodisc can connect to a smartphone via Bluetooth Low Energy and store and communicate the temperature changes in the wrist via our "Temperature Detection App" to our central server (for our present version, see IJzerman et al., 2017a). The sensors are affordable and our software open source.

To apply these sensors for measurement in research during EFT, couples can wear bracelets with a temperature sensor that connects to their own mobile phones via Bluetooth Low Energy. In order to be able to classify the thermoregulatory dynamics, therapists and researchers can then assess their clients' co-thermoregulation in their day-to-day life and connect these to clients' discrete emotional states via smartphone apps (e.g., through the SurveyMonkey or MoodiMoodi, app, etc.), and proximity to one another via Bluetooth connection or GPS.

Beside the BlueMaestro TempoDisc, a multitude of sensors are becoming available to measure peripheral temperature, such as a *thermodo* (a tiny thermometer one can plug into one's smartphone), and several skin thermometer gadgets (Coxworth, 2013; see **Table 1** for a list of several possible technological sensors to be used in research and therapy). The challenges to create STT are obvious, but become resolvable: beyond needing to interpret just how thermoregulation relates to discrete emotional states, it is unclear *how quickly* a thermoregulatory response to the other is most efficacious. Furthermore, *how strongly* can and should a partner respond to the other's distress? It is clear that the understanding of many of the mechanisms we outline here are in their beginning stages. But the exploratory approaches we pointed to are an important first step to be able to create accurate descriptions of what are high quality relationships. *Data-driven approach* will help us classify which thermoregulatory responses relate to "healthy" relationships versus relationships for those in need of therapy.

For this, we favor prediction over explanation: by deriving predictions from data, we can thereafter start formulating theories on how to manipulate temperature and how to craft interventions. One powerful exploratory approach that allows for classification of co-regulatory systems and making predictions from data is *supervised machine learning* (Breiman, 2001; IJzerman et al., 2016; Yarkoni and Westfall, 2016). Machine learning relies on explorative algorithms to *learn* from data, deriving complex patterns as accurately and reliably as possible. Machine learning helps to deeper understand data and reduces, for example, problems of *under-* or *overfitting,* or the problem to apply models that are overly simplistic or complex and also prevents us from applying linearity where none exists (Boulesteix et al., 2012). Supervised machine learning can thus help us generate patterns where we have no reasonable predictions *a priori*. Such exploratory approaches thus hold great promise *specifically* for real world problems such as how to integrate STT into EFT. Thus, instead of having fixed hypotheses, patterns not defined *a priori* can be detected and hypotheses derived thereafter.

Supervised machine learning thus help *classify* which co-thermoregulatory patterns relate to successful relationship outcomes, and will help define what is responsive and what is

TABLE 1 | Specifications of possible sensor devices to be used in co-thermoregulation research and therapy.

	Range (°C)	Accuracy (°C)	Resolution (°C)	Data Transfer Method	Independently Verified?	Data saved on own server only? If no, where?
Tempodisc	−25 to 75	±0.5	Unknown	Bluetooth Low Energy to smartphone app	No	No, BlueMaestro server
iButton	−20 to 85	±0.5	0.625	BlueDot receptor attached to PC	Yes	Yes
Tokyo University Sensors	25 to 50	±0.1	Unknown	Unknown	No	Unknown
MIT Band-Aid	Unknown	Unknown	Unknown	Unknown	No	Unknown
YSI 400	0 to 60	±0.1	Unknown	Unknown	Yes	Yes
Thermocouple Type T	−270 to 400	±1	Unknown	Wired	Yes	Yes
Thermistor	−100 to 300	±0.1	Unknown	Wired	Yes	Yes
Cyberglove II	10 to 45	3	<1	Wireless USB plug	No	Yes

unresponsive co-thermoregulation. Applying this approach to psychological science, IJzerman et al. (2017b) classified complex social integration as one of the most important predictors of core body temperature. Using a similar approach, researchers and therapists can easily identify whether responsive co-thermoregulation is one of the most important predictors of relationship quality (or not), and which types of oscillation patterns are ideal for high quality relationships. We suggest supervised over unsupervised machine learning, as the proposed research provides a so-called "supervisory signal" (e.g., whether people stay together or what they perceive the quality of their relationship to be).

Prior to intervention, several of such studies are needed to understand exactly how (and whether) communal relationships are facilitated through co-thermoregulation and how (and whether) interventions should be crafted to trigger *responsive co-thermoregulation* in couples that suffer from *unresponsive co-thermoregulation*. Research needs to be conducted to understand *how* therapists can intervene to train couples to show greater responsive co-thermoregulation in case the therapist decides he or she should help the couple. But whether this is efficacious, whether this is helpful in the relationship, and whether this is helpful to the individual needs to be researched carefully through collaborations between researchers and therapists. Finally, measuring subjective experience and expressive behavior are of course crucial to fully appreciate the *relative* contribution of STT in comparison to other therapies (see, for example, the development of an algorithm for our baby app that can detect and record the crying of infants; Lavner et al., 2016).

Intervention

Once exploratory approaches are finished, protocols for therapy can be tested in to-be designed randomized controlled trials (RCTs). Such RCTs can lead to interventions, through tactile technological devices, and we believe these could be available in the near future. One of the most promising devices for intervention is the "Wristify," a wristband that can manipulate peripheral temperature. In our own research, we currently use a design inspired by the Wristify, with actuators integrated into a bracelet that holds a Peltier element (controlled through an Arduino Uno with a Velleman VMA23 Motor Shield) that can apply alternating pulses of hot or cold to the skin with a range of 0.4°C per second. The pulse provides a strong subjective experience of feeling warmed or cooled. Because the wristband can be worn and controlled through Bluetooth Low Energy, with sufficient understanding of co-regulatory dynamics, apps can be designed to apply interventions in daily life. These interventions can be tailor made by the therapist for the client and controlled and monitored from a distance. We suspect interventions will be focused on enhancing a more permanent perception of the relationships' predictability (i.e., the communality) through *associative learning* (Beckes et al., 2013).[9] The Wristify is but one of the technologies; we have summarized some relevant technologies for intervention in **Table 2**. Besides wristbands to warm up or cool down, several companies have been experimenting with game controllers using temperature feedback (Dillow, 2010; Fincher, 2012).

Thus, through actuators built into a wearable device, unresponsive co-thermoregulation could be manipulated to be responsive so as to support couples that have relationship problems. One option might be to give warm (or cold) pulses to one's skin, like the wrist, with a tactile device when one's partner is sad (or otherwise shows a peripheral temperature drop) to upregulate one's temperature that we suspect will help regulate one's partner.[10] We again stress that the exact mechanisms are still unclear and that STT should not be integrated into relationship therapy until a number of exploratory and confirmatory studies have been conducted.

THE RISKS OF RELYING ON BIG DATA AND FURTHER ETHICAL CONCERNS

With such potential for rapid change and advances, we also see considerable risks. First, careful (theoretical) interpretation of data is a dire necessity and not *just* relying on automatic

[9] Although some have suggested that full-body warmth treatments can be effective against psychopathologies (Janssen et al., 2016), others have voiced their criticisms (Fried, 2016), and rightfully so. Social Thermoregulation Therapy is not *just* about warmth, but by associating warmth with predictability in the right social situations.

[10] We again would like to stress that it will not *just* be thermoregulation that will help regulate the partner, but we postulate that thermoregulation is causally linked to other emotional states that allow for more direct regulation mechanisms.

TABLE 2 | Specifications of possible actuator devices to be used in co-thermoregulation research and therapy.

	Speed / Efficiency (°C)	Method of Manipulation	Independently Verified?
Wristify	0.4 per second	(warm/cool) pulses	Yes
MIT Band-Aid	Unknown	Unknown	No
Thermosuit	−3 core temperature in 30 min	Full-body suit with waterfilled tubes	Yes
Sensor and actuator by University of Illinois	Unknown	Warming skin on top of vein	No
Electronic Skin	Unknown	Microheater on skin	Yes
Climaware Wrist Wrap	8 to 43 in a few seconds	Cools/heats wrist	No
Sony – Temperature feedback motion controller	Unknown	Cold/hot grip in hand, also fan could expel cold/hot air	No
Powerclaw haptic gloves	Unknown	Gloves with actuators	No

classification through machine learning. Without interpretation, automated processes may become unfair to one of the relationship partners, or evidence may be misguided based on pre-existing biases in past research. For example, suppose stigmatized couples or couples from lower socio-economic status do not benefit from co-regulation as others, researchers may infer that they are unable to co-regulate. However, it could instead be that the inability to co-regulate or to benefit from it is caused by perceived threat in the environment, rather than an inherent inability. An intimate collaboration between therapists and researchers to interpret complex data through a theoretical lens will be required to prevent such mishaps.

Furthermore, even though therapy may become cheaper and the threshold to seek therapy lower, future clients may fear intrusions of their privacy, with manipulations of their personal life in ways they do not desire – for good reasons. Leaked records of *Big Data* now total over 30,000 records (World's Biggest Data Breaches, 2016), while, amongst others, pharmaceutical companies (Hardekopf, 2015), real estate companies (Shamah, 2015; Ward, 2015), web shops (Marr, 2015), and Google (Marr, 2015; Van Rijmenam, n.d.) make use of *Big Data* for commercial interest in ways not necessarily for the interest of the consumer. Furthermore, rumors of the 2016 American election being manipulated by Russian hackers or companies like Twitter being brought offline through an attack on everyday wireless devices are real and legitimate concerns. One could only imagine the nightmares associated with an industry focused on manipulating and controlling one's social network. Thus, forethought for how to handle data from therapy is required and solid privacy and security protocols need to be created (Liu and Kuhn, 2010).

As a first step, the European Union now has adopted a code of conduct on privacy for mobile health applications, which specifies general guidelines for data storage (e.g., not store exact age of birth), including the "right to identity" and specifies what to do in case of data breaches (European Commission, 2017). How to prevent data breaches is still in its infancy, and the discussion on data breaches should become an important part of being able to use *Big Data* for STT.

Beyond legitimate concerns about novel technologies and questions of privacy, people may also be wary to start a therapy using novel technological devices, as fear and distrust tend to emerge at the introduction of novel technologies (q.v., Marshall, 2014; Wilson, n.d.). To avoid this, therapists need to foresee and be responsive to users' fears and developers need to design the technologies (a) to anticipate and consider the expectations, fears, and values of therapists and clients (b) to most naturally integrate them into clients' daily lives (q.v., Bartneck et al., 2007; Mori et al., 2012; Canepari et al., 2015).

To help integrate such technologies, it will be helpful to create educational material such as introductory videos demonstrating how and why the devices are used, while test booths can be created for potential new participants to test devices and instruct clients before starting the study or therapy. This allows the user to maintain control over the intervention and feel empowered to stop its usage when desired. Further, manipulations in day-to-day life of one's body may feel intrusive and may let the client wonder whether the relationship *is* still authentic, and importantly: *perceived* as authentic. Another relevant question for the balancing of benefits, risks, and costs of an intervention is whether couples can be aided by technological devices to, from there on, sustain the responsive co-thermoregulation on their own, or whether they will need the technology as a constant aiding device in times of need?

Responsible Implementation of Technology in Therapy

A key question that emerges is how to responsibly introduce such technology in the therapeutic relationship. Besides the effect monitoring may have on the person's behavior and feelings, implementation may also have effects on relationships between client(s) and therapist. Importantly, the therapist using this intervention must be sensitive in understanding the potential effects of the device on the single client, the couple, as well as on the relationship between the client/couple and the therapist. Does the technology support a trustful therapeutic relation or hinder it? How can the therapist understand whether the intervention is helpful versus harmful? Here, concerns about authenticity, naturalness, and autonomy may continue to arise. These need to be addressed by the therapist before using the devices, and they should be considered in the design phase of the devices already. And there are practical challenges with the usage in continuously monitoring in real life: what if, for example, the therapist's responsibility when he or she suspects or even notices through suspicious patterns from the fine-grained data that her client is cheating on his or her significant other?

CONCLUSION

There are still a number of questions that need to be answered before one can intervene through STT. We nevertheless aimed to provide a convincing case for its need. We have first shown considerable evidence that thermoregulation is still key to people's modern social lives and we have discussed existing evidence on co-regulation. From there, we integrated the literatures, arguing that responsive *co-thermoregulation* is a crucial feature of a healthy emotional social life. We have discussed the limitations of what we know and don't know, and the path to crafting a responsible STT. Clearly, research in the area of co-thermoregulation is still in its beginning stages. However, with the current theoretical knowledge and advancements within technology and statistical analyses, like actuator and sensor technologies and supervised machine learning, new research can be conducted with greater reliability, accuracy, and replicability. We suspect that STT can become an important part of how we improve our relationships, and that STT will become integrated into EFT for maximal effectiveness.

Technologies have become available and researchers sensitively need to help channel the implementation of these technologies, discussing the benefits and perils to allow responsible innovation. First exploratory studies – combining teams of researchers from different disciplines with therapists – need to be conducted to assess how, in whom, when, and where co-thermoregulation works. Based on this, RCTs should be designed using haptic technologies (see **Table 2**) to see whether and how, when, and with whom interventions are possible and beneficial when deemed necessary. This challenge is worthwhile because loving and warm relationships are not only pleasant, but will lead to a longer, happier, and healthier life.

AUTHOR CONTRIBUTIONS

HIJ and EH: wrote the first drafts of the paper. SN and TP: provided several critical revisions of the paper and helped writing the paper, and HIJ: finished the final version of the paper. HIJ: mostly wrote the revisions after the first submission, where TP and SN: provided several critical revisions.

FUNDING

The preparation of this article was supported by a VENI grant (016.145.049) and a Cor Wit Fonds grant both awarded to HIJ.

REFERENCES

Anderson, M. L. (2010). Neural reuse: a fundamental organizational principle of the brain. *Behav. Brain Sci.* 33, 245–313. doi: 10.1017/S0140525X10000853

Aram, S., Troiano, A., and Pasero, E. (2012). "Environment sensing using smartphone," in *Proceedings of the IEEE Sensors Applications Symposium, SAS 2012*, Brescia, 110–113. doi: 10.1109/SAS.2012.6166275

Argyle, M. (1992). "Benefits produced by supportive social relationships," in *The Meaning and Measurement of Social Support*, eds H. O. F. Veiel and U. Baumann (Washington, DC: Hemisphere Publishing Corp), 13–32.

Bach, V., Telliez, F., and Libert, J.-P. (2002). The interaction between sleep and thermoregulation in adults and neonates. *Sleep Med. Rev.* 6, 481–492. doi: 10.1053/smrv.2001.0177

Bartneck, C., Kanda, T., Ishiguro, H., and Hagita, N. (2007). "Is the uncanny valley an uncanny cliff?," in *Proceedings of the IEEE International Workshop on Robot and Human Interactive Communication*, Jeju, 368–373. doi: 10.1109/ROMAN.2007.4415111

Beckes, L., and Coan, J. A. (2011). Social baseline theory: the role of social proximity in emotion and economy of action. *Soc. Pers. Psychol. Compass* 5, 976–988. doi: 10.1111/j.1751-9004.2011.00400.x

Beckes, L., Coan, J. A., and Morris, J. P. (2013). Implicit conditioning of faces via the social regulation of emotion: ERP evidence of early attentional biases for security conditioned faces. *Psychophysiology* 50, 734–742. doi: 10.1111/psyp.12056

Beckes, L., IJzerman, H., and Tops, M. (2015). Toward a radically embodied neuroscience of attachment and relationships. *Front. Hum. Neurosci.* 9:266. doi: 10.3389/fnhum.2015.00266

Benzinger, T. H. (1969). Clinical temperature: new physiological basis. *JAMA* 209, 1200–1206. doi: 10.1001/jama.1969.03160210032008

Bertram, B. C. (1980). Vigilance and group size in ostriches. *Anim. Behav.* 28, 278–286. doi: 10.1016/S0003-3472(80)80030-3

Boulesteix, A.-L., Janitza, S., Kruppa, J., and König, I. (2012). Overview of random forest methodology and practical guidance with emphasis on computational biology and bioinformatics. *Wiley Interdiscip. Rev.* 2, 493–507. doi: 10.1002/widm.1072

Bowlby, J. (1969). *Attachment and Loss: Attachment*, Vol. 1. New York, NY: Basic Books.

Breiman, L. (2001). Random forests. *Mach. Learn.* 45, 5–32. doi: 10.1023/A:1010933404324

Butler, E. A., and Randall, A. K. (2013). Emotional coregulation in close relationships. *Emot. Rev.* 5, 202–210. doi: 10.1177/1754073912451630

Canepari, Z., Cooper, D., and Cott, E. (2015). *The Uncanny Lover*. Available at: http://www.nytimes.com/video/technology/100000003731634/the-uncanny-lover.html

Coan, J. A., and Sbarra, D. A. (2015). Social baseline theory: the social regulation of risk and effort. *Curr. Opin. Psychol.* 1, 87–91. doi: 10.1016/j.copsyc.2014.12.021

Coan, J. A., Schaefer, H. S., and Davidson, R. J. (2006). Lending a hand: social regulation of the neural response to threat. *Psychol. Sci.* 17, 1032–1039. doi: 10.1111/j.1467-9280.2006.01832.x

Coxworth, B. (2013). *Thermodo Adds a Thermometer to Your Smartphone*. Available at: http://www.gizmag.com/thermodo-smartphone-thermometer/26579/

Dillow, C. (2010). *A Video Game Controller that Stimulates with Hot and Cold Sensations*. Available at: http://www.popsci.com/technology/article/2010-07/video-game-controller-stimulates-hot-and-cold-sensations

Dufau, S., Duñabeitia, J. A., Moret-Tatay, C., McGonigal, A., Peeters, D., and Grainger, J. (2011). Smart phone, smart science: how the use of smartphones can revolutionize research in cognitive science. *PLoS ONE* 6:e24974. doi: 10.1371/journal.pone.0024974

Ebensperger, L. A. (2001). A review of the evolutionary causes of rodent group-living. *Acta Theriol.* 46, 115–144. doi: 10.1007/BF03192423

Ebersole, C. R., Atherton, O. E., Belanger, A. L., Skulborstad, H. M., Allen, J. M., and Brown, E. R. (2016). Many labs 3: evaluating participant pool quality across the academic semester via replication. *J. Exp. Soc. Psychol.* 67, 68–82. doi: 10.1016/j.jesp.2015.10.012

Ein-Dor, T., Coan, J. A., Reizer, A., Gross, E. B., Dahan, D., and Zohar, A. H. (2015). Sugarcoated isolation: evidence that social avoidance is linked to higher basal glucose levels and higher consumption of glucose. *Front. Psychol.* 6:492. doi: 10.3389/fpsyg.2015.00492

Ekman, P. (1993). Facial expression and emotion. *Am. Psychol.* 48, 384–392. doi: 10.1037/0003-066x.48.4.384

Ekman, P., Levenson, R. W., and Friesen, W. V. (1983). Autonomic nervous system activity distinguishes among emotions. *Science* 221, 1208–1210. doi: 10.1126/science.6612338

European Commission (2017). *Draft Code of Conduct on Privacy for Mobile Health Applications*. Brussels: European Commission.

Fincher, J. (2012). *Sony Patents Temperature Feedback Games Controller*. Available at: http://www.gizmag.com/sony-controller-temperature-feedback/24599/

Fitzsimons, G. M., Finkel, E. J., and vanDellen, M. R. (2015). Transactive goal dynamics. *Psychol. Rev.* 122, 648–673. doi: 10.1037/a0039654

Fried, E. (2016). *Hyperthermia as Depression Treatment*. Available at: http://eiko-fried.com/hyperthermia-as-depression-treatment/

Gilbert, C., Robertson, G., Le Maho, Y., Naito, Y., and Ancel, A. (2006). Huddling behavior in emperor penguins: dynamics of huddling. *Physiol. Behav.* 88, 479–488. doi: 10.1016/j.physbeh.2006.04.024

Gottman, J. M., and Levenson, R. W. (1992). Marital processes predictive of later dissolution: behavior, physiology, and health. *J. Pers. Soc. Psychol.* 63, 221–233. doi: 10.1037/0022-3514.63.2.221

Greenberg, L. S. (2004). Emotion–focused therapy. *Clin. Psychol. Psychother.* 16, 3–16. doi: 10.1002/cpp.388

Greenman, P. S., and Johnson, S. M. (2013). Process research on emotionally focused therapy (EFT) for couples: linking theory to practice. *Fam. Process* 52, 46–61. doi: 10.1111/famp.12015

Guyton, A. C. (1991). *Textbook of Medical Physiology*. Philadelphia, PA: W.B. Saunders.

Hardekopf, B. (2015). *The Big Data Breaches of 2014*. Available at: http://www.forbes.com/sites/moneybuilder/2015/01/13/the-big-data-breaches-of-2014/#1e977af53a48

Henriksen, R. E., Torsheim, T., and Thuen, F. (2014). Loneliness, social integration and consumption of sugar-containing beverages: testing the social baseline theory. *PLoS ONE* 9:e104421. doi: 10.1371/journal.pone.0104421

Hensel, H. (1974). Thermoreceptors. *Annu. Rev. Physiol.* 36, 233–249. doi: 10.1146/annurev.ph.36.030174.001313

Himms-Hagen, J. (1979). Obesity may be due to a malfunctioning of brown fat. *Can. Med. Assoc. J.* 121, 1361–1364.

Himms-Hagen, J. (1989). Brown adipose tissue thermogenesis and obesity. *Prog. Lipid Res.* 28, 67–115. doi: 10.1016/0163-7827(89)90009-X

Himms-Hagen, J. (1990). Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB J.* 4, 2890–2898.

Holt-Lunstad, J., Smith, T. B., and Layton, J. B. (2010). Social relationships and mortality risk: a meta-analytic review. *PLoS Med.* 7:e1000316. doi: 10.1371/journal.pmed.1000316

Hong, J., and Sun, Y. (2012). Warm it up with love: the effect of physical coldness on liking of romance movies. *J. Consum. Res.* 39, 293–306. doi: 10.1086/662613

House, J. S., Landis, K. R., and Umberson, D. (1988). Social relationships and health. *Science* 241, 540–545. doi: 10.1126/science.3399889

Huang, X. I., Zhang, M., Hui, M. K., and Wyer, R. S. Jr. (2014). Warmth and conformity: the effects of ambient temperature on product preferences and financial decisions. *J. Consum. Psychol.* 24, 241–250. doi: 10.1016/j.jcps.2013.09.009

IJzerman, H., Coan, J. A., Wagemans, F. M. A., Missler, M. A., Beest, I., van Lindenberg, S., et al. (2015a). A theory of social thermoregulation in human primates. *Front. Psychol.* 6:464. doi: 10.3389/fpsyg.2015.00464

IJzerman, H., Gallucci, M., Pouw, W. T., Weiβgerber, S. C., Van Doesum, N. J., and Williams, K. D. (2012). Cold-blooded loneliness: social exclusion leads to lower skin temperatures. *Acta Psychol.* 140, 283–288. doi: 10.1016/j.actpsy.2012.05.002

IJzerman, H., and Hogerzeil, L. J. (2017). "People as penguins: thermoregulation as part of the human essence," in *Oxford Handbook of Human Essence*, eds M. van Zomeren and J. Dovidio (Oxford: Oxford University Press). Available at: https://papers.ssrn.com/sol3/Papers.cfm?abstract_id=2844963

IJzerman, H., Janssen, J. A., and Coan, J. A. (2015b). Maintaining warm, trusting relationships with brands: increased temperature perceptions after thinking of communal brands. *PLoS ONE* 10:e0125194. doi: 10.1371/journal.pone.0125194

IJzerman, H., Karremans, J. C., Thomsen, L., and Schubert, T. W. (2013). Caring for sharing. *Soc. Psychol.* 44, 160–166. doi: 10.1027/1864-9335/a000142

IJzerman, H., Ligtenberg, W., and Verbeke, W. (2017a). *The Social Thermoregulation App*. Available at: https://github.com/wligtenberg/closeness-android

IJzerman, H., Lindenberg, S., Dalgar, I., Weissgerber, S. C., Vergara, R. C., and Zickfeld, J. (2017b). *The Human Penguin Project: Social Integration Protects Against Cold Climates*. Available at: https://osf.io/preprints/psyarxiv/6b7ne

IJzerman, H., and Semin, G. R. (2009). The thermometer of social relations: mapping social proximity on temperature: research article. *Psychol. Sci.* 20, 1214–1220. doi: 10.1111/j.1467-9280.2009.02434.x

IJzerman, H., and Semin, G. R. (2010). Temperature perceptions as a ground for social proximity. *J. Exp. Soc. Psychol.* 46, 867–873. doi: 10.1016/j.jesp.2010.07.015

IJzerman, H., Szymkow, A., and Parzuchowski, M. (2016). Warmer hearts, and warmer, but noisier rooms: communality does elicit warmth, but only for those in colder ambient temperatures–commentary on Ebersole et al. (2016). *J. Exp. Soc. Psychol.* 67, 88–90. doi: 10.1016/j.jesp.2015.12.004

Inagaki, T. K., Irwin, M. R., and Eisenberger, N. I. (2015). Blocking opioids attenuates physical warmth-induced feelings of social connection. *Emotion* 15, 494. doi: 10.1037/emo0000088

Janssen, C. W., Lowry, C. A., Mehl, M. R., Allen, J. J. B., Kelly, K. L., Gartner, D. E., et al. (2016). Whole-body hyperthermia for the treatment of Major Depressive Disorder. *JAMA Psychiatry* 53706, 1–7. doi: 10.1001/jamapsychiatry.2016.1031

Johnson, S. M. (1999). "Emotionally focused couple therapy: straight to the heart," in *Short-Term Couple Therapy*, ed. J. Donovan (New York, NY: Guilford Press), 13–42.

Johnson, S. M., Hunsley, J., Greenberg, L., and Schindler, D. (1999). Emotionally focused couples therapy: status and challenges. *Clin. Psychol.* 6, 67–79. doi: 10.1093/clipsy.6.1.67

Johnson, S. M., Moser, M. B., Beckes, L., Smith, A., Dalgleish, T., and Coan, J. A. (2013). Soothing the threatened brain: leveraging contact comfort with emotionally focused therapy. *PLoS ONE* 8:e79314. doi: 10.1371/journal.pone.0079314

Krebs, J. R., and Davies, N. B. (1993). *An Introduction to Behavioural Ecology*. New York, NY: John Wiley & Sons.

Lakoff, G., and Johnson, M. (1999). *Philosophy in the Flesh: The Embodied Mind and its Challenge to Western Thought*. New York, NY: Basic Books.

Lavner, Y., Cohen, R., Ruinskiy, D., and IJzerman, H. (2016). "Baby cry detection in domestic environment using deep learning," in *Proceedings of the ICSEE International Conference on the Science of Electrical Engineering*, Rochester, NY. doi: 10.1109/icsee.2016.7806117

LeBel, E. P., Borsboom, D., Hasselman, F., Stahl, C., Kievit, R., and Campbell, L. (2016). *Overcoming Experimental Psychology's Crisis by Studying Humans at the Intra-Individual (Rather Than Inter-Individual) Level*. Available at: Retrieved from https://docs.google.com/document/d/1qTd87KBhhNcqemdkcr0qcrYvJrLh4MBT4RKaz-S7Wx4/edit

LeBel, E. P., and Campbell, L. (2013). Heightened sensitivity to temperature cues in individuals with high anxious attachment: Real or elusive phenomenon? *Psychol. Sci.* 24, 2128–2130. doi: 10.1177/0956797613486983

Lee, P., Greenfield, J. R., Ho, K. K., and Fulham, M. J. (2010). A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 299, E601–E606. doi: 10.1152/ajpendo.00298.2010

Lee, Y.-G., Jeong, W. S., and Yoon, G. (2012). Smartphone-based mobile health monitoring. *Telemed. J. E Health* 18, 585–590. doi: 10.1089/tmj.2011.0245

Liu, S., and Kuhn, R. (2010). Data loss prevention. *IT Prof.* 12, 10–13. doi: 10.1109/MITP.2010.52

Lubar, J. F., Swartwood, M. O., Swartwood, J. N., and O'Donnell, P. H. (1995). Evaluation of the effectiveness of EEG neurofeedback training for ADHD in a clinical setting as measured by changes in TOVA scores, behavioral ratings, and WISC-R performance. *Biofeedback Self Regul.* 20, 83–99. doi: 10.1007/BF01712768

Mandler, J. M. (1992). How to build a baby: II. Conceptual primitives. *Psychol. Rev.* 99, 587–604. doi: 10.1037/0033-295X.99.4.587

Marr, B. (2015). *Big Data: The Top 10 Commercial Hadoop Platforms*. Available at: https://www.linkedin.com/pulse/big-data-top-10-commercial-hadoop-platforms-bernard-marr [accessed May 11, 2016].

Marshall, G. (2014). *Twelve Technologies that Scared the World Senseless*. Available at: http://www.techradar.com/news/world-of-tech/12-technologies-that-scared-the-world-senseless-1249053 [accessed May 11, 2016].

McFarland, R., Henzi, S. P., Barrett, L., Wanigaratne, A., Coetzee, E., Fuller, A., et al. (2015). Thermal consequences of increased pelt loft infer an additional utilitarian function for grooming. *Am. J. Primatol.* 78, 456–461. doi: 10.1002/ajp.22519

McFarland, R. A. (1985). Relationship of skin temperature changes to the emotions accompanying music. *Biofeedback Self Regul.* 10, 255–267. doi: 10.1007/BF00999346

Messer, M., Imhoff, R., and IJzerman, H. (2017). *Temperature Cues Bias the Memory of Trustworthiness of Faces*. Available at SSRN: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2847556.

Mizukami, K., Kobayashi, N., Ishii, T., and Iwata, H. (1990). First selective attachment begins in early infancy: a study using telethermography. *Infant Behav. Dev.* 13, 257–271. doi: 10.1016/0163-6383(90)90034-6

Mori, M., MacDorman, K. F., and Kageki, N. (2012). The uncanny valley. *IEEE Robot. Autom. Mag.* 19, 98–100. doi: 10.1109/MRA.2012.2192811

Nummenmaa, L., Glerean, E., Hari, R., and Hietanen, J. K. (2014). Bodily maps of emotions. *Proc. Natl. Acad. Sci. U.S.A.* 111, 646–651. doi: 10.1073/pnas.1321664111

Nuñez-Villegas, M., Bozinovic, F., and Sabat, P. (2014). Interplay between group size, huddling behavior and basal metabolism: an experimental approach in the social degu. *J. Exp. Biol.* 217, 997–1002. doi: 10.1242/jeb.096164

Paolini, D., Alparone, F. R., Cardone, D., van Beest, I., and Merla, A. (2016). "The face of ostracism": the impact of the social categorization on the thermal facial responses of the target and the observer. *Acta Psychol.* 163, 65–73. doi: 10.1016/j.actpsy.2015.11.001

Park, Y., and Chen, J. V. (2007). Acceptance and adoption of the innovative use of smartphone. *Ind. Manage. Data Syst.* 107, 1349–1365. doi: 10.1108/02635570710834009

Parry, B. L. (2007). Sleep disturbances at menopause are related to sleep disorders and anxiety symptoms. *Menopause* 14, 812–814. doi: 10.1097/gme.0b013e318127188b

Pechlivanova, D., Tchekalarova, J., Nikolov, R., and Yakimova, K. (2010). Dose-dependent effects of caffeine on behavior and thermoregulation in a chronic unpredictable stress model of depression in rats. *Behav. Brain Res.* 209, 205–211. doi: 10.1016/j.bbr.2010.01.037

Pouw, W., Flore, P. C., and IJzerman, H. (2012). *Measuring Temperature with the iButton–a Quick Guide*. Available at: http://www.academia.edu/1557698/Measuring_Temperature_with_the_iButton_A_Quick_Guide

Proffitt, D. R. (2006). Embodied perception and the economy of action. *Perspect. Psychol. Sci.* 1, 110–122. doi: 10.1111/j.1745-6916.2006.00008.x

Raison, C. L., Hale, M. W., Williams, L., Wager, T. D., and Lowry, C. A. (2015). Somatic influences on subjective well-being and affective disorders: the convergence of thermosensory and central serotonergic systems. *Front. Psychol.* 5:1580. doi: 10.3389/fpsyg.2014.01580

Satinoff, E. (1978). Neural organization and evolution of thermal regulation in mammals. *Science* 201, 16–22. doi: 10.1126/science.351802

Satinoff, E. (1982). "Are there similarities between thermoregulation and sexual behavior?," in *The Physiological Mechanisms of Motivation*, ed. D. W. Pfaff (New York, NY: Springer), 217–251.

Schilder, J. D., IJzerman, H., and Denissen, J. J. (2014). Physical warmth and perceptual focus: a replication of IJzerman and Semin (2009). *PLoS ONE* 9:e112772. doi: 10.1371/journal.pone.0112772

Schnall, S., Zadra, J. R., and Proffitt, D. R. (2010). Direct evidence for the economy of action: Glucose and the perception of geographical slant. *Perception* 39, 464–482. doi: 10.1068/p6445

Shamah, D. (2015). *Big Data a Big Deal in Commercial Real Estate, Say CrediFi*. Available at: http://www.timesofisrael.com/big-data-a-big-deal-in-commercial-real-estate-says-credifi/ [accessed May 11, 2016].

Shellock, F. G., Riedinger, M. S., and Fishbein, M. C. (1986). Brown adipose tissue in cancer patients: possible cause of cancer-induced cachexia. *J. Cancer Res. Clin. Oncol.* 111, 82–85. doi: 10.1007/BF00402783

Song, W., Yu, H., Liang, C., Wang, Q., and Shi, Y. (2012). "Body monitoring system design based on android smartphone," in *Proceedings of the World Congress on Information and Communication Technologies (WICT)*, Trivandrum, doi: 10.1109/WICT.2012.6409247

Szymkow, A., Chandler, J., IJzerman, H., Parzuchowski, M., and Wojciszke, B. (2013). Warmer Hearts, warmer rooms: focusing on positive communal but not agentic traits increases estimates of ambient temperature. *Soc. Psychol.* 44, 167–176. doi: 10.1027/1864-9335/a000147

Van Acker, B. B., Kerselaers, K., Pantophlet, J., and IJzerman, H. (2016). Homelike thermoregulation: how physical coldness makes an advertised house into a home. *J. Exp. Soc. Psychol.* 67, 20–27. doi: 10.1016/j.jesp.2015.10.008

Van den Heuvel, C. J., Ferguson, S. A., Gilbert, S. S., and Dawson, D. (2004). Thermoregulation in normal sleep and insomnia: the role of peripheral heat loss and new applications for digital thermal infrared imaging (DITI). *J. Therm. Biol.* 29, 457–461. doi: 10.1016/j.jtherbio.2004.08.014

Van Marken Lichtenbelt, W. D., Daanen, H. A., Wouters, L., Fronczek, R., Raymann, R. J., Severens, N. M., et al. (2006). Evaluation of wireless determination of skin temperature using iButtons. *Physiol. Behav.* 88, 489–497. doi: 10.1016/j.physbeh.2006.04.026

Van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M. A. F. L., Kemerink, G. J., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508. doi: 10.1056/NEJMoa0808718

Van Rijmenam, M. (n.d.). *How Google Applies Big Data to Know you–Infographic*. Available at: from https://datafloq.com/read/google-applies-big-data-infographic/385 [accessed May 11, 2016].

Vess, M. (2012). Warm thoughts attachment anxiety and sensitivity to temperature cues. *Psychol. Sci.* 23, 472–474. doi: 10.1177/0956797611435919

Vuorenkoski, V., Wasz-Hockert, O., Koivisto, E., and Lind, J. (1969). The effect of cry stimulus on the temperature of the lactating breast of primipara. A thermographic study. *Experientia* 25, 1286–1287. doi: 10.1007/BF01897502

Ward, M. (2015). *How Big Data is Transforming Real Estate*. Available at: http://www.cnbc.com/2015/08/04/how-big-data-is-transforming-real-estate.html [accessed May 11, 2016].

Whitsett, D. D., and Shoda, Y. (2014). An approach to test for individual differences in the effects of situations without using moderator variables. *J. Exp. Soc. Psychol.* 50, 94–104. doi: 10.1016/j.jesp.2013.08.008

Wiebe, S. A., and Johnson, S. M. (2016). A review of the research in emotionally focused therapy for couples. *Fam. Process* 55, 390–407. doi: 10.1111/famp.12229

Wilson, L. (n.d.). *11 Examples of Fear and Suspicion of New Technology*. Available at: http://lenwilson.us/11-examples-of-fear-and-suspicion-of-new-technology/ [accessed May 11, 2016].

World's Biggest Data Breaches (2016). Available at. http://www.informationisbeautiful.net/visualizations/worlds-biggest-data-breaches-hacks/ [accessed July 10, 2016].

Yarkoni, T., and Westfall, J. (2016). *Choosing Prediction Over Explanation in Psychology: Lessons From Machine Learning*. Available at: http://jakewestfall.org/publications/Yarkoni_Westfall_choosing_prediction.pdf

Zhang, Y., and Risen, J. L. (2014). Embodied motivation: using a goal systems framework to understand the preference for social and physical warmth. *J. Pers. Soc. Psychol.* 107, 965. doi: 10.1037/a0038153

Zhong, C. B., and Leonardelli, G. J. (2008). Cold and lonely: does social exclusion literally feel cold? *Psychol. Sci.* 19, 838–842. doi: 10.1111/j.1467-9280.2008.02165.x

Ziegler, L. H., and Cash, P. T. (1938). A study of the influence of emotions and affects on the surface temperature of the human body. *Am. J. Psychiatry* 95, 677–696. doi: 10.1176/ajp.95.3.677

Zwebner, Y., Lee, L., and Goldenberg, J. (2013). The temperature premium: warm temperatures increase product valuation. *J. Consum. Psychol.* 24, 251–259. doi: 10.1016/j.2013.11.003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read, for greatest visibility

COLLABORATIVE PEER-REVIEW
Designed to be rigorous – yet also collaborative, fair and constructive

FAST PUBLICATION
Average 85 days from submission to publication (across all journals)

COPYRIGHT TO AUTHORS
No limit to article distribution and re-use

TRANSPARENT
Editors and reviewers acknowledged by name on published articles

SUPPORT
By our Swiss-based editorial team

IMPACT METRICS
Advanced metrics track your article's impact

GLOBAL SPREAD
5'100'000+ monthly article views and downloads

LOOP RESEARCH NETWORK
Our network increases readership for your article

Frontiers
EPFL Innovation Park, Building I • 1015 Lausanne • Switzerland
Tel +41 21 510 17 00 • Fax +41 21 510 17 01 • info@frontiersin.org
www.frontiersin.org

Find us on