Cold face test-induced increases in heart rate variability are abolished by engagement in a social cognition task

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Abstract

The vagus nerve is a major constituent in the bidirectional relationship between the heart and the

prefrontal cortex. This study investigated the role of the vagus in social cognition using the cold

face test (facial cooling) to stimulate the vagus nerve and increase prefrontal inhibitory control.

Heart Rate Variability (HRV) was measured to index parasympathetic outflow while social

cognition ability was tested using the Reading the Mind in the Eyes Test (RMET). Healthy males

(n=25) completed the RMET under two conditions: with and without facial cooling. Results

indicated that although facial cooling increased HRV at rest, there was no improvement on the

RMET during the facial cooling condition. Interestingly, completing the RMET with facial

cooling abolished this increase in HRV, suggesting interference along the vagal reflex arc. These

results are consistent with the involvement of a common cortico-subcortical circuit in autonomic

and cognitive processes, important for emotion recognition.

Key words: Heart rate variability; Social cognition; Vagal function; Cold face test.

1. Introduction

Optimal social behavior is characterized by accurate social cognition ability, defined as the interpretation and perception of the intentions and behaviors of others (Fiske & Taylor, 2013). This process involves both central ('brain') and autonomic ('heart') integration (Thayer & Lane, 2009). Whilst the medial prefrontal cortex (Amodio & Frith, 2006) and amygdala (Davis & Whalen, 2001) are key central brain areas involved in these processes, the role of the Autonomic Nervous System (ANS) in social cognition has increasingly become a focus of investigation (Appelhans & Luecken, 2006; Quintana, Kemp, Alvares, & Guastella, 2013).

The neurovisceral integration model provides an important framework for understanding the relationship between these complex systems in social cognition (Thayer & Lane, 2009). The model proposes that an adaptive neural network – including 'the Central Autonomic Network'-is involved in the self-regulation of behavioral, emotional, and cognitive processes (Benarroch, 1993; Thayer & Lane, 2000). Optimal functioning within this network promotes flexible adaptation to changing environmental demands that reflects good emotional regulation, crucial for social cognition. Specifically, the prefrontal cortex exerts inhibitory control over subcortical activity to regulate these processes in a cortico-subcortical circuit (Thayer & Lane, 2009). This bidirectional pathway includes the myelineated vagus nerve, which exerts parasympathetic control over the sinoatrial node, the heart's pacemaker, along with the stellate ganglion, which exerts sympathetic control of the sinoatrial node (Benarroch, 1993; Porges, 2003; Thayer & Lane, 2000).

The autonomic output of this network can be indexed via heart rate variability (HRV), a measure of beat-to-beat variation in the heart over time (Berntson et al., 1997). High frequency (HF; 0.15–0.4 Hz) heart rate oscillations are strongly associated with cardiovagal activity

(Akselrod et al., 1981; Berntson et al., 1997; Camm et al., 1996), and provide an index of parasympathetic cardiac input to the sinoatrial node and efficient cardiac vagal control from subcortical regions (Berntson, Cacioppo, & Quigley, 1993). Consistent with the neurovisceral integration model, neuroimaging data supports a link between cortico-subcortical areas involved in social and emotional regulation, and HRV. For example, Lane et al. (2009) found that increased regional cerebral blood flow to the ventromedial prefrontal cortex and insula during emotion perception and recollection tasks correlated with increased HF-HRV. Additionally, an association between the dorsomedial prefrontal cortex, involved in emotion recognition, and greater autonomic cardiac control was identified by a recent meta-analysis (Thayer, Åhs, Fredrikson, Sollers III, & Wager, 2012). Together, these findings support the relationship between prefrontal cortical activity and parasympathetic cardiac regulation in social cognition, as indexed by HRV.

Characterizing the role of the neural circuits involved in social cognition and autonomic function is of further interest given that reduced HRV has been linked to a number of psychiatric disorders with notable social cognition or social functioning impairments (Alvares et al., 2013; Ieda et al., 2013; Kemp et al., 2010; Malpas, Whiteside, & Maling, 1991; Quintana, McGregor, Guastella, Malhi, & Kemp, 2013). Interestingly, HRV changes during social interactions may provide a useful marker for effective social interaction skill in children (Shahrestani, Stewart, Quintana, Hickie, & Guastella, 2014). We recently showed that HRV was positively associated with social cognition performance in a healthy population, whereby those with higher HF-HRV performed better on the Reading the Mind in the Eyes Task (RMET; Quintana, Guastella, Outhred, Hickie, & Kemp, 2012), an established test of 'theory of mind' (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). The RMET requires participants to indicate what

emotion an individual is displaying based on viewing photographs of the eye region alone. It has been used to test emotion recognition ability of individuals with autism (Baron-Cohen et al., 2001) and depression (Lee, Harkness, Sabbagh, & Jacobson, 2005). This finding is consistent with the observed social cognition impairments in those psychiatric disorders associated with reduced HRV. However, it is yet to be determined whether autonomic activity, as indexed by HRV, is causally related to poor social cognition.

Previous studies have demonstrated that HRV can be increased experimentally via non-invasive vagal stimulation using the 'cold face test' (Khurana & Wu, 2006; La Marca et al., 2011). The cold face test (from here referred to as 'facial cooling') involves applying a cold stimulus to the face. This invokes the diving reflex (Butler & Jones, 1997) and then subsequent bradycardia via the trigeminal system. Facial cooling is used to index the integrity of the trigeminal-brainstem-vagal pathways, and interference at any point of this reflex arc can reduce or abolish the characteristic cardiovascular effects (Khurana, Watabiki, Hebel, Toro, & Nelson, 1980). For example, patients with brain stem lesions or peripheral vagus nerve dysfunction exhibit impaired cardiovascular responses to facial cooling (Bannister & Oppenheimer, 1972; Baumert & Sacre, 2013; Benarroch & Parisi, 2000; Kristensson, Olsson, & Sourander, 1971). Importantly, stimulation of the diving reflex does not appear to significantly influence blood pressure (Allen, Shelley, & Boquet Jr, 1992) or respiration rate (Hayashi, Ishihara, Tanaka, Osumi, & Yoshida, 1997), which are important considerations when experimentally manipulating the cardiorespiratory system given their relationships with heart rate (Elstad, Walløe, Chon, & Toska, 2011; Hirsch & Bishop, 1981; Quintana & Heathers, 2014).

Previous research in modifying HRV has shown increased HRV, through physical fitness, was associated with better executive function, supporting the hypothesized association

between HRV and prefrontal activity (Hansen, Johnsen, Sollers, Stenvik, & Thayer, 2004). Specifically to facial cooling, stimulating the trigeminal-brainstem-vagal system acts on the cortico-subcortical circuit that is involved in cognitive and affective processes (Thayer, Hansen, Saus-Rose, & Johnsen, 2009). However, the influence that experimentally increased cardiovagal activity has on social cognition, and in particular, emotion perception, is yet to be fully examined. We have previously proposed (Guastella et al., 2013) that this pathway may be important in mediating the effect of social-cognitive interventions, such as oxytocin nasal spray, to enhance social cognition and behavior. More direct evidence would provide a clearer understanding of autonomic and central functions mediating optimal social behavior, a crucial association given the functional impact of social cognition impairments in psychiatric illness (Bauminger, 2002; Couture, Penn, & Roberts, 2006).

Thus, we aimed to examine whether increasing Parasympathetic Nervous System (PNS) activity improves social cognition performance in healthy males. We have selected a restricted population group to maintain high homogeneity (that is, all male, within a narrow age range, exhibiting average physical and mental health), to investigate these psychophysiological phenomenon, free of known confounds on autonomic physiology. Firstly, we hypothesized that facial cooling would increase PNS outflow to the heart, indexed by greater HRV, in comparison to no facial cooling. Secondly, we predicted that this increase in PNS function during facial cooling would result in better performance on the social cognition task, relative to no facial cooling.

2. Material and methods

2.1. Participants

Twenty-five male volunteers were recruited from advertisements placed in local classifieds. The University of Sydney Human Research Ethics Committee provided approval (project number: 2013/641). Exclusion criteria included: current or history of psychiatric illness determined by the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon, & Williams, 2007), current use of any medication, and self-reported major physical health or heart problems. Participants were asked to refrain from alcohol and illicit substances 24 hours prior to testing, and from smoking, food and drink (including caffeine) three hours before testing, to reduce the influence of such substances on autonomic physiology.

2.2. Instruments

2.2.1. Heart Rate Variability

All resting baseline heart rate measurements were recorded when participants were seated in a relaxed position with their eye gaze directed at a stationary dot stimulus presented on a computer monitor. The Polar RS800CX (Polar Electro Oy, Kempele, Finland) heart rate monitoring system was used to measure Inter-Beat Intervals (IBI) for the entire experiment (approximately 45 minutes) at 1000Hz. The monitor wirelessly collected HRV data via a two-lead chest strap worn by the participants. The validity of Polar monitors to measure R-R intervals has been reported as comparable to electrocardiogram (ECG) with intra class correlations (95% confidence interval values were > 0.75) and Bland-Altman limits of agreement demonstrating exceptional agreement between the two instruments (Weippert et al., 2010).

2.2.2. Reading the Mind in the Eyes Test (RMET)

Participants completed a split version of the original 36-item RMET (Baron-Cohen et al., 2001). This split version, created for this study to reduce the impact of practice effects with repeated administrations, contains the original 36 images of the eye region of different faces with the corresponding four response options for each image (e.g., panicked). These were split into two versions with 18-items per set that took approximately 4 minutes to complete. Items in each set were matched according to gender of face and weighted difficulty of the item as calculated from normative data from the original test (Quintana et al., 2012). Split half reliability was moderate at .563, comparable to internal consistency values reported for the RMET (Vellante et al., 2013).

2.2.3. Facial Cooling (FC)

A FC headband, made of thin cotton, was strapped to the participants' head with a pouch (24 x 11cm) containing a gel icepack ('Freeza Pak', Jackeroo, KMART Australia) covering the entire forehead. The icepacks (23 x 10.5cm) were kept at 0 - 1°c for the FC (La Marca et al., 2011) condition and at room temperature (22 - 23°c) for the 'No Facial Cooling' (NFC) condition. Both conditions used the same headband. The time course for facial cooling effects on heart rate occur relatively quickly, approximately 5 – 10 seconds, with maximum effects occurring at approximately 35 to 50 seconds and a slow decline in effect following the stimulus removal (Khurana & Wu, 2006). Since these effects occur quickly and the effects are sustained, the length of HRV recordings (2-min) were deemed sufficient to assess changes in HRV.

2.2.4. Factors influencing HRV

To assess factors that influence HRV, including smoking (Barutcu et al., 2005), depression (Carney et al., 2001; Kemp et al., 2010), anxiety (Thayer, Friedman, & Borkovec, 1996), stress (Dishman et al., 2000), and alcohol consumption (Quintana, McGregor, et al., 2013; Thayer, Hall, Sollers III, & Fischer, 2006), a separate battery of questionnaires were completed. This included the Depression Anxiety and Stress Scale (DASS-21; Lovibond & Lovibond, 1995), Alcohol Use Disorders Identification Test (AUDIT; Babor, Korner, Wilber, & Good, 1987), and the State-Trait Anxiety Inventory (STAI -Y2; Spielberger, Gorsuch, Lushene, & Vagg, 1983). Participants also self-reported smoking habits. Body Mass Index (BMI) was calculated using the standard equation of mass (kg) divided by their height (m) squared.

The Visual Analogue Scale- pain (VAS; Duncan, Bushnell, & Lavigne, 1989) and the State-Trait Anxiety Inventory 6 (STAI-6; Marteau & Bekker, 1992) were used before and after every HRV recording, as brief measures to assess any changes in state anxiety and/or head pain due to facial cooling. The STAI-6 has good reliability (α = .82) and concurrent validity with the 6-item mean comparable to the full 20-item STAI-Y1 (Marteau & Bekker, 1992). Moreover, the validity of the shortened STAI has been reviewed and deemed specifically appropriate for research settings (Kruyen, Emons, & Sijtsma, 2013).

2.3. Procedure

All participants were tested in the same room between the hours of 12:00 – 19:00. A room temperature of 22 - 23°c was consistently maintained for all experiments. See Figure 1 for a timeline of the experiment's procedures. Upon completion of the questionnaires, participants' height and weight measurements were taken to calculate BMI. Participants were also asked to

empty their bladder (Mehnert, Knapp, Mueller, Reitz, & Schurch, 2009) before being fitted with the Polar chest strap, which remained on the participant for the duration of testing. Baseline HRV measurements were recorded for 10 minutes in a seated rest position consisting of 2-minute rest with no IBI recording, 2-minute baseline recording, 1-minute break, 2-minute NFC recording, 1 minute break and 2-minute FC recording. The break periods were utilized to apply the relevant facial cooling headband to the forehead of the participant. Participants then completed the RMET under both conditions (FC and NFC), presented in a computer generated randomized order. Participants completed the STAI-6 and VAS before and after every HRV recording.

Insert figure one about here

2.4. Data analysis

Raw IBI data from the Polar device was extracted into Kubios (version 2.0, 2008, Biosignal Anaylsis and Medical Imaging Group, University of Kuopio, Finland, MATLAB). All raw data was filtered using a low automatic filter and visually inspected for artifacts by the investigator (FI), before calculating average HR from R-R intervals and HF-ab (0.15-0.4Hz; absolute units) using the Fast Fourier transform. Preliminary analyses were conducted to ensure the assumptions of normality, linearity, multicollinearity and homoscedasticity were not violated. According to the Kolmogorov-Smirnov statistic, HRV was positively skewed (p = .02). Thus, a logarithmic transformation to base-10 was then applied after which no significant skew was evident (p = .20). Pearson's product moment correlations were used to examine the relationships between RMET scores without facial cooling, HRV, age, BMI, depression, anxiety, stress, alcohol use and trait anxiety. A 2 (FC and NFC) x 2 (rest and RMET) repeated measures ANOVA was employed to investigate the main effect and interaction of facial cooling and task

conditions, on HRV. A follow up test was conducted to investigate the effect of task condition on HRV during FC. Partial eta Squared (η_p^2) was used as a measure of effect size (.01 = small, .06 = medium and .14 = large) (Cohen, 1973). A paired samples t-test was used to compare RMET performance during testing for both conditions. Two separate 2 (FC and NFC) x 2 (rest and RMET) repeated measures ANOVA were employed to assess the changes in the VAS and STAI-6 scores after facial cooling and task conditions.

3. Results

3.1. Participants

Participant characteristics, including heart rate values, are presented in Table 1. In addition to the SCID, which ruled out the presence of psychiatric illness, the mean scores of the DASS, STAI and AUDIT were below clinical cut offs. Pearson's bivariate correlations indicate that all covariates had no significant correlation with HF-HRV, whilst significant correlations were identified between RMET during NFC and DASS-A scores (table 2).

Insert table one about here

3.2. Effect of facial cooling and task condition on HRV

A significant overall main effect of facial cooling on HRV was found. HRV during the FC condition was significantly higher than that of the NFC (F (1, 24) = 13.88, p = .001, η_p^2 = .37) averaged across task condition. There was also a significant interaction effect between the facial cooling condition and task condition (F (1, 24) = 6.49, p = .018, η_p^2 = .21). At baseline, HRV under FC (M= 3.06, SD= 0.36) was significantly higher than NFC (M= 2.82, SD= 0.44).

However, during the RMET, HRV under FC (M= 2.91, SD= 0.36) was not significantly different to NFC (M= 2.88, SD= 0.36). HRV under FC at baseline was significantly greater than during the RMET (F (1, 24) = 7.217, p = .01, η_p^2 = .23). Thus, at rest FC significantly increased HRV when compared to NFC; this effect was absent during the RMET condition (Figure 1).

Insert figure two about here

3.3. Effect of facial cooling on social cognition performance

No significant differences in RMET scores between FC (M=14.20, SD=2.20) and NFC (M: 14.24, SD: 2.19) conditions (t (24) = -0.08, p = .94) were found.

3.4.Effect of facial cooling on state anxiety and pain

In regards to STAI-6 scores, there was a significant overall main effect of facial cooling (F (1, 24) = 12.68, p < .00, η_p^2 = .35) and task condition (F (1, 24) = 5.49, p = 0.28, η_p^2 = .19) on state anxiety. This indicated state anxiety was lower following FC, and was lower following rest compared to the RMET. However, a significant interaction effect (F (1, 24) = 4.36, p = .05, η_p^2 = .15) indicated that the decreases in state anxiety during facial cooling were greatest at baseline (M= -0.84, SD= 1.41), with no change observed following the RMET (M= 0.00, SD= .76). In regards to the pain scores, a significant main effect was found amongst the FC condition (F (1, 24) = 17.71, p < .00, η_p^2 = .43) indicating that there was a significantly greater increase in pain ratings after FC compared to NFC.

Insert table 2 about here

4. Discussion

This study demonstrates that HRV can be increased experimentally by facial cooling. Whilst there was no evidence to suggest that facial cooling subsequently altered social cognition ability, the results suggest that the effect of facial cooling on HRV is moderated by engagement in the social cognition task. When participants in the current study were completing the RMET, the hypothesized increases in HRV under facial cooling were not observed. This cannot be explained by a decrease in HRV whilst completing the task, as HRV during task completion was comparable to HRV during no facial cooling at rest. These results are important for understanding the role of HRV in the relationship between ANS function and central processes integral to optimal social cognition.

Increased vagal activity is primarily responsible for the increases in HRV observed during facial cooling at rest (Ryan, Hollenberg, Harvey, & Gwynn, 1976). The application of the cold stimulus stimulates trigeminal afferent pathways to central areas, which exert greater inhibitory control over vagal efferent pathways from the brainstem to the heart (Khurana et al., 1980). The increases in HRV observed in this study are consistent with the expected intact trigeminal-brainstem-vagal function of the participants recruited for the present study. Since facial cooling increased vagally mediated HRV, our previous work (Quintana et al., 2012) linking high HRV with better social cognition performance would suggest that participants should have improved on the RMET during facial cooling. Despite this expectation, no such effect was observed in the present study as the facial cooling manipulation failed to successfully increase HRV during the social cognition tasks. Thus, we were unable to test whether increases in HRV during the social cognition test causally result in improved social cognition performance.

Whilst facial cooling increased HRV at rest, no effect of facial cooling was observed during the social cognition task. An abolished cardiovascular effect during facial cooling could imply interference along the trigeminal-brainstem-vagal pathway has occurred (Khurana et al., 1980). In the present study, any interference is unlikely to be attributed to lesions along afferent and/or efferent pathways as these deficits were absent at rest, thus interference may have occurred at another point along this reflex arc. This explanation is consistent with the inhibitory hypothesis of the neurovisceral integration model which proposes that a common corticosubcortical circuit is involved in psychological and physiological regulation (Thayer et al., 2009). In this view, facial cooling at rest increased the vagal activity in this inhibitory circuit; however, completing the RMET abolished this effect, and the subsequent increases in vagally mediated HRV. This explanation implicates common resources involved in autonomic control and cognitive ability, which is consistent with the positive relationship observed between vagally mediated HRV and cognitive function (Hansen, Johnsen, Sollers, Stenvik, & Thayer, 2004). Further investigations should characterize the specific effect that the social element of the task is having on HRV during facial cooling by employing a range of cognition tasks. This would delineate whether the effect of facial cooling on the hypothesized cortico-subcortical circuit is specific to social cognition or more generally to broader cognitive processes.

An alternative explanation for the lack of an increase in HRV during facial cooling whilst completing the RMET may be habituation to the cold face test. Some evidence has shown that repeated facial immersion procedures or cold pressor tasks, similar to facial cooling used in this experiment, leads to attenuated responses (Zbrożyna & Westwood, 1992), but not uniformly (Durel et al., 1993). Whilst, this may account for the differences in HF-HRV observed in the second application of the facial cooling mask, the habituation described previously occurred after

repeated facial immersion and warmer facial cooling temperatures. Since the present study applied a more intense stimulus, and for shortened period of time, any potential habituation effects would be more limited.

The effect of cognitive load may have prevented an increase in HRV whilst completing the RMET. Evidence suggests that performing advanced neuropsychological tests for a prolonged period (30 minutes or greater) to induce mental fatigue can reduce parasympathetic activity (Mizuno et al., 2011; Tanaka, Mizuno, Tajima, Sasabe, & Watanabe, 2009). However, it is unlikely that the use of the RMET in this present study would induce the same level of mental fatigue as there was no time limit imposed on task completion, and we used a shortened version of the RMET. Nevertheless, these findings provide further insight into the complex cortico-subcortical processes involved in autonomic regulation, which is critical to understanding the relationship between these neural mechanisms and social dysfunction in psychiatric illnesses.

This study has some limitations. Firstly, we did not measure respiration rate, a factor identified as having an indirect effect on HRV (Quintana & Heathers, 2014), and a potential confound since breathing patterns may have been affected by facial cooling (Brick, 1966). However, others have shown that the dive reflex does not affect respiration (Hayashi et al., 1997; Stemper, Hilz, Rauhut, & Neundörfer, 2002), and that heart rate during facial cooling with or without breath holding does not differ (Kawakami, Natelson, & DuBois, 1967). It will be important for future research to monitor and analyze respiratory frequency to clarify the influence of the dive reflex on respiration and its relationship with HRV. Secondly, we identified significant effects of facial cooling on pain and state anxiety, sources of potential confound. Small decreases in subjective state anxiety ratings were observed following facial cooling. Such an effect is expected considering that increases in HRV are commonly associated with 'rest and

digest', low arousal and feeling calm (Goldie, McGregor, & Murphy, 2010). Given that this effect is a result of the facial cooling manipulation, we could conclude that facial cooling is responsible for these observed state anxiety changes. Whilst the alternate relationship could be argued (that is, reduced state anxiety leads to increased HRV), significant increases in head pain during facial cooling suggest otherwise. As previous research has suggested that increased pain actually leads to reductions in HRV (Appelhans & Luecken, 2008), we suggest that the state anxiety effects are a result of the facial cooling and increased HRV. Whilst the effects of anxiety and pain could have been controlled for in the analysis, the small size of this study is a notable limitation, and was the primary reason for excluding pain and anxiety covariates in the analysis of HRV during the RMET task (Van Breukelen & Van Dijk, 2007).

A number of methodological strengths add to the significance of the present study. The use of a repeated-measures design, high homogeneity of the included sample (that is, participants were male, within a narrow age range, exhibiting good physical and mental health), and strict control of a number of known influences on human physiology (that is, emptied bladder, no food, drink or substances prior to study, testing during a similar time of day), increase our confidence in the observed effects of facial cooling on HRV and subsequent discussion. By implementing such control, we have demonstrated the reliable use of a non-invasive cold face test to increase HRV at rest, and provided further insight into the important cortico-subcortical mechanisms responsible for autonomic cardiac control and socio-cognitive processes.

In summary, this study demonstrates that facial cooling increases HRV. The observed increase was abolished by completing a social-cognitive task, providing indirect evidence for the involvement of a common cortico-subcortical circuit involved in autonomic regulation and psychological processes. Although further investigations are warranted to clarify this finding in

other samples and with other tests of social cognition, this preliminary evidence emphasizes the importance of considering the mutual action of the heart and brain in an individual's ability to respond to changing environmental demands (Thayer & Lane, 2009). Understanding these mechanisms is crucial for determining the underlying causes of social cognition deficits that may be important for directing targeted interventions to the affected areas.

References

- Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A., & Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science*, *213*(4504), 220-222.
- Allen, M. T., Shelley, K. S., & Boquet Jr, A. J. (1992). A comparison of cardiovascular and autonomic adjustments to three types of cold stimulation tasks. *International Journal of Psychophysiology*, 13(1), 59-69.
- Alvares, G. A., Quintana, D. S., Kemp, A. H., Van Zwieten, A., Balleine, B. W., Hickie, I. B., & Guastella, A. J. (2013). Reduced heart rate variability in social anxiety disorder: associations with gender and symptom severity. *PLoS One*, 8(7), e70468. doi: 10.1371/journal.pone.0070468
- Amodio, D. M., & Frith, C. D. (2006). Meeting of minds: the medial frontal cortex and social cognition. *Nature Reviews Neuroscience*, 7(4), 268-277.
- Appelhans, B. M., & Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Review of general psychology*, 10(3), 229.
- Appelhans, B. M., & Luecken, L. J. (2008). Heart rate variability and pain: Associations of two interrelated homeostatic processes. *Biological psychology*, 77(2), 174-182. doi: http://dx.doi.org/10.1016/j.biopsycho.2007.10.004
- Babor, T. F., Korner, P., Wilber, C., & Good, S. P. (1987). Screening and early intervention strategies for harmful drinkers: Initial lessons from the Amethyst Pproject. *Drug Alcohol Rev*, *6*(4), 325-339.
- Bannister, R., & Oppenheimer, D. (1972). Degenerative diseases of the nervous system associated with autonomic failure. *Brain*, *95*(3), 457-474.

- Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., & Plumb, I. (2001). The "Reading the mind in the eyes" test revised version: A study with normal adults, and adults with asperger syndrome or high-functioning autism. *Journal of Child Psychology and Psychiatry*, 42(2), 241-251.
- Barutcu, I., Esen, A. M., Kaya, D., Turkmen, M., Karakaya, O., Melek, M., . . . Basaran, Y. (2005). Cigarette smoking and heart rate variability: dynamic influence of parasympathetic and sympathetic maneuvers. *Annals of noninvasive electrocardiology*, 10(3), 324-329.
- Baumert, M., & Sacre, J. W. (2013). *Heart rate complexity and cardiac sympathetic*dysinnervation in patients with type 2 diabetes mellitus. Paper presented at the

 Engineering in Medicine and Biology Society (EMBC), 2013 35th Annual International

 Conference of the IEEE.
- Bauminger, N. (2002). The facilitation of social-emotional understanding and social interaction in high-functioning children with autism: Intervention outcomes. *Journal of autism and developmental disorders*, 32(4), 283-298.
- Benarroch, E. E. (1993). *The central autonomic network: functional organization, dysfunction, and perspective.* Paper presented at the Mayo Clinic Proceedings.
- Benarroch, E. E., & Parisi, J. E. (2000). Involvement of the ventrolateral medulla in parkinsonism with autonomic failure. *Neurology*, *54*(4), 963-968.
- Berntson, Bigger, J. T., Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., . . . van der Molen, M. W. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology*, *34*(6), 623-648.

- Berntson, Cacioppo, J. T., & Quigley, K. S. (1993). Respiratory sinus arrhythmia: autonomic origins, physiological mechanisms, and psychophysiological implications.

 *Psychophysiology, 30(2), 183-196.
- Brick, I. (1966). Circulatory responses to immersing the face in water. *J Appl Physiol*, *21*(1), 33-36.
- Butler, P. J., & Jones, D. R. (1997). Physiology of diving of birds and mammals. *Physiological Reviews*, 77(3), 837.
- Camm, A., Malik, M., Bigger, J., Breithardt, G., Cerutti, S., Cohen, R., . . . Kleiger, R. (1996).

 Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*, *93*(5), 1043-1065.
- Carney, R. M., Blumenthal, J. A., Stein, P. K., Watkins, L., Catellier, D., Berkman, L. F., . . . Freedland, K. E. (2001). Depression, heart rate variability, and acute myocardial infarction. *Circulation*, *104*(17), 2024-2028.
- Cohen, J. (1973). Eta-squared and partial eta-squared in fixed factor ANOVA designs. *Educational and psychological measurement*.
- Couture, S. M., Penn, D. L., & Roberts, D. L. (2006). The functional significance of social cognition in schizophrenia: a review. *Schizophrenia bulletin*, *32*(suppl 1), S44-S63.
- Davis, M., & Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Molecular psychiatry*, 6(1), 13-34.
- Dishman, R. K., Nakamura, Y., Garcia, M. E., Thompson, R. W., Dunn, A. L., & Blair, S. N. (2000). Heart rate variability, trait anxiety, and perceived stress among physically fit men and women. *International Journal of Psychophysiology*, *37*(2), 121-133.

- Duncan, G. H., Bushnell, M. C., & Lavigne, G. J. (1989). Comparison of verbal and visual analogue scales for measuring the intensity and unpleasantness of experimental pain. *Pain*, *37*(3), 295-303.
- Durel, L. A., Kus, L. A., Anderson, N. B., Mcneilly, M., Llabre, M. M., Spitzer, S., . . . Schneiderman, N. (1993). Patterns and stability of cardiovascular responses to variations of the cold pressor test. *Psychophysiology*, *30*(1), 39-46.
- Elstad, M., Walløe, L., Chon, K. H., & Toska, K. (2011). Low-frequency fluctuations in heart rate, cardiac output and mean arterial pressure in humans: what are the physiological relationships? *Journal of hypertension*, *29*(7), 1327-1336.
- First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. (2007). 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.
- Goldie, J., McGregor, C., & Murphy, B. (2010). *Determining levels of arousal using*electrocardiography: a study of HRV during transcranial magnetic stimulation. Paper

 presented at the Engineering in Medicine and Biology Society (EMBC), 2010 Annual

 International Conference of the IEEE.
- Guastella, A. J., Hickie, I. B., McGuinness, M. M., Otis, M., Woods, E. A., Disinger, H. M., . . . Banati, R. B. (2013). Recommendations for the standardisation of oxytocin nasal administration and guidelines for its reporting in human research.

 *Psychoneuroendocrinology, 38(5), 612-625.
- Hansen, A., Johnsen, B., Sollers, J., III, Stenvik, K., & Thayer, J. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining.

- European Journal of Applied Physiology, 93(3), 263-272. doi: 10.1007/s00421-004-1208-0
- Hayashi, N., Ishihara, M., Tanaka, A., Osumi, T., & Yoshida, T. (1997). Face immersion increases vagal activity as assessed by heart rate variability. *European journal of applied physiology and occupational physiology*, 76(5), 394-399.
- Hirsch, J. A., & Bishop, B. (1981). Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *American Journal of Physiology-Heart and Circulatory Physiology*, *241*(4), H620-H629.
- Ieda, M., Miyaoka, T., Wake, R., Liaury, K., Tsuchie, K., Fukushima, M., . . . Horiguchi, J.
 (2013). Evaluation of autonomic nervous system by salivary alpha-amylase level and heart rate variability in patients with schizophrenia. *Eur Arch Psychiatry Clin Neurosci*.
 doi: 10.1007/s00406-013-0411-6
- Kawakami, Y., Natelson, B. H., & DuBois, A. (1967). Cardiovascular effects of face immersion and factors affecting diving reflex in man. *J Appl Physiol*, 23(6), 964-970.
- Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., & Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biological psychiatry*, 67(11), 1067-1074.
- Khurana, R. K., Watabiki, S., Hebel, J., Toro, R., & Nelson, E. (1980). Cold face test in the assessment of trigeminal-brainstem-vagal function in humans. *Annals of neurology*, 7(2), 144-149.
- Khurana, R. K., & Wu, R. (2006). The cold face test: a non-baroreflex mediated test of cardiac vagal function. *Clin Auton Res*, *16*(3), 202-207. doi: 10.1007/s10286-006-0332-9

- Kristensson, K., Olsson, Y., & Sourander, P. (1971). CHANGES IN THE VAGUS NERVE IN DIABETES MELLTTUS. *Acta Pathologica Microbiologica Scandinavica Section A Pathology*, 79(6), 684-685.
- Kruyen, P. M., Emons, W. H., & Sijtsma, K. (2013). Shortening the S-STAI: Consequences for research and clinical practice. *Journal of Psychosomatic Research*.
- La Marca, R., Waldvogel, P., Thorn, H., Tripod, M., Wirtz, P. H., Pruessner, J. C., & Ehlert, U. (2011). Association between Cold Face Test-induced vagal inhibition and cortisol response to acute stress. *Psychophysiology*, *48*(3), 420-429. doi: 10.1111/j.1469-8986.2010.01078.x
- Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L., & Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *Neuroimage*, 44(1), 213-222.
- Lee, L., Harkness, K. L., Sabbagh, M. A., & Jacobson, J. A. (2005). Mental state decoding abilities in clinical depression. *J Affect Disord*, 86(2), 247-258.
- Lovibond, P. F., & 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. *Behaviour research and therapy*, 33(3), 335-343.
- Malpas, S. C., Whiteside, E. A., & Maling, T. J. (1991). Heart rate variability and cardiac autonomic function in men with chronic alcohol dependence. *Br Heart J*, 65(2), 84-88.
- Marteau, T. M., & Bekker, H. (1992). The development of a six-item short-form of the state scale of the Spielberger State—Trait Anxiety Inventory (STAI). *British Journal of Clinical Psychology*, 31(3), 301-306.

- Mehnert, U., Knapp, P. A., Mueller, N., Reitz, A., & Schurch, B. (2009). Heart rate variability:

 An objective measure of autonomic activity and bladder sensations during urodynamics.

 Neurourology and urodynamics, 28(4), 313-319.
- Mizuno, K., Tanaka, M., Yamaguti, K., Kajimoto, O., Kuratsune, H., & Watanabe, Y. (2011).

 Mental fatigue caused by prolonged cognitive load associated with sympathetic hyperactivity. *Behav Brain Funct*, 7, 17.
- Porges, S. W. (2003). The polyvagal theory: Phylogenetic contributions to social behavior. *Physiology & Behavior*, 79(3), 503-513.
- Quintana, D. S., Guastella, A. J., Outhred, T., Hickie, I. B., & Kemp, A. H. (2012). Heart rate variability is associated with emotion recognition: direct evidence for a relationship between the autonomic nervous system and social cognition. *Int J Psychophysiol*, 86(2), 168-172. doi: 10.1016/j.ijpsycho.2012.08.012
- Quintana, D. S., & Heathers, J. A. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Front Psychol*, 5.
- Quintana, D. S., Kemp, A. H., Alvares, G. A., & Guastella, A. J. (2013). A role for autonomic cardiac control in the effects of oxytocin on social behavior and psychiatric illness. *Front Neurosci*, 7, 48. doi: 10.3389/fnins.2013.00048
- Quintana, D. S., McGregor, I. S., Guastella, A. J., Malhi, G. S., & Kemp, A. H. (2013). A Meta-Analysis on the Impact of Alcohol Dependence on Short-Term Resting-State Heart Rate Variability: Implications for Cardiovascular Risk. *Alcoholism: Clinical and Experimental Research*, 37(s1), E23-E29.

- Ryan, C., Hollenberg, M., Harvey, D. B., & Gwynn, R. (1976). Impaired parasympathetic responses in patients after myocardial infarction. *The American journal of cardiology*, 37(7), 1013-1018.
- Shahrestani, S., Stewart, E. M., Quintana, D. S., Hickie, I. B., & Guastella, A. J. (2014). Heart rate variability during social interactions in children with and without psychopathology: a meta-analysis. *Journal of Child Psychology and Psychiatry*.
- Spielberger, C. D., Gorsuch, R. L., Lushene, R. E., & Vagg, P. R. (1983). State-Trait Anxiety Inventory (STAI). *BiB* 2010, 180.
- Stemper, B., Hilz, M., Rauhut, U., & Neundörfer, B. (2002). Evaluation of cold face test bradycardia by means of spectral analysis. *Clinical Autonomic Research*, *12*(2), 78-83.
- Tanaka, M., Mizuno, K., Tajima, S., Sasabe, T., & Watanabe, Y. (2009). Central nervous system fatigue alters autonomic nerve activity. *Life sciences*, 84(7), 235-239.
- Thayer, J. F., Åhs, F., Fredrikson, M., Sollers III, J. J., & 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. *Neuroscience & Biobehavioral Reviews*, *36*(2), 747-756.
- Thayer, J. F., Friedman, B. H., & Borkovec, T. D. (1996). Autonomic characteristics of generalized anxiety disorder and worry. *Biological psychiatry*, *39*(4), 255-266.
- Thayer, J. F., Hall, M., Sollers III, J. J., & Fischer, J. E. (2006). Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: Evidence for impaired inhibitory control of the HPA axis in heavy drinkers. *International Journal of Psychophysiology*, *59*(3), 244-250.
- Thayer, J. F., Hansen, A. L., Saus-Rose, E., & Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration

- perspective on self-regulation, adaptation, and health. *Annals of Behavioral Medicine*, 37(2), 141-153.
- Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J Affect Disord*, 61(3), 201-216.
- Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart–brain connection: Further elaboration of a model of neurovisceral integration. *Neuroscience & Biobehavioral Reviews*, 33(2), 81-88.
- Van Breukelen, G. J., & Van Dijk, K. R. (2007). Use of covariates in randomized controlled trials. *Journal of the International Neuropsychological Society*, 13(05), 903-904.
- Vellante, M., Baron-Cohen, S., Melis, M., Marrone, M., Petretto, D. R., Masala, C., & Preti, A. (2013). The "Reading the Mind in the Eyes" test: systematic review of psychometric properties and a validation study in Italy. *Cognitive neuropsychiatry*, *18*(4), 326-354.
- Weippert, M., Kumar, M., Kreuzfeld, S., Arndt, D., Rieger, A., & Stoll, R. (2010). Comparison of three mobile devices for measuring R–R intervals and heart rate variability: Polar S810i, Suunto t6 and an ambulatory ECG system. *European Journal of Applied Physiology*, 109(4), 779-786.
- Zbrożyna, A. W., & Westwood, D. M. (1992). Cardiovascular responses elicited by simulated diving and their habituation in man. *Clinical Autonomic Research*, *2*(4), 225-233.

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Table 1. Participant characteristics (N = 25).

| Variable | Mean | SD |
|-----------------|----------------------|------|
| Age (range) | 23.96 (20 – 30years) | 2.19 |
| BMI | 24.18 | 3.28 |
| Depression | 1.84 | 1.93 |
| Anxiety | 1.20 | 1.35 |
| Stress | 3.76 | 2.48 |
| Alcohol use | 8.92 | 4.09 |
| Trait Anxiety | 33.48 | 6.63 |
| Smoker (yes/no) | (1/24) | |
| HR FC | 63.91 | 5.87 |
| HR NFC | 66.48 | 5.82 |
| HR RMET FC | 65.08 | 5.42 |
| HR RMET NFC | 65.56 | 5.13 |

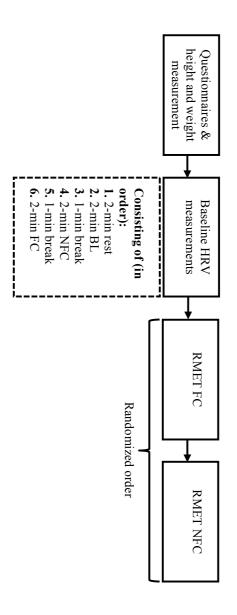
Note. **BMI**= Body mass index, **HR FC** = Heart Rate during Facial Cooling at rest, **HR NFC** = Heart Rate during No Facial Cooling at rest, **HR RMET FC** = Heart Rate during Facial Cooling whilst completing RMET, **HR RMET NFC** = Heart Rate during No Facial Cooling whilst completing RMET,

Table 2. Correlation coefficients among participant measures (N = 25).

| | Age | BMI | Depression | Anxiety | Stress | Alcohol | Trait | HF |
|----------------|------|------|------------|---------|--------|---------|-------------------|-----------|
| | | | , | | | use | Anxiety HRV BL | HRV BL |
| BMI | 150 | | | | | | | |
| Depression | 189 | 118 | | | | | | |
| Anxiety | 236 | .227 | .411* | | | | | |
| Stress | 025 | .049 | .516** | .152 | | | | |
| Alcohol use | .246 | 016 | .252 | .326 | .216 | | | |
| Trait Anxiety | 042 | .051 | .569** | .160 | .244 | .092 | | |
| HF-HRV BL | .221 | .096 | .029 | 028 | .038 | .372 | 245 | |
| RMET NFC score | .192 | 097 | 357 | 521** | 029 | .027 | .034 | .112 |
| | | | | | | | | |

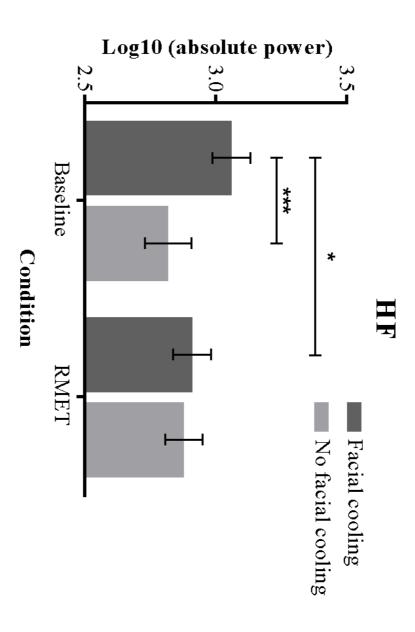
during No Facial Cooling Note. * P<0.05, ** p<0.01. BMI=Body Mass Index, HF-HRV BL = High frequency HRV at baseline, RMET NFC = RMET score

Figure 1



Cooling condition, NFC = No Facial Cooling condition. Figure 1: A timeline depicting the different phases of the experiment. BL = Baseline, HRV = Heart Rate Variability, FC = Facial

Figure 2



each condition see table 1. ***= p value < 0.001, *= p value < 0.05. RMET = Reading the mind in the eyes task; HF = High frequency. For heart rate values for Figure 2: The effect of facial cooling on HRV; at baseline and during the RMET. Error bars depict standard error of the mean.