Final Scientific Report (Project: 88/16)

Project title: The Interoceptive Self: Transcutaneous Vagus Nerve Stimulation as a new tool to investigate heart-brain interactions

PI: Ruben Azevedo, Manos Tsakiris (co-PI)

Other researchers: Valerio Villani

Institution: Royal Holloway University of London

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Project Aims

The aim of this project was to investigate the potential of Transcutaneous Vagus Nerve Stimulation (tVNS) in bringing the interoceptive body into conscious awareness and in modulating the experience of emotional feelings.

Interoception is the body-to-brain axis of sensations originating from the internal body and its visceral organs that signal their physiological state. The interoceptive body reveals itself to its owner mainly through feelings and emotions, and in more dramatic cases through illness. Its function is only to a certain extent dependent on cortical control, and more often than not operates below the level of conscious awareness. While the interoceptive mind is increasingly becoming the focus of attention, the precise underlying mechanisms are still largely unknown and empirical evidence is scarce, mostly because interoception is not easily amenable to experimental manipulation as we have little control over the inputs to the system. However, recent advances provide us with the opportunity to affect the processing of cardiac signals, and as a result interoception, in a non-invasive and controlled way.

The vagus nerve, i.e. the X cranial nerve, is part of the parasympathetic division of the autonomic nervous system and one of the most important communication pathways between the body and the brain (Cameron, 2002; Critchley & Harrison, 2013) conveying afferent signals from the major internal organs, including the heart. This information is funnelled into the NTS of the brainstem, a major interoceptive hub for homeostatic control with direct ascending projections to the monoaminergic neuromodulatory system (Critchley & Harrison, 2013). However, while the functional importance of the vagus nerve in the conduction and regulation of autonomic signals is well documented, experimental evidence of its role in bringing these signals into consciousness is still lacking.
In 2000, Ventureyra (2000) developed tVNS, a non-invasive technique, consisting in the application of mild electrical current to the auricular branch of the vagus nerve whose fibres are found in the tragus and the cavum conchae of the auricle (Peuker & Filler, 2002). Since then, several fMRI studies confirmed that tVNS modulates brain activity in those areas identified by direct stimulation of the vagus (achieved through an electrical device implanted in the upper chest), such as brainstem regions, thalamus, amygdala and insula (Dietrich et al., 2008; Kraus et al., 2007; Kraus et al., 2013; Badran et al., 2018a), many of them known to be involved in the regulation of autonomic activity. Research on tVNS initially focused on investigating the possible clinical applications of this stimulation, but there is now a growing interest in tVNS as a tool to modulate cognitive and emotional processes (Colzato et al, 2017; Ventura-Bort et al., 2018). For instance, recent studies

have shown that tVNS enhances emotion recognition from others' faces and bodies (Colzato et al, 2017; Sellaro et al, 2018) and may accelerate fear extinction (Burger et al, 2016; but see Genheimer et al., 2017). However, it is still unclear whether these effects are mediated by changes in the representation of physiological activity. Research investigating the impact of tVNS on autonomic activity has presented, so far, conflicting evidence. Some studies found tVNS modulation of physiological indices, such as heart rate and heart rate variability (HRV) and blood pressure (Clancy et al, 2015; Antonino et al, 2017; Badran et al., 2018b) while other studies failed to observe such effects (e.g. Burger et al., 2016; Colzato et al., 2017). Indeed, despite the fundamental role of the vagus nerve in the communication and regulation of physiological activity, little attention has been given to its effects in the processing of the actual physiological signals. In this project we explored potential of tVNS in bringing the interoceptive body into conscious awareness and in modulating the experience of emotional feelings. The main implications of this research are threefold: i) to further our understanding on the precise mechanisms underlying the brain and body dialogue, ii) to establish tVNS as a method in the study of the brain-body interactions, and iii) to understand how greater awareness of the inner body shapes the ways in which the self-experiences emotions and the social world.

To achieve this, we proposed to carry out 4 experimental studies and write a theoretical article providing a critical review of the current state of the literature and suggesting future directions (Review 1):

Study 1 investigated the role of the vagus nerve in the conscious awareness of interoceptive signals. For that, participants carried out two task designed to measure interoceptive accuracy, i.e. the ability to correctly identify heartbeats, during active and sham tVNS.

Study 2 (originally Study 3) looks into how tVNS modulates the impact that physiological states have in emotional appraisal. Previous research has shown that time-locking the presentation of brief stimuli to coincide with different phases of the cardiac cycle, i.e. systole and diastole, allows studying the influence of afferent cardiac signals in the processing of certain types of stimuli, most notably fear (Garfinkel et al, 2014; Azevedo et al., 2017). Specifically, the processing of threat cues is upregulated during systole, when the representation in the brain of afferent cardiac signals is maximal, compared to during diastole, when the representation of cardiac signals is minimal. We have used this approach to investigate if and how tVNS modulates the impact of physiological signals in emotional appraisal.

Study 3 aims at establishing the effectiveness of new stimulation parameters and identifying the precise mechanisms underlying the influence of tVNS in cognition and emotional experience. For that, we applied short bursts (3 secs of active and sham stimulation (randomly) coinciding with stimulus presentation in an (emotional) attentional blink task, which is known to be mediated by the locus coeruleus-norepinephrine system.

Study 4 looks into the potential impact of tVNS on the way in which we relate to others. For that we used an established empathy for pain paradigm (Azevedo et al, 2013; Azevedo et al., 2014) during active and sham stimulation. Subjective ratings of first- and third-person emotional experience and pupil dilation, an index of physiological activity, were collected as measures of empathic reactivity.

Project alterations

The project has suffered two main alterations, both of them highlighted in the progress report and approved by BIAL.

Alteration 1: Originally, we planned to carry out a study investigating the impact of tVNS on emotional appraisal. However, since our project application several papers have been published showing the impact of tVNS on performance on emotion recognition tasks (Colzato et al, 2017; Sellaro et al, 2018) and impact on the extinction of conditioned fear (Burger et al, 2017; Genheimer et al., 2017). Because these studies partially overlap with originally-named-Study 2 we decided not to carry out this study and write a critical literature review that can serve as guideline for research on role of tVNS on cognitive and emotional processes.
Alteration 2: Motivated by results obtained in Study 2 and by the knowledge and experienced gained with studies 1-2, we decided to carried out an additional study (Study 3) aimed at: i) exploring novel stimulation parameters and ii) identifying the precise mechanisms underlying the influence of tVNS in the emotional experience.

Methods and Results

Stimulation (Studies 1-2): A single blind, sham-controlled, within-subjects design was used. All participants carried out two identical experimental sessions, differing only on the stimulation type: active or sham tVNS. The session order was randomized. Stimulation was delivered using the Transcutaneous Electrical Nerve Stimulation device (V-TENS Plus; https://bodyclock.co.uk/) with a custom-built clip electrode (cf. Clancy et al, 2014). During active tVNS, the electrode was placed on the anterior wall of the external ear canal corresponding to the participant's tragus. Sham tVNS was performed by placing the electrodes on the left earlobe, an area of the auricle which is known to be free of vagal endings (Peuker & Filler, 2002). Current was applied continuously with the following parameters: pulse width=250μs, frequency=25Hz. The intensity of stimulation was individually tailored to a level just above the participant's perceptual threshold. To achieve this, the experimenter slowly increased the amplitude until the participant reported some sensations (e.g., tingling), which could be barely detected and did not cause pain nor discomfort.

Stimulation (Studies 3-4): A double blind, sham-controlled, event-related within-subjects design was used (one single session). Stimulation consisted in short bursts (2-3secs) of active and sham stimulation (randomly) coinciding with stimulus presentation. Stimulation was delivered with Digitimer 7A (https://digitimer.com/). Target locations (active= tragus; sham= ear lobe) and stimuli intensity were delivered as in studies 1-2, but stimulation parameters were adjusted according to recent recomendations (Badran et al, 2018x): pulse width=500μs, frequency=10Hz.

Study 1

Active and sham tVNS stimulation was applied while participants performed the two most widely used tasks to measure interoceptive accuracy: the heartbeat counting task (HCT; see Schandry et al., 1981) and the heartbeat detection task (HDT; see Whithead et al., 1977). Following recent guidelines (Kletckner et al., 2015) we have collected data from a total of 51 participants but data from 5 participants was excluded from analyses due to technical issues during recording.
Data from both tasks was analysed with mixed-model regressions using the lme4 v1.1 -17 package (Bates et al, 2015) available for R software (R Core Team, 2013) with participant's ID as a priori random factor, i.e. the model allowed subject-specific intercepts, and stimulation type (1=tVNS; 0=Sham) as a dummy predictor. Several additional variables – session number, heart-rate variability during stimulation, heart-rate, BMI, age, gender and average reported stimulation sensations – were tested as covariates.

Results in the HDT showed improved performance during Active tVNS (mean=0.58; sd=0.11) compared to Sham (mean=0.55; sd=0.091) stimulation ($\chi 2=6.86$, $p=0.009$; see Figure 1A).

Conversely, performance on the HCT did not differ ($\chi2=0.75$, $p=0.39$) between tVNS (mean=27.7, sd=15.3) and Sham (mean=29.9, sd=16.4; see Figure 1B) stimulation. Both heart-rate during stimulation ($\chi2=6.9$, $p=0.008$) and reported stimulation sensations ($\chi2=5.76$, $p=0.016$) were found to be negative predictors of accuracy.

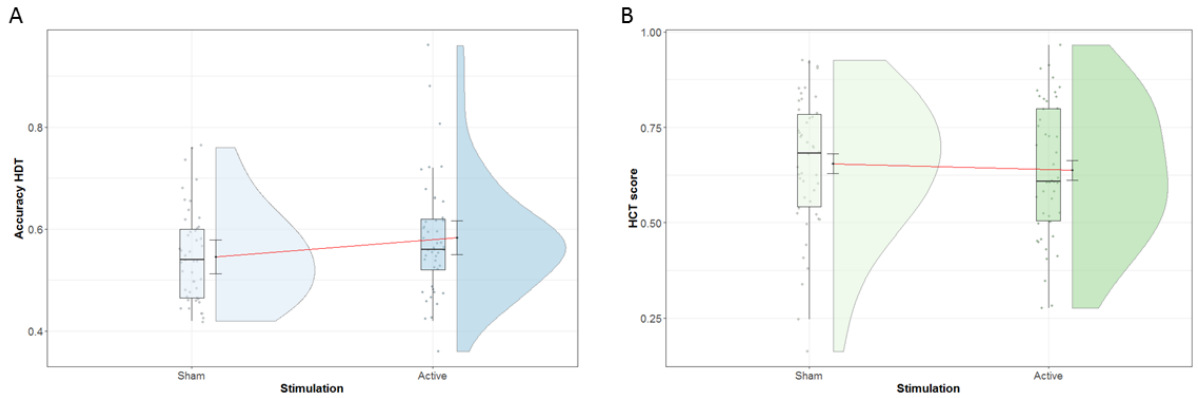

Figure 1. Accuracy in the A) heartbeat discrimination task (HDT) and in the B) heartbeat counting task (HCT) as a function of stimulation type. The raincloud plots provide a comprehensive descriptive representation of accuracy scores during Sham (lighter colour) and Active (darker colour) stimulation. The red lines correspond to the fitted values, with error bars, and represent the main effect of stimulation that was found to be significant in the HDT but not in the HCT.

Study 2

Participants rated the intensity of fearful and neutral facial expressions during active- and sham-tVNS. Stimuli were presented for 100ms either during cardiac diastole (coinciding with the ECG's R-wave) or cardiac systole (300ms after the ECG's R-wave). Average pupil dilation (2000-3000ms after stimulus onset) in response to the stimuli were taken as measures of physiological arousal.

Repeated measures ANOVA with 2 Cardiac Cycle (Systole; Diastole) x 2 Emotion (Fear; Neutral) x 2 Stimulation (Active; Sham) as within-factors were used to analyse the data. To carry out post-hoc comparisons responses to neutral stimuli were subtracted from those to fearful stimuli in the corresponding Cardiac Cycle and Simulation conditions. Planned comparisons, with two-tailed paired t-tests, were carried out to test compare responses within Cardiac Cycle and Stimulation (Bonferroni correction applied).

Results on the intensity ratings revealed a main effect of emotion ($F(1,35)=169.41$, $p<0.001$) and a significant Cardiac Cycle x Emotion x Stimulation interaction ($F(1,35)=5.05$, $p=0.031$) suggesting a tVNS modulation of emotional processing as a function of cardiac cycle. Planned post-hoc analyses revealed that participants rated fearful expressions as more intense at systole (vs diastole) only during active ($t =2.68$, $p=0.044$) and not during sham ($t =0.35$, $p>0.05$) stimulation. No other comparison was significant (ps>0.05)
Similarly, a significant the Cardiac Cycle x Emotion x Stimulation interaction ($F(1,35)=4.22$, $p=0.048$) was found for the pupil responses. However, post-hoc analyses were not conclusive on the directios of the effects (ts<2.2. ps>0.05) .No other main effect or interaction was found (ps>0.05).

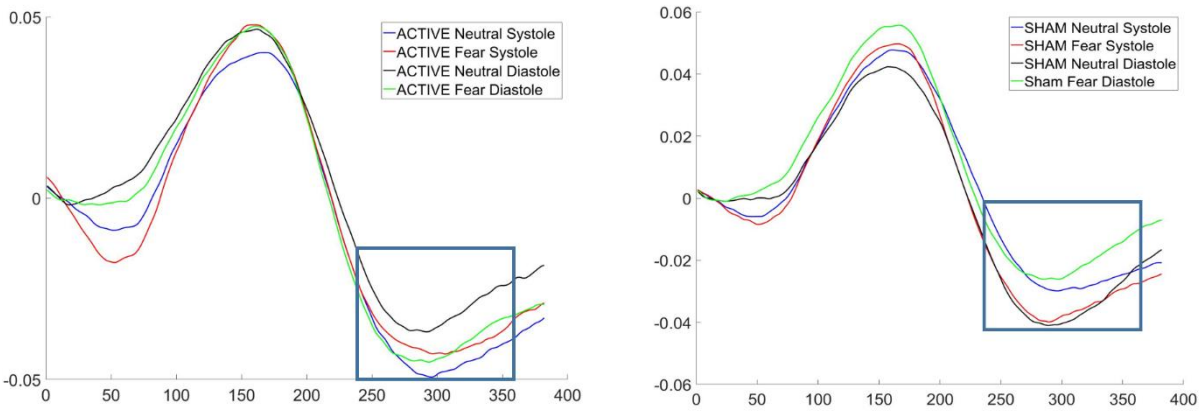

Figure 2. Pupil responses to stimuli in each condition. Y-axis: pupil diameter (mm) difference from baseline; X-axis: time since stimuli onset. Each time point is a sample collected on a 120Hz eye-tracker. The average dilation from 2000-3000ms after stimuli onset (values inside the square) were submitted to analysis.

Study 3

Stimulation was delivered using a Digitimer DS7A (https://digitimer.com/), controlled by Matlab, with a custom-built clip electrodes. As in the previous studies, we stimulated the participants' left tragus and left earlobe for the active and sham stimulations, respectively, and the intensity of stimulation was individually tailored to a level just above the participant's perceptual threshold. Short bursts (i.e. 3 seconds) of Active or Sham stimulation (pulse width=500µs, frequency=10Hz (Badran et al, 2018b)) were delivered, in a random fashion, during stimuli presentation in an (emotional) attentional blink task (see Garfinkel et al., 2014 study 1). In this version of the task, while the first target (T1) is always a face with neutral expression and the second target (T2) can be a face with fearful, happy or neutral expression. The dependent variable in this study is accuracy in the identification T2 in each condition of the (emotional) attentional blink task: 2 Stimulation (Active tVNS; Sham tVNS) x 2 Emotion (Neutral T2; Fear T2; Happy T2). The prediction was that these new stimulation parameters deliver effective tVNS, as reflected in the modulation of behaviour in an (emotional) attentional blink task. Specifically, we predicted that tVNS would increase the detection of T2, either irrespective of facial expression or specifically T2 with emotional facial expressions.

The target sample size was 40, estimated (with Gpower) by averaging the suggested sample sizes obtained using the effect sizes of two studies investigating the effect of tVNS on emotion recognition (Colzato et al., 2017; Sellaro et al., 2018). Data from 45 participants was collected.

Repeated measures ANOVA with 2 Stimulation (Active tVNS; Sham tVNS) x 3 Emotion (Fear; Neutral; Happy) as within-factors were used to analyse the data. Results revealed a significant main effect of stimulation ($F(1,44)=4.81$, $p=0.034$) but no main effect of Emotion ($F(1,44)=0.79$, $p=0.49$) nor Stimulation X Emotion interaction ($F(1,44)=1.64$, $p=0.200$). Equivalent results were observed when analysing only the first 40 participants, corresponding to the target sample size, (main effect of stimulation: $F(1,39)=5.64$, $p=0.023$). Because the interaction was not significant, no post-hoc analyses were carried out.

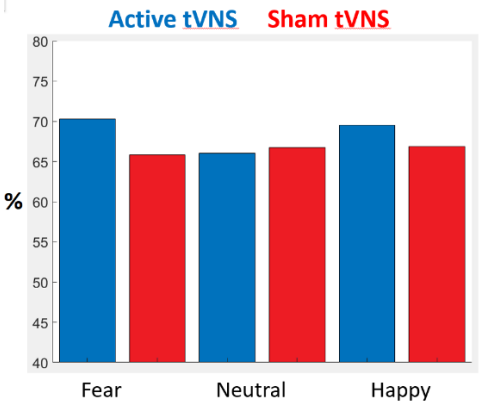

Figure 3. Percentage of correct T2 detections in each condition of the attentional blink task. Blue bars correspond to trials during active tVNS and red bars to trials during sham tVNS.

Study 4

Participants were presented with short video clips (~3000ms) depicting faces or hands being gently stimulated either with a cotton bud (Touch condition) or pricked with a needle (Pain condition). In half of the trials they were asked to rate in a VAS "how intense was the sensation felt by the target" (other-condition) or "how intense was the distress reaction they felt watching the video" (self-condition). In addition to subjective ratings, we also measured pupil dilation as an index of autonomic reactivity. As in study 3, short bursts of Active or Sham stimulation were delivered, in a random fashion, during stimuli presentation to investigate the role of tVNS on, other- and self-oriented, empathic responses to other's pain. We predicted that tVNS will modulate empathic responses, i.e. physiological responses, to others pain. This was an exploratory study and therefore we had no specific predictions on the direction of the effects.

The target sample size was 40, estimated (with Gpower) by averaging the suggested sample sizes obtained using the effect sizes of two studies investigating the effect of tVNS on emotion recognition (Colzato et al., 2017; Sellaro et al., 2018). Data from 45 participants was collected but data from 4 participants was excluded from analyses due to noisy pupil dilation data.

Two repeated measures ANOVAs with 2 Stimulation (Active tVNS; Sham tVNS) x 2 Emotion (Pain; Touch) as within-factors were used to analyse the pupil data and subjective ratings. For the physiological responses, results revealed only a significant main effect of Emotion ($F(1,40)=121.74$, $p<0.001$) but no main effect of Stimulation ($F(1,40)=0.036$, $p=0.85$) nor Stimulation X Emotion interaction ($F(1,40)=0.49$, $p=0.49$). Because the interaction was not significant, no post-hoc analyses were carried out. Likewise, only the effect of Emotion ($F(1,40)=732.44$, $p<0.001$) was found to be significant for the subjective ratings. All other $Fs<0.21$, $ps>0.65$.

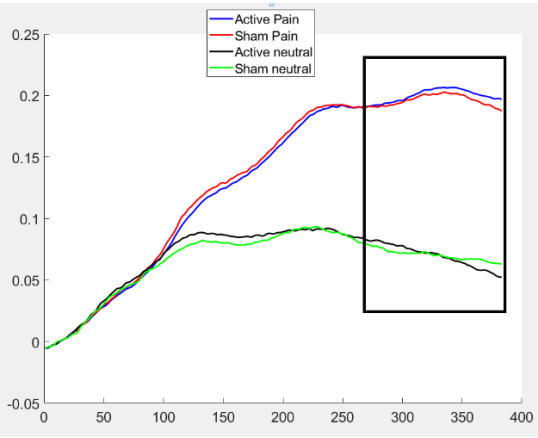

Figure 4. Pupil responses to stimuli in each condition. Y-axis: pupil diameter (mm) difference from baseline; X-axis: time since stimuli onset. Each time point is a sample collected on a 120Hz eye-tracker. The average dilation from 2000-3200ms after stimuli onset (values inside the rectangle) were submitted to analysis.

Review 1

We are currently writing a theoretical paper consisting on a critical review of the available empirical evidence for the effects of tVNS on cognition and emotional processing.
The key aspects covered by this work are: i) the effects of the tVNS on neurophysiological responses, ii) on the autonomic nervous system and iii) on cognitive and emotional processes; iv) the mechanisms underlying tVNS and v) future directions and recommendations.
Importantly, in this work also reviews the different stimulation parameters and experimental protocols adopted in each study and discuss the importance of adopting standard procedures. We note, for example, that the contradictory effects of tVNS on the autonomic activity observed in the literature (e.g. Clancy et al., 2014; Colzato et al., 2017) seem to be dependent on the stimulation site, i.e. right tragus vs left conchae or tragus. We also note the results obtained in Study 3 and argue that adopting more flexible event-related stimulation approaches will be crucial to advance experimental research.
Regarding the mechanisms underlying the effects of tVNS on cognition and emotion, we highlight two, non-mutually exclusive, proposals. One of the dominant proposals posits that the effects of tVNS are due to the modulation of noradrenergic system through stimulation of the locus-coeruleus. Indeed, some studies have found that tVNS modulates performance in cognitive tasks thought to be mediated by noradrenergic system (Sellaro et al., 2015; Ventura-Bort et al., 2018). Our Study 3 may offers additional support for this hypothesis by showing tVNS modulation on the attentional blink, a phenomenon believed to rely on the noradrenergic system. Another possibility is that tVNS impacts emotion experience through its modulation in autonomic processing. Indeed, our studies 1-2, provide convincing support for this hypothesis.

Discussion

While the anatomical afferent pathways conveying visceral information to the brain are fairly well understood, the neurophysiological mechanisms underlying the conscious perception of cardiac signals remain subject to debate. Some authors argue for a somatosensory pathway, whereby heartbeat sensations are mainly the result of the stimulation of somatosensory afferents on the skin (Khalsa et al., 2009). Others defend the importance of a visceral route, highlighting the role of baroreceptors in the conduction of cardiac afferent signals via the vagus and glossopharyngeal nerves to the brainstem nuclei and thereafter to cortical regions such as the insula. By showing an improvement in IAcc during non-invasive vagus nerve stimulation (Study 1), our findings argue in

favour of the latter. Indeed, our results provide one of the most compelling pieces of empirical evidence, so far, in support of a visceral pathway mediating the conscious perception of cardioceptive signals. While the functional importance of the vagus nerve in the conduction and regulation of autonomic signals is well documented, experimental evidence of its role in bringing these signals into consciousness is lacking. Our findings, relying on non-invasive stimulation of the vagus, provide the first tentative evidence on the role that the vagus nerve may have in consciously perceiving interoceptive states.

Moreover, Study 2 revealed that tVNS modulates the impact that afferent physiological activity has on the processing of emotional stimuli, i.e. threat-signalling cues. The paradigm used capitalizes on natural fluctuations in cardiac afferent signalling, i.e. the firing of arterial barorceptors, to identify specific body-to-brain effects on cognition (Garfinkel et al, 2014; Azevedo et al., 2017). Here, we show that the tVNS modulates the influences the impact of arterial baroreceptors in threat processing. While post-hoc analyses did not allow to draw conclusive interpretations on the direction of this modulation, our results provide relevant evidence for the mechanisms underlying tVNS on emotional processing.

Several fMRI studies have shown that tVNS modulates activity in several brain regions related with the processing of afferent vagal signals and interoception, such as the NTS, thalamus, precentral gyrus and insular cortex (e.g. Badran et al., 2018b; Dietrich et al., 2008; Yakunina et al, 2016) and the locus coeruleus-norepinephrine system (Ruffoli et al, 2017). Similarly to other non-invasive brain stimulation techniques, tVNS effects might be explained by modulation of neural activity and consequent shifts in the signal to noise ratio in the system (Miniussi, Harris, & Ruzzoli, 2013). Importantly, the effects of the stimulation depend on the state of the recruited neural populations (i.e. state dependency) and thus on the task's characteristics. Here, it is likely that tVNS modulation of brain activity within vagal and interoceptive regions, e.g. NTS or insula, increased the signal-to-noise ratio enhancing the sensitivity to heartbeat sensations during the interoceptive accuracy task (Study 1) and during the processing of threat cues (Study 2). Moreover, Study 3 also suggests modulation of the locus coeruleus-norepinephrine system. Importantly, it also shows that these tVNS induced neural states are transient and can be capitalized upon with event-related stimulation protocols. This may constitute a valuable tool for experimental research on tVNS. Rapid event-related protocols allow isolating and testing discrete cognitive processes that otherwise could be undifferentiated or obscured by prolonged tVNS-induced state changes. Two important fields of knowledge are likely to benefit greatly from this approach. Firstly, event-related tVNS can be very useful for brain-body interactions research by modulating the central representation of afferent physiological signals. Secondly, it may be very important for research investigating the role of the locus coeruleus-norepinephrine system in cognition. Because the locus coeruleus-norepinephrine system appears to exhibit two modes of function, i.e. phasic and tonic (Aston-Jones and Cohen, 2005), only event-related stimulation protocols may be able to effectively explore the tVNS modulation on this neural system.

In study 4, we explored if and how tVNS modulates one of the most fundamental dimensions of human social behaviour, that is empathic reactivity, or in other words, the ability to share and understand others' affective states. Participants were presented, during active and sham tVNS, with videos of people experiencing painful or non-painful stimulation while we recorded their physiological responses and subjective ratings of the target's experience. However, we found no tVNS modulation neither on physiological responses nor on subjective reports. These results suggest that: i) either tVNS does not modulate empathic reactivity others' pain; ii) or that our experimental paradigm was not sensitive enough to measure tVNS effects. It is possible, for example, that the little variability in the stimuli used, in terms of sensations depicted and perceived intensity, promoted quick habituation and precluded tVNS from being observed. However, because existing evidence does suggest that tVNS improves emotion recognition (Colzato et al, 2017), future studies might use different types of stimuli to further investigate this issue.

In sum, we introduce procedure to manipulate interoceptive and emotional processing with large potential for experimental research and clinical practice. The non-invasiveness of this method and the fact that it is does not seem to rely on the modulation of higher-order cognitive factors, such as attention or psychological stress, makes it a valuable tool for the study of body-brain interactions and the impact of afferent cardiac signals in healthy and clinical populations. Future studies may want to investigate how tVNS can promote interoceptive learning or be integrated with other therapeutic techniques, e.g. mindfulness, for the treatment of metal health conditions (Khalsa et al, 2017). The findings reported here enhance our understanding of the mechanisms underlying the conscious perception of heartbeats, in the processing of emotional stimuli, and demonstrate the potential of tVNS as an important tool to investigate brain-body interactions.

References

Antonino et al (2017). Non-invasive vagus nerve stimulation acutely improves spontaneous cardiac baroreflex sensitivity in healthy young men - A randomized placebo-controlled trial. Brain Stimulation, 10 875-881

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus–norepinephrine function: Adaptive gain and optimal performance. Annual Review of Neuroscience, 28, 403-450

Badran et al (2018a). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: A concurrent taVNS/fMRI study and review. Brain Stimulation, 11, 492-500

Badran et al (2018b). Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. Brain stimulation.

Bates, D. et al. (2015) Fitting linear mixed-effects models using lme4. arXiv 1406.5823

Burger, A., Verkuil, B., Van Diest, I., Van Der Does, W., Thayer, J., & Brosschot, J. (2016). The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans. Neurobiology of Learning and Memory, 132, 49-56.

Cameron O (2001) Interoception: The inside story - a model for psychosomatic processes. Psychosom Med 63: 697–710.

Clancy JA, Mary DA, Witte KK, Greenwood JP, Deuchars SA, Deuchars J. (2014). Noninvasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. Brain Stimul 7(6):871e7.

Colzato, L. S., Sellaro, R., & Beste, C. (2017). Darwin revisited: The vagus nerve is a causal element in controlling recognition of other's emotions. Cortex; a Journal Devoted To the Study of the Nervous System and Behavior, 92, 95-102.

Critchley, H.D. et al. 2004.Neural systems supporting interoceptive awareness. Nat. Neurosci. 7: 189–195.

Critchley, H.D. & N.A. Harrison. 2013. Visceral influences on brain and behavior. Neuron 77: 624–638.

Dietrich S, Smith J, Scherzinger C, Hofmann-Preiß K, Freitag T, Eisenkolb A, et al. (2008). A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by

functional MRI/Funktionelle Magnetresonanztomographie zeigt Aktivierungen des Hirnstamms und weiterer zerebraler Strukturen unter transkutaner Vagusnervstimulation. Biomed Tech/Biomed Eng53(3):104e11.

Kraus T, Hosl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. J Neural Transm 2007;114(11):1485e93.

Kraus T, Kiess O, Hosl K, Terekhin P, Kornhuber J, Forster C. CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canalea pilot study. Brain Stimul 2013;6(5):798e804.

Peuker, E. T., & Filler, T. J. (2002). The nerve supply of the human auricle. Clinical Anatomy (New York, N.Y.), 15(1), 35 -37.

Ventureyra, E. C. (2000). Transcutaneous vagus nerve stimulation for partial onset seizure therapy. Child's Nervous System, 16(2),101-102.

Whitehead, W., Drescher, V. M., Heiman, P., & Blackwell, B. (1977). Relation of heart rate control to heartbeat perception. Biofeedback and Self Regulation, 2, 371–392.

Schandry, R. (1981). Heart beat perception and emotional experience. Psychophysiology, 18(4), 483–488.

Kleckner, I. R., Wormwood, J. B., Simmons, W. K., Barrett, L. F., & Quigley, K. S. (2015). Methodological recommendations for a heartbeat detection-based measure of interoceptive sensitivity. Psychophysiology,52(11), 1432–1440.

Ruffoli, R., Giorgi, F.S., Pizzanelli, C., Murri, L., Paparelli, A. & Fornai, F. (2011) The chemical neuroanatomy of vagus nerve stimulation. J. Chem. Neuroanat., doi:10.1016/j.jchemneu.2010.12.002.

Yakunina N, Kim SS, Nam EC. Optimization of transcutaneous vagus nerve stimulation using functional MRI. Neuromodulation Technol Neural Interface 2017 Apr 1;20(3):290-300.

Genheimer et al (2017). Reinstatement of contextual conditioned anxiety in virtual reality and the effects of transcutaneous vagus nerve stimulation in humans. Scientific Reports

Khalsa SS, Adolphs R, Cameron OG, Critchley HD, Davenport PW., Feinstein JS, et al. (2017). Interoception and mental health: a roadmap. Biol Psychiatry 3:501–13

Khalsa SS, Rudrauf D, Feinstein JS, Tranel D (2009): The pathways of interoceptive awareness. Nat Neurosci 12:1494–1496.