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Research Report

Darwin revisited: The vagus nerve is a causal element in controlling recognition of other's emotions

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ABSTRACT

Charles Darwin proposed that via the vagus nerve, the tenth cranial nerve, emotional facial expressions are evolved, adaptive and serve a crucial communicative function. In line with this idea, the later-developed polyvagal theory assumes that the vagus nerve is the key phylogenetic substrate that regulates emotional and social behavior. The polyvagal theory assumes that optimal social interaction, which includes the recognition of emotion in faces, is modulated by the vagus nerve. So far, in humans, it has not yet been demonstrated that the vagus plays a causal role in emotion recognition. To investigate this we employed transcutaneous vagus nerve stimulation (tVNS), a novel non-invasive brain stimulation technique that modulates brain activity via bottom-up mechanisms. A sham/placebo-controlled, randomized cross-over within-subjects design was used to infer a causal relation between the stimulated vagus nerve and the related ability to recognize emotions as indexed by the Reading the Mind in the Eyes Test in 38 healthy young volunteers. Active tVNS, compared to sham stimulation, enhanced emotion recognition for easy items, suggesting that it promoted the ability to decode salient social cues. Our results confirm that the vagus nerve is causally involved in emotion recognition, supporting Darwin's argumentation.

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1. Introduction

In his seminal book "The Expression of Emotions in Man and Animals" (1872/1965), Darwin was the first to propose that emotion expressions are controlled by a bidirectional neural communication between the heart and the brain via the so-called "pneumogastric" nerve. Nowadays known as the vagus nerve, this is the tenth cranial nerve and represents a key component of the parasympathetic nervous system. Notably, the vagus nerve is the longest cranial nerve. Given that it passes via the neck and thorax to the abdomen, the vagus nerve has the broadest distribution in the body. It comprises somatic and visceral afferent fibers, as well as general and special visceral efferent fibers. According to Darwin, emotional facial expressions are evolved and adaptive and serve a crucial communicative function. Unfortunately, at that time, the understanding of the neuroanatomy and phylogeny of the nervous system was limited and Darwin's intuition has been further developed only a century later by Porges (2001; 2003; 2007) in his influential polyvagal theory. This theory proposes that the vagus nerve is the key phylogenetic substrate that regulates emotional and social behavior. Interestingly, mammals are the only vertebrates characterized by a myelinated vagus that can rapidly regulate the nervous system to foster engagement and disengagement with the environment. According to the polyvagal theory two functionally distinct branches of the vagus fulfill different evolutionary responses in mammals. Whereas, the more primitive branch (The Dorsal Vagal Complex) is supposed to provoke immobilization behaviors (e.g., feigning death), the more evolved branch (The Ventral Vagal Complex) has been proposed to be related to social communication and self-soothing behaviors (Porges, 2001; 2003; 2007). The polyvagal theory assumes that optimal social interaction, which includes the recognition of emotion in faces, is modulated by the vagus nerve. In line with this idea, it has been found that the vagal tone (as indexed by heart rate variability) is a reliable marker of one's ability to respond to and recognize social cues: resting-state heart rate variability was positively associated with performance on Reading the Mind in the Eyes Test (RMET) (Quintana, Guastella, Outhred, Hickie, & Kemp, 2012). The RMET requires participants to assess someone's emotions based on images of the eye region and performance on this test has been found to be poor among patients suffering from pathologies associated with dysfunction of the vagus nerve, such as autism (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001) and depression (Lee, Harkness, Sabbagh, & Jacobson, 2005). Unfortunately, the nature of the studies outlined above is correlative and, so far, in humans it has not yet been demonstrated that the vagus nerve plays a causal role in emotion recognition.

The aim of the current study therefore is to examine the causal involvement of the vagus nerve and the recognition of someone's emotions based on images of the eye region, as indexed by the RMET. In order to do that, we employed transcutaneous (through the skin) vagus nerve stimulation (tVNS), a novel non-invasive brain stimulation technique that modulates brain activity via bottom-up mechanisms (Ventureyra, 2000). That is, the propagation of the afferent signal from the vagus nerve travels from peripheral nerves toward the brain stem and from there to higher cortical structures (Shiozawa et al., 2014; Vonck et al., 2014). As pointed out by Shiozawa et al. (2014) the vagus nerve innervates the nucleus tractus solitarious bilaterally, which is connected to the locus coeruleus. tVNS, proposed for the first time by Ventureyra (2000), is safe and accompanied only by minor side effects such as tingling or itching sensation under the electrodes. Several studies employing high intensity tVNS have not revealed any major side-effects (Dietrich et al., 2008; Kraus et al., 2007; Bauer et al., 2016). Given the right vagal nerve has efferent fibers to the heart, tVNS is safe to be performed only in the left ear (Kreuzer et al., 2012). tVNS acts via the auricular branch of the vagus nerve (ABVN) which supplies the skin of the concha in the human ear (Peuker & Filler, 2002) allowing for a reliable transcutaneous electrical stimulation of the nerve fibers in this area. The stimulation activates the thick-myelinated Aβ fibers of the ABVN which project directly to the nucleus of the solitary tract in the brainstem.

Following Kraus et al. (2007), a clever way to create a sham condition using tVNS is by attaching the stimulation electrodes to the center of the left ear lobe, which is free of cutaneous vagal innervation, see Fig. 1 (Peuker & Filler, 2002). By doing this, the participants perceive the exact same minor side effects of the active stimulation and they are not able to disentangle the active from the sham stimulation.

In contrast to imaging techniques, which are only correlational, by means of tVNS we are able to infer a causal relation between the stimulated vagus nerve and the related ability to recognize emotions as indexed by performance on the RMET (van Leusden, Sellaro, & Colzato, 2015). tVNS has been found to reliably activate the vagus nerve. In a seminal study Fallgatter et al. (2003) stimulated the tragus and demonstrated, by means of early acoustic evoked potentials, that active tVNS, compared to sham, produced a clear and reliable vagus sensory evoked potential in healthy participants. Further, two functional magnetic resonance imaging (MRI) studies in healthy humans have found that tVNS increased activation in the brainstem region including the locus coeruleus and nucleus of the solitary tract, indicating that tVNS is able to effectively stimulate vagal afferents to the brainstem (Dietrich et al., 2008; Frangos, Ellrich, & Komisaruk, 2015).

In sum, if the vagus nerve is involved in the process of emotion recognition as hypothesized by Darwin (1872/1965) and Porges (2001; 2003; 2007), we would expect that active tVNS, compared to sham stimulation, will enhance performance of the recognition of someone's emotions based on images of the eye region.

2. Material and methods

2.1. Participants

Thirty-eight Leiden University undergraduate students (30 females, 8 males, mean age = 22.29 years, range 18–26) participated in the experiment. Participants were recruited via

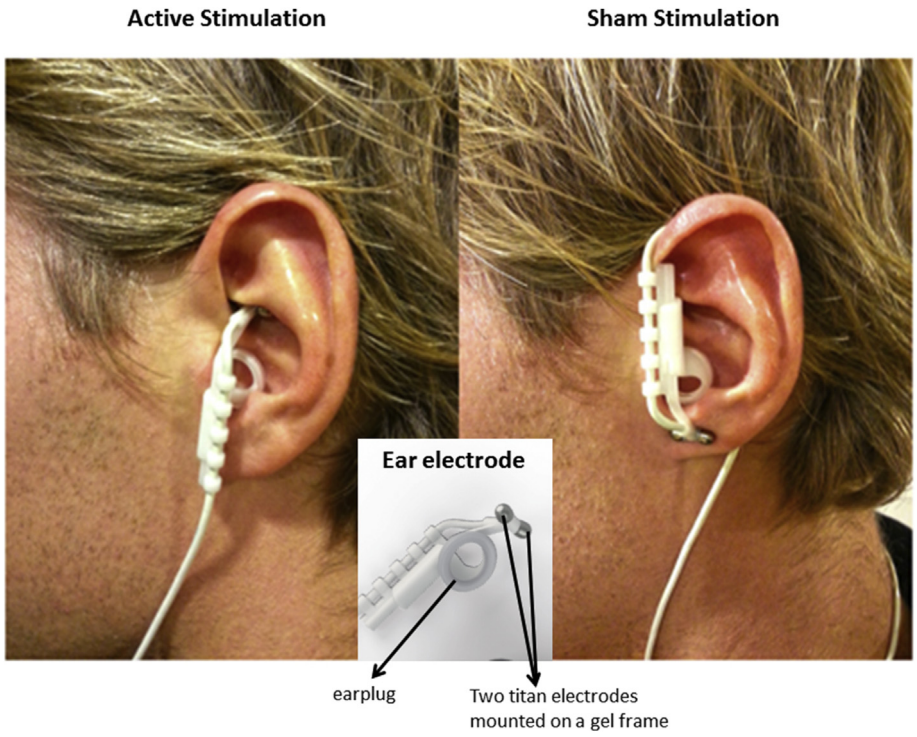

Fig. 1 – tVNS is delivered via a single ear electrode. This ear electrode consists of an earplug that is placed in the auricle like an earphone and two titan electrodes mounted on a gel frame that allow to generate and transfer electric impulses from the stimulator to the surface of the skin. For the active stimulation (left-side panel), the two titan electrodes are placed on the outer auditory canal of the left ear. For the sham stimulation (right-side panel), this two electrodes are placed on the center of the left ear lobe.

an on-line recruiting system and were offered either course credits or a financial reward of 10 euros for participating in a study on the effects of brain stimulation on decision-making. Once recruited, all participants were screened individually by the same lab-assistant using the Mini International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998). The M.I.N.I. is a short, structured interview of about 15 min that screens for several psychiatric disorders and drug use, and it is often used in clinical and pharmacological research (Colzato, Kool, & Hommel, 2008; Colzato et al., 2012).

Following previous published protocols (Beste et al., 2016; Sellaro et al., 2015; Steenbergen et al., 2015) participants were considered suitable to participate in this study if they fulfilled the following criteria: (i) age between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no history of brain surgery, tumors, or intracranial metal implantation; (v) no chronic or acute medications; (vi) no pregnancy; (vii) no susceptibility to seizures or migraine; (viii) no pacemaker or other implanted devices. All participants were naïve to tVNS. Prior to the testing session, they received a verbal and written explanation of the procedure and of the typical adverse effects (i.e., itching and tingling skin sensation, skin-reddening, and headache). No information was provided about the different types of stimulation (active *vs* sham) or about the hypotheses concerning the experiment. The study conformed to the ethical standards of the Declaration of Helsinki and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research). Written informed consent was obtained from all participants.

2.2. *Apparatus and procedure*

A single-blind, sham/placebo-controlled, randomized cross-over within-subjects design with counterbalanced order of conditions was used to assess the effect of on-line (i.e., stimulation overlapping with the critical task) tVNS on RMET performance in healthy young volunteers.

To control for nonspecific effects of tVNS on arousal, mood, heart rate and blood pressure and to control for empathy baseline levels and eventual autistic traits in our healthy sample of participants, we assessed these variables directly by means of suitable questionnaires and devices described below.

All participants took part in two sessions (active *vs* sham) and were tested individually. In both sessions, upon arrival, participants were asked to rate their mood on a 9 × 9 pleasure × arousal grid (Russell, Weis, & Mendelsohn, 1989) with values ranging from −4 to 4. Heart rate (HR) and systolic and diastolic blood pressure (SBP and DBP) were collected from the non-dominant arm with an OSZ 3 Automatic Digital Electronic Wrist Blood Pressure Monitor (Speidel & Keller) for the first time (T1). Immediately after, participants were asked to fill in some questionnaires: the interpersonal reactivity index (IRI) and autism spectrum quotient (AQ) in session 1, and the empathy quotient (EQ), in session 2.

Twenty minutes after the onset of stimulation, participants again rated their mood and HR, SBP, and DBP were collected for the second time (T2). Afterwards, they performed the RMET (Baron-Cohen et al., 2001), which lasted for about 15 min. After completing the RMET, mood, HR, SBP, and DBP were measured for the third time (T3) and the stimulation was terminated. After completion of each session, participants were asked to complete a tVNS adverse effects questionnaire requiring them to rate, on a 5-point (1–5) scale, how much they experienced (1) headache, (2) neck pain, (3) nausea, (4) muscle contraction in face and/or neck, (5) stinging sensation under the electrodes, (6) burning sensation under the electrodes, (7) uncomfortable (generic) feelings, and (8) other sensations and/or adverse effects. None of the participants reported major complaints or discomfort during or after tVNS.

At the end of the second session participants were debriefed and compensated for their participation. We did explicitly assess participants' blinding by asking them if they could guess the stimulation received and no one reported to be aware of it.

The IRI is a self-report questionnaire that assesses perceived individual differences in the tendency to be empathetic. It consists of 28 Likert-type items on a response scale with five alternatives ranging from 0 (does not describe me well) to 4 (describes me very well). It comprises four subscales assessing affective (empathic concern and personal distress) and cognitive (fantasy and perspective taking) components of empathy (Davis, 1980, 1983).

The AQ is a self-report questionnaire aimed at detecting the presence of autistic traits in normal adult populations (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). The questionnaire is made of 50 items that, taken together, provide a measure of the degree to which an adult with normal intelligence has traits associated with the autistic spectrum (range 0–50, higher scores indicating autistic-like behavior).

The EQ is a self-report questionnaire designed to assess empathy in normal adult populations (Baron-Cohen & Wheelwright, 2004). It comprises 60 questions (20 items are filler questions) that, taken together, provide an overall measure of cognitive perspective taking, affective empathy, and social skills (range 0–80, higher scores indicating more empathy).

2.3. *Transcutaneous vagus nerve stimulation (tVNS)*

We used the NEMOS® tVNS neurostimulating device. This device consists of a stimulation unit connected to a dedicated ear electrode. The ear electrode comprises an earplug that is placed in the auricle like an earphone, and two titan electrodes mounted on a gel frame that allow to generate and transfer electric impulses from the stimulator to the surface of the skin, see Fig. 1. Following the protocol of previous studies for optimal stimulation (Beste et al., 2016; Sellaro et al., 2015; Steenbergen et al., 2015), the tVNS® device was programmed to a stimulus intensity at .5 mA, delivered with a pulse width of 200–300 μs at 25 Hz. Stimulation alternated between on and off periods every 30 sec. In the active condition, the stimulation electrodes were applied to the outer auditory canal. In the sham (placebo) condition, the stimulation electrodes were placed on the center of the left ear lobe. Indeed, the ear lobe has been found to be free of cutaneous vagal innervation (Fallgatter et al., 2003; Peuker & Filler, 2002) and a recent fMRI study showed that this sham condition, as compared to active stimulation, produced no activation in the cortex and brain stem (Kraus et al., 2013).

Importantly, following safety criteria to avoid cardiac side effects, the stimulation was always applied to the left ear (Nemeroff et al., 2006; Cristancho, Cristancho, Baltuch, Thase, & O'Reardon, 2011). Indeed, although efferent fibers of the vagus nerve modulate cardiac function, such a modulation seems to relate only to the efferent vagal fibers connected to the right ear (Nemeroff et al., 2006). Consistently, a clinical trial showed no arrhythmic effects of tVNS when applied to the left ear (Kreuzer et al., 2012).

2.4. *Reading the mind in the eyes task (RMET)*

Participants were tested on a computerized version of the RMET – revised (Baron-Cohen et al., 2001), using the E-Prime 2.0 software system (Psychology Software Tools, Inc., Pittsburgh, PA). The test consisted of 36 black-and-white photos of a human's eye region, presented one by one along with four adjectives (one target word and three foil words) arranged around the eye region. On each trial, participants were instructed to choose which of the four words better described what the person in the picture was thinking or feeling, by clicking on it with the mouse. The 36 target pictures were preceded by an additional picture that served as an example. Participants were asked to respond as fast as possible, but no time limit was imposed. Whenever necessary, participants were allowed to consult a glossary including a brief definition of each word. Performance on this test is measured by computing the percentage of correct responses.

2.5. *Statistical analyses*

Following previous studies (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Guastella et al., 2010), the 36 items were divided in two subsets of easy and difficult items (see Table 1). The two subsets were created based on the median-split of item difficulty obtained from a pilot study involving 30 healthy participants (21 females, 9 males, mean age = 22.97 years), with no history of psychiatric/neurological disorders and drug use. For each participant, and for both the active and the sham stimulations, the percentage of correct answers for the easy and difficult items was calculated. To examine whether active tVNS, as compared to sham (placebo) stimulation, improved RMET performance, a repeated-measures analysis of variance (ANOVA) was carried out with the percentage of correct answers as dependent variable and session (active *vs* sham) and item difficulty (easy *vs* difficult) as within-participant factors.

Mood (i.e., pleasure and arousal scores), HR, SBP, and DBP were analyzed separately by means of repeated-measures ANOVAs with effect of time (first *vs* second *vs* third measurement) and session (active *vs* sham) as within-participant factors.

A significance level of $p < .05$ was adopted for all statistical tests. Fisher LSD post hoc tests were performed to clarify mean differences.

Table 1 – Subdivision of RMET items in two subsets of easy and difficult items. This subdivision reflects the median-split of item difficulty derived from the results obtained in a pilot study involving 30 healthy participants. Items are defined in terms of their corresponding number in the original test, as described in Baron-Cohen et al. (2001a). Accuracy scores are reported within parentheses.

Easy items	Difficult items
1 (90.0%)	2 (63.3%)
3 (86.7%)	5 (76.7%)
4 (83.3%)	7 (30.0%)
6 (80.0%)	10 (43.3%)
8 (90.0%)	12 (73.3%)
9 (90.0%)	13 (63.3%)
11 (83.3%)	14 (66.7%)
18 (90.0%)	15 (63.3%)
20 (90.0%)	16 (76.7%)
21 (93.3%)	17 (43.3%)
22 (80.0%)	19 (73.3%)
24 (80.0%)	23 (50.0%)
25 (80.0%)	27 (60.0%)
26 (80.0%)	28 (66.7%)
31 (90.0%)	29 (43.3%)
36 (90.0%)	30 (76.7%)
	32 (73.3%)
	33 (73.3%)
	34 (66.7%)
	35 (60.0%)

3. Results

3.1. *Interpersonal reactivity index (IRI), autism spectrum quotient (AQ) and empathy quotient (EQ)*

Because of technical problems, two participants did not fill in the AQ, one participant did not fill in the EQ, and one participant did not fill in the IRI.

For all questionnaires, participants scores were in the normal range: $IRI_{perspective\ taking}$ (19.51, SEM = .7); $IRI_{fantasy\ scale}$ (19.32, SEM = .7); $IRI_{emphatic\ concern}$ (20.62, SEM = .6); $IRI_{personal\ distress}$ (12.70, SEM = .7); AQ (17.22, SEM = .9); EQ (48.43, SEM = 1.7).

3.2. *RMET*

ANOVA revealed a significant main effect of item difficulty, $F(1,37) = 127.86$, $p < .001$, $\eta^2_p = .78$: accuracy was significantly higher for the easy (85.0, SEM = 1.3) than for the difficult (63.8, SEM = 2.0) items. The main effect of session was not significant, $F(1,37) = 1.11$, $p = .30$, $\eta^2_p = .03$: participants showed comparable accuracy in the sham (73.4, SEM = 1.9) and in the active (75.3, SEM = 1.4) sessions. Crucially, a significant interaction involving item difficulty and session was found, $F(1,37) = 246.97$, $p = .026$, $\eta^2_p = .13$. Post-hoc analyses indicated that, for the difficult items, no significant difference occurred between sham and active sessions ($p = .67$); whereas, for the easy items, accuracy was significantly higher in the active than in the sham session ($p = .007$), see Fig. 2.

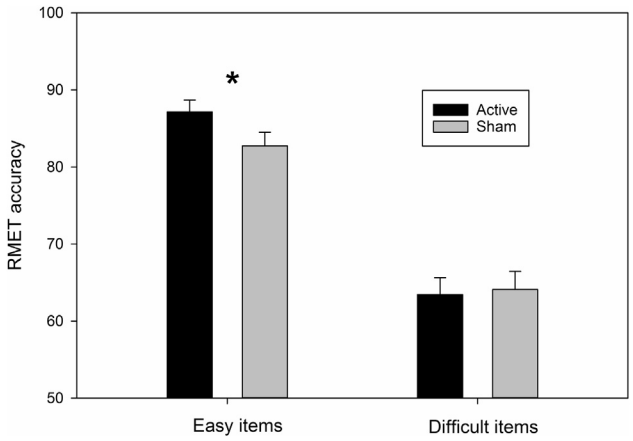

Fig. 2 – Percentage of correct answers (i.e., accuracy) on the RMET for the active and sham sessions as a function of item difficulty. Asterisks indicate significant ($^*p < .05$) differences between active and sham sessions. Vertical capped lines atop bars indicate standard error of the mean.

3.3. *Mood and physiological measurements*

Table 2 provides an overview of the outcomes for mood and physiological measurements. ANOVAs revealed a significant main effect of time for SBP, $F(2,74) = 3.50$, $p = .035$, $\eta^2_p = .09$, HR, $F(2,74) = 7.54$, $p = .001$, $\eta^2_p = .17$, and arousal, $F(2,74) = 3.41$, $p = .038$, $\eta^2_p = .08$, but not for DBP, $F(2,74) = 1.63$, $p = .20$, $\eta^2_p = .04$, and pleasure, $F(1,37) = .71$, $p = .49$, $\eta^2_p = .02$. Post-hoc analyses showed that SBP (117.3 *vs* 113.7 *vs* 113.8) and HR (81.2 *vs* 77.7 *vs* 74.9) levels were lower at the second ($p_s \leq .02$) and third ($p_s \leq .03$) measurements, as compared to the first one. Arousal values (3.4 *vs* 3.3 *vs* 2.9) dropped significantly at the third measurement, as compared to the first ($p = .01$), but not to the second one ($p = .07$). Importantly, pleasure, arousal, HR, DBP, SBP, did not significantly differ between the two sessions. Indeed, neither the main effects of session nor the two-way interactions involving session and time were significant, $F_s \leq 2.7$, $p_s \geq .08$, $\eta^2_{ps} \leq .07$.

4. Discussion

We can conclude that online stimulation of the vagus nerve enhances recognition of someone's emotions based on images of the eye region when item difficulty was taken into account. Indeed, when actively stimulated, participants were better in inferring people's emotional state, but only when confronted with easy items and not when presented with difficult items. This suggests that active tVNS improved the ability to recognize salient social cues, without affecting the recognition of subtler social cues. If one considers that people's fight/flight response strategies are more likely to rely on salient and recognizable social cues, the present findings fit with the proposed role of the vagus nerve to regulate social engagement via emotion recognition (Porges, 2001; 2003; 2007). This

Table 2 – Pleasure and arousal scores, mean heart rate values (in beats per minute), systolic and diastolic blood pressure (in mmHg), as a function of time [first (T1) *vs* second (T2) *vs* third (T3) measurement] for the active and the sham sessions. Standard errors are shown in parentheses.

	T1		T2		T3	
	Active	Sham	Active	Sham	Active	Sham
Pleasure	4.4 (.5)	4.5 (.4)	4.4 (.4)	4.4 (.4)	4.1 (.4)	4.5 (.5)
Arousal	3.5 (.5)	3.3 (.4)	3.2 (.5)	3.4 (.5)	2.9 (.4)	2.9 (.4)
Heart rate	84.1 (2.8)	78.3 (2.2)	79.4 (2.3)	75.2 (2.5)	74.3 (2.8)	75.5 (2.4)
Systolic blood pressure	118.2 (2.0)	116.5 (2.0)	112.8 (2.4)	114.6 (2.0)	113.5 (2.0)	114.2 (1.9)
Diastolic blood pressure	71.2 (1.6)	74.3 (1.5)	71.6 (2.1)	69.6 (1.7)	70.6 (1.8)	71.0 (1.5)

is not to deny, however, that more research is needed to support this conclusion. Nevertheless, given the causal nature of the brain stimulation technique employed in the current study, our results provide a first direct demonstration for a causal link between the vagus nerve and emotion recognition as hypothesized by Darwin (1872/1965) and Porges (2001; 2003; 2007).

Our findings are in line with previous correlative studies. First, Quintana et al. (2012) found that participants with higher resting-state heart rate variability (an index of vagal tone) were better in identifying the emotion expressed on the RMET after adjusting for item difficulty. Second, patients suffering from pathologies associated with dysfunction in the vagus nerve, such as autism (Cheshire, 2012) and depression (Rush et al., 2005), also demonstrate worse performance on the RMET (Baron-Cohen et al., 2001; Lee et al., 2005). In particular, a recent study with a large sample size ($n = 118$) that considered item difficulty, has shown that participants suffering from autistic spectrum disorder (ASD) scored low on easy items in the RMET (Baribeau et al., 2015). Therefore, the potential of tVNS to enhance the ability to decode the affective mental state of others might be helpful for people suffering from ASD by diminishing social ambiguity and promoting social communication. So far only invasive vagus nerve stimulation has been tried in single cases of autism with comorbid epilepsy, with the goal of reducing seizures but not with the aim of ameliorating social information processing (Hull, Madhavan, & Zaroff, 2015; Sansa et al., 2011).

Given that we controlled for nonspecific effects of tVNS on arousal, mood, heart rate and blood pressure and we made sure participants showed normal empathy baseline levels and no autistic traits, we can rule out an explanation of our results in these terms.

Future studies need to understand whether the current results could be extended to emotion recognition of other part of the body. That is, whether the effect of tVNS can be generalized to the recognition of someone's emotions based on images of whole faces (as indexed for example by the Facial Expression Recognition Task; Young et al., 1997) and whole bodies (for instance by means of The Bodily Expressive Action Stimulus Test; De Gelder & Van den Stock, 2011). Further, given the putative role of the vagus nerve in emotional biasing (Martin, Denburg, Tranel, Granner, & Bechara, 2004), it also seems relevant to investigate whether vagus nerve may be casually involved in the Emotional Egocentricity Bias, that is, the degree to which empathic judgments are biased by one's own emotions if they are incongruent to those of the person we empathize with (Silani, Lamm, Ruff, & Singer, 2013).

Finally, there are some limitations of the current study that warrant discussion. First, it would have been optimal to have linked the implementation of tVNS with appropriate physiological assays, such as the vagus-evoked potentials (Fallgatter et al., 2003). Moreover, it would be useful to look into the activation of the left inferior frontal gyrus, a cortical correlate of the recognition of someone's emotions based on images of the eye region (Dal Monte et al., 2014).

Notwithstanding these limitations, our observations provide direct evidence for the idea that the vagus nerve plays a causal role in the recognition of someone's emotions based on images of the eye region. Therefore, these findings may represent an important step in stimulating research to further extend our understanding of the specific role of the vagus nerve in the general processing of emotions. Further, our results support the idea that tVNS is a promising noninvasive brain stimulation technique for enhancing social and affective processes in healthy humans.

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