Connecting minds and sharing emotions through human mimicry

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Chapter 7

The effect of transcutaneous vagus nerve stimulation on pupil mimicry – trust link

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All data, code, and materials that are associated with this paper and used to conduct the analyses are accessible on the Leiden University archiving platform DataverseNL.

Abstract

During social encounters, people make eye contact to invite approach and foster bonding. In these moments, if both partners' pupils align and simultaneously dilate, pupil mimicry boosts trust. So far, little is known about the neuromodulation of this pupil mimicry-trust link, but it has been proposed that the locus coeruleus–norepinephrine (LC-NA) system might be at play. In this experiment, we investigate the role of the LC-NA system in the pupil contingent trust formation by using transcutaneous vagus nerve stimulation (tVNS); a method that has been proposed to increase norepinephrine concentrations in the brain and which we expected would induce pupil dilation. Participants' pupil sizes and investments were measured as they played trust games with partners whose pupils changed in size. Using a single-blind, sham-controlled, within-subject design, we also manipulated the background luminance of stimuli to induce pupil dilation without targeting the LC-NA system. The results revealed that neither tVNS nor a dark stimulus modulated pupil mimicry, which demonstrates that pupil mimicry is a robust phenomenon resistant to mechanistic manipulation. Moreover, in support of our hypothesis, active as compared to sham stimulation decreased trust in partners with static pupils compared to dilating pupils. These results support the theory that the vagal nerve plays a causal role in the recognition of eye signals. However, since tVNS did not modulate participants' overall (non-baseline-corrected) pupil size, we conclude that behavioral influences induced by tVNS cannot be fully attributed to the LC-NA system. We discuss a potential alternative neurological pathway through which tVNS influences trust along with implications for future investigation using this method.

Keywords: Norepinephrine, Locus coeruleus, Pupil diameter, Pupil mimicry, Trust

Introduction

Over the past decade in the field of cognitive neuroscience, there has been an increased interest in pupillary responses as reflections of cognitive states. Apart from responding to changes in ambient light, pupils dilate with activation of the locus coeruleus–norepinephrine (LC-NE) system (Aston-Jones, Chiang, & Alexinsky, 1991; Eldar, Cohen, & Niv, 2013; Jepma & Nieuwenhuis, 2011). What makes pupil size particularly interesting is that, in contrast to other autonomic responses (e.g. skin conductance, heart rate), pupils are visible to the human eye if one's eyes are light enough, which is why they have the potential to modulate social interactions (Kret, 2015; Procházková & Kret, 2017). For example, previous research has shown that people with large pupils are perceived more positively than people with small pupils (Hess, 1975; Hess & Fischer, 2013; Kret, Fischer & De Dreu, 2015; Kret, 2017; Kret & De Dreu, 2017). Moreover, existing evidence has shown that people mimic the pupil size of others (Harrison, Wilson & Critchley, 2007; Kret et al., 2015; Kret & De Dreu, 2017). Apart from human adults, this phenomenon has been found in infants (Aktar, Raijmakers & Kret, 2020; Fawcett, Wesevich & Gredebäck, 2016) and chimpanzees (Kret, Tomonaga & Matsuzawa, 2014), which implies that pupil mimicry might be an innate and evolutionarily old phenomenon. Intriguingly, prior research has shown that pupil dilation mimicry is positively related to measures of trust (Kret et al., 2015; Kret & De Dreu, 2017; Procházková et al., 2018; Wehebrink, Koelkebeck, Piest, de Dreu, & Kret, 2018). In this previous work, participants played trust games with partners whose pupils were manipulated to dilate, remain static, or constrict (Kret et al., 2015). Results revealed that when participants mimicked their partner's dilating pupils, they trusted their partner more than when they did not mimic. In order to better understand the functional significance of the relationship between pupil dilation mimicry and trust (and the order of their effects), it is essential zoom in on the underplaying mechanisms.

Previous neuroimaging research has shown that observed pupil size in another person is processed by the amygdala (Amemiya and Ohtomo, 2012; K. E. Demos et al., 2008). An individual's own pupil size positively correlates with norepinephrine, which the locus coeruleus (a nucleus situated in the brainstem) elicits during arousal (Lavín et al., 2014; Sara and Bouret, 2012). We propose that when a partner's pupils dilate, the observer's amygdala sends excitatory projections to the LC that make the observer's pupils dilate as well (pupil-mimicry occurs, Figure 1). The LC-NE system in the brain further influences cortical areas engaged in decision-making (Donner and Nieuwenhuis, 2013; Eldar et al., 2013; Lavín et al., 2014; Sara and Bouret, 2012). When NE concentration in the brain is high, communication between distinct areas of the brain increases, which consequently biases individuals towards their dominant modes of thought and behavior, potentially facilitating appropriate behavioral and physiological responses to perceived stimuli. Considering that humans have a natural tendency to perceive large pupils as more positive (Hess, 1975; Hess & Fischer, 2013; Kret et al., 2015; Kret, 2017; Kret & De Dreu, 2017), the positive association between large pupils and trust is likely to increase with NE release - which can be noninvasively tracked by measuring pupil size. From this perspective, it makes sense that when participants mimic partners' dilating pupils, trust increases. In support of this theoretical model, in our recent fMRI study we showed that when participants perceived partners' dilating or constricting pupils, their visual brain areas (V5) related to luminance changes became active (Procházková et al., 2018). However, when subjects mimicked partners' dilating pupils compared to when they did not mimic, the neural activity in social brain regions (Theory of Mind network; Temporo-Parietal Junction and anterior cingulate cortex) increased. This evidence further supports the view that pupil mimicry stimulates higher cognitive functions involved in social cognition, and that the association between large pupils and trust may become pronounced with higher concentrations of NE (reflected in pupil diameter). Nevertheless, since the neurological underpinnings of pupil mimicry have been thus far tested only with correlational measures (e.g. fMRI), the causal role of the noradrenergic system and pupil mimicry in pupil-contingent trust has not been established.

The present study investigates the causal role of LC-NA in the pupil mimicry-trust linkage by manipulating participants' pupil size with two methods: transcutaneous vagus nerve stimulation (tVNS) and by global luminance manipulation (Figure 1). tVNS is a non-invasive method that has been proposed to increase NE concentrations in the brain (Follesa et al., 2007; Hassert et al., 2004; Roosevelt et al., 2006). The NE increase is believed to be a result of the anatomical connections between the vagus nerve and the LC - the noradrenergic supply center of the brain (Assenza et al., 2017;

Frangos et al., 2015; Hulsey et al., 2017; Samuels and Szabadi, 2008), which further modulates emotional and social areas in the cortex (Capone et al., 2015; Dietrich et al., 2008; Frangos and Komisaruk, 2017; Kraus et al., 2013; Yakunina et al., 2017). Apart from invasive LC recordings, pupil size is proposed to be the most reliable marker of LC-NE activity under constant luminance (Joshi, Li, Kalwani, & Gold, 2016). Due to close correlations between the activity of LC neurons and fluctuations in pupil size (Aston-Jones, et al. 2005), a consequence of stimulating this system could be an increase in pupil dilation (but see; Burger, Van der Does, Brosschot, & Verkuil, 2020; Warren et al., 2019). Moreover, the prepotent pathways between the brainstem and theory of mind (ToM) network in the cortex may become more enhanced during tVNS and boost trust when individual's and partner's pupils dilate. In contrast to pupillary responses related to NE release, a global luminance manipulation should only influence pupil size and therefore have no impact on pupil dilation mimicry.

In sum, while both luminance and tVNS manipulations are expected to **(a)** increase participants' pupil size. Due to their distinct underlying mechanisms, they should have different effects on pupil mimicry and pupil contingent trust. Specifically, in line with prior literature (Harrison, Wilson & Critchley, 2007; Kret et al., 2015; Kret & De Dreu, 2017), we expected participants to mimic their partners pupil sizes. Since pupil dilation (as a proxy of NE release) biases individuals towards their dominant predispositions (Donner and Nieuwenhuis, 2013; Eldar et al., 2013), and large pupils are perceived as more positive (Hess, 1975; Hess & Fischer, 2013; Kret et al., 2015; Kret, 2017; Kret & De Dreu, 2017), we hypothesized that if tVNS it should enhance the positive association between dilated pupils and trust. In other words, we expect that tVNS will **(b)** enhance pupil mimicry and (**c)** make subjects' pupil sizes and investments more dependent on partners' pupil size changes (compared to sham and luminance conditions). Finally, since tVNS is believed to increase NE in the brain, **(d)** tVNS should boost the pupil dilation mimicry-trust linkage by enhancing the neural connectivity between the brainstem and ToM regions. The objectives are summarized in Figure 1.

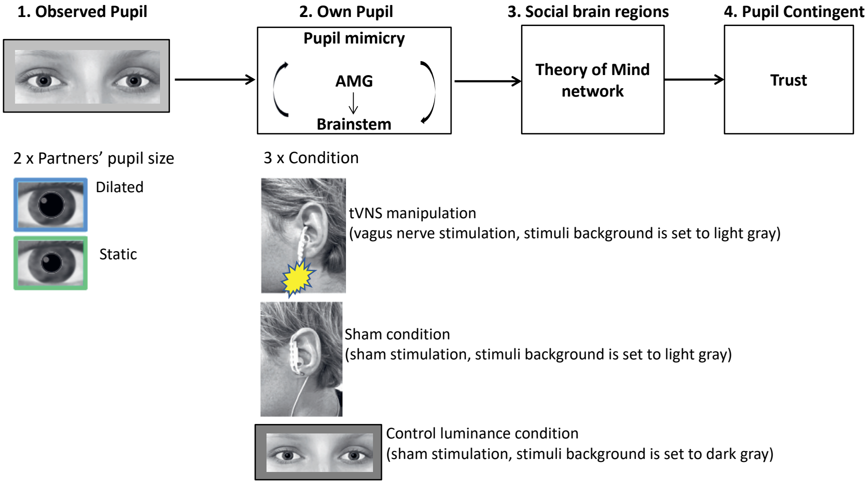

Figure 1: Theoretical model of neurological mechanisms underlying pupil mimicry-trust linkage: (1) observed pupil change (dilating vs. static partners' pupils) activate the amygdala (Amemiya and Ohtomo, 2012; K. E. Demos et al., 2008). (2) which projects to the locus coeruleus–norepinephrine (LC-NE) system that (3) modulates widespread cortical activation including neural regions involved in trust decisions. Pupil contingent trust is a result of prepotent pathways between the brainstem - theory of mind (ToM) network in the cortex, which becomes enhanced when individual's own pupil dilates. **Conditions:** Transcutaneous vagus nerve stimulation (tVNS) is a novel non-invasive brain stimulation technique. By applying an electrode to the outer ear to deliver electrical impulses to the auricular branch of the vagus nerve, the afferent fibers of Arnold's nerve are excited and the signal penetrates from peripheral nerves to the brainstem and, ultimately, to LC (Colzato et al., 2017; Frangos et al., 2015). To provide a control situation, we further included a luminance condition in which participants' pupils were manipulated to dilate as a consequence of a darker stimuli background.

Results

(a) The effect of tVNS and luminance manipulations on pupil size

Before we evaluated the effect of the tVNS and luminance (a darker stimulus background) manipulations on mimicry, we checked whether participants' non-baseline corrected pupil size increased in response to the luminance and tVNS manipulation. In the first multilevel model (see Methods) we tested the effect of condition coded as −1 (luminance), 0 (sham), 1 (tVNS) on participants' pupil size (without baseline correction). A main effect of condition was observed $F(2, 114607) = 11.070$, $p < .0001$, Table S1). Post-hoc pairwise comparisons show that participants' mean pupil size was significantly larger in the luminance condition compared to the control condition ($p < 0.0001$). However, there was no difference in participants' pupil size between the tVNS and control condition ($p > 0.05$) (Supplementary Fig. 1).

These results show that our luminance manipulation worked in that it increased participants' pupil size as we anticipated. We also expected that the tVNS manipulation would boost norepinephrine levels, and therefore, would also increase participants' pupil sizes. This did not occur, so from this analysis, we cannot infer whether tVNS had any effect on participants, a point we return to below.

(b) The effect of tVNS and luminance manipulations on pupil mimicry

In our previous work we have shown that participants' pupil sizes enlarge when looking into the eyes of another person with dilating compared to static or constricting pupils. In a second multilevel model, we investigated whether pupil mimicry (comparing partners' static and dilating pupils) is modulated by our *tVNS and* luminance manipulation. In addition to the fixed factor condition, we added partners' pupil size coded as 0 (static), and 1 (dilating) and partner pupil size × condition as predictors of participants' baseline-corrected pupil size. Results revealed evidence of pupil mimicry with an effect of partner pupil size, $F(1, 106385) = 13.483$, $p < .0001$, (Fig 3. and Table S2) and a Partner Pupil Size × Linear Trend interaction, $F(1, 106385) = 5.840$, $p < .016$), which shows that participants' pupils were largest and increased fastest over stimulus-presentation time when partners' pupils dilated as compared to remained static. We did not find significant effects of condition, or the interaction between

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condition and partner pupil size, which implies that pupil mimicry was not influenced by our tVNS or luminance manipulation.

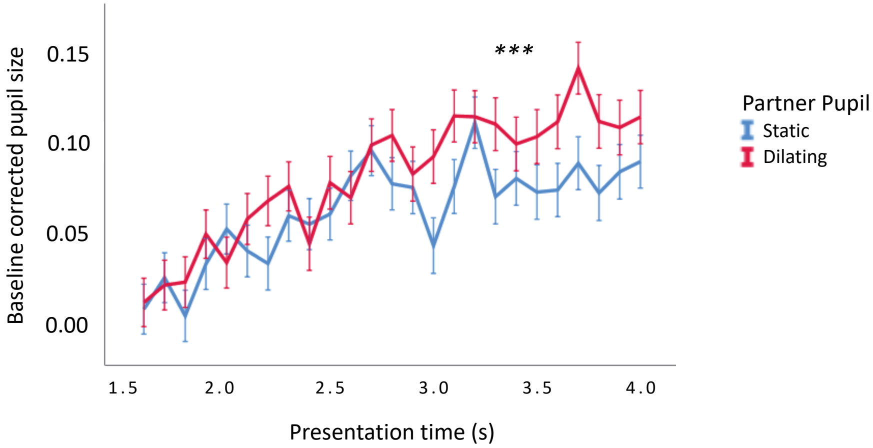

Figure 3: The effect of tVNS and background luminance manipulations on pupil mimicry. Error bar = ± 1 standard error. *** $p < 0.001$. Time (in seconds) is from 1.5 sec after stimulus onset, that is, the moment at which partners' pupils started to dilate or not, to 4 seconds, which was stimulus offset.

(c) The effect of partners' pupil size, tVNS and luminance manipulations on trust-related investments

Next, we investigated whether our two manipulations influenced participants' trust. A third multilevel model including condition, partner pupil size, and their interaction as fixed factors predicted participants' trial-by-trial investments. We found no main effects of partners' pupils on condition. However, we did find a significant interaction between partner pupil size and condition $F(1, 5280) = 3.268$, $p = .038$, (Figure 4, Table S3) revealed that participants' trust was lower when looking into partners' eyes with static pupil size, but this effect was only observed under tVNS ($p = 0.003$) and not significant in the other two conditions.

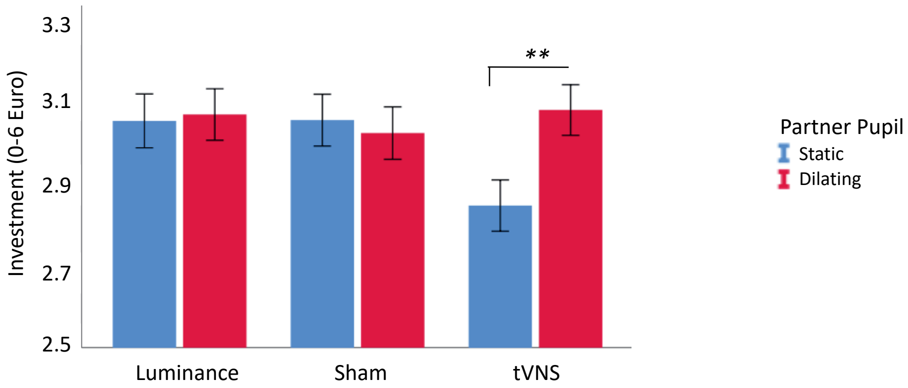

Figure 4: The effect of tVNS and luminance manipulations and partners' pupil size on trust-related investments. Error bar = ± 1 standard error. ** $p < 0.005$.

(d) Does pupil dilation mimicry modulate changes in trust

In a final analysis, we tested whether pupil dilation mimicry modulates pupil-contingent trust (see Methods for details). Contrary to our expectations and prior literature (Kret et al., 2015; Kret & De Dreu, 2017; Procházková et al., 2018; Wehebrink, Koelkebeck, Piest, de Dreu, & Kret, 2018), we did not find a main effect of pupil dilation mimicry ($p > 0.05$), or an interaction effect of condition and participant's dilation mimicry on trust ($p > 0.05$, Table S4).

Discussion

Previous research has shown that looking into the eyes of someone with large or dilating pupil sizes boosts trust, especially when observers' pupils mimic those of the observer (Kret et al., 2015; Kret & De Dreu, 2017; Procházková et al., 2018; Wehebrink, Koelkebeck, Piest, de Dreu, & Kret, 2018). In the current study, the left vagus nerve was stimulated to gain insight into the role of the LC-NA system in this pupil dilation mimicry-trust linkage. Our results are threefold. First, we observe that participants mimic partners' pupil size independent of our manipulations (luminance, sham, tVNS). Second, active as compared to sham stimulation lowered trust in partners with static compared to dilating pupils. Third, we found no evidence for a relationship between pupil dilation mimicry and pupil-contingent trust. In the following section, we discuss each result in detail in the context of the existing literature.

First, we observe that participants mimicked their partners' pupil size independent of our manipulations (luminance, sham, tVNS). Previous studies have shown that tVNS increases activity in the LC-NA system (Chen and Williams, 2012; Dorr and Debonnel, 2006; Follesa et al., 2007; Frangos et al., 2015; Groves et al., 2005; Hassert et al., 2004; Hulsey et al., 2017; Manta et al., 2013; Roosevelt et al., 2006; Vonck et al., 2014). Concerning these findings, we anticipated that tVNS would increase participants' level of arousal and as a consequence, that this would be reflected by enlarged pupil sizes. In the current study we aimed to pull apart the putative effects of *arousal* from potential effects of *luminance* on pupil contingent trust. Specifically, we anticipated that a low luminance stimulus background would increase baseline pupil size but have no influence on pupil mimicry. Pupil dilation mimicry, on the other hand, has been predicted to increase with levels of neural arousal (gain) induced by tVNS activity. In contrast to this hypothesis, participants' pupil sizes did not differ between the tVNS and the sham condition. In the luminance condition where pupil size was successfully manipulated and increased as a result of a darker stimulus background, pupil mimicry was unaffected, contrary to our predictions. This shows that manipulating participants' pupil size, at least to the extent we did (an increase of 19%), does not modulate pupil mimicry. The null effect of tVNS on pupil size/pupil mimicry contradicts prior studies that imply that vagus nerve stimulation (iVNS) increases norepinephrine (NE) via the locus coeruleus (LC) activation (Chen and Williams, 2012; Dorr and Debonnel, 2006; Follesa et al., 2007; Groves et al., 2005; Hassert et al., 2004; Hulsey et al., 2017; Manta et al., 2013; Roosevelt et al., 2006; Vonck et al., 2014). Instead, this result aligns with previous research that also found no impact of tVNS on pupil size (Burger et al., 2020; Keute et al., 2019; Warren et al., 2019). In addition, here we demonstrate that pupil mimicry is a resilient effect that is not easily disrupted by mechanistic manipulation.

Our second key finding shows that tVNS lowered participants' trust in partners with static pupil sizes**.** This result confirms the third hypothesis suggesting that tVNS will make subjects' investments more dependent on partners' pupil size changes. Previous neuroimaging work has shown that activity in the LC-NA system modulates activity in social and emotional areas in the cortex (Dietrich et al., 2008; Kraus et al., 2013; Yakunina et al., 2017). Accordingly, our recent fMRI research revealed an association between these areas and pupil dilation mimicry (Procházková et al., 2018), which fosters the view that phasic pupillary responses (reflective of NE increase) upregulate neocortical networks involved in trust. Based on these findings, we predicted that tVNS would enhance communication between the brainstem and the ToM network and modulate the positive association between large pupils and trust. In support of this theory, we find that tVNS reduced trust in partners with static pupils compared to dilating pupils and therefore strengthen the association between average-sized pupils and lower levels of trust. Yet, tVNS did not boost trust in partners with dilating pupils. An explanation for why tVNS lowered participants' trust to partners' static pupils instead of increasing trust to dilating pupils is highly speculative. A plausible explanation is that tVNS influences human behavior via an alternative neurochemical pathway. Converging evidence from animal and clinical studies imply that apart from NE, tVNS increases levels of GABA (Ben-Menachem et al., 1995) and acetylcholine (ACh) involved in inhibitory and parasympathetic processes (Borovikova et al., 2000). GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the adult vertebrate brain that plays a key role in the neuromodulation of response selection (Bar-Gad et al., 2003) and cortical inhibition in healthy adults (Capone et al., 2015). Moreover, tVNS has been shown to reduce sympathetic activity and produce a shift toward parasympathetic functions (e.g., slowing heart rate; Clancy et al., 2014). It is, therefore, possible that tVNS modulates social cognition via activation of parasympathetic processes instead of noradrenergic/sympathetic responses. In support of this interpretation, it has been shown that parasympathetic activity measured by heart rate variability predicts individuals' ability to read others' emotions from their eyes (Quintana et al., 2012). Thus, a possible explanation is that tVNS may inhibit trust in partners with static and average-sized pupils (slightly aversive stimuli, especially in the context of other partners with dilating pupils) compared to dilating pupils (positive stimuli) via activation of the GABAergic system. Such an interpretation would also account for the lack of tVNS effects on pupil size.

Another finding that merits interpretation is why partners' pupils did not influence trust during sham and luminance conditions. Previous research has shown that looking into the eyes of someone with large or dilating pupil sizes boosts trust (Kret et al., 2015; Kret & De Dreu, 2017; Procházková et al., 2018; van Breen et al., 2018; Wehebrink et al., 2018). Yet here we only found such pupil contingent trust effects when the participant's vagus nerve was stimulated. The lack of a main effect is not in complete contradiction to previous research. In some of our earlier studies, partners' dilating pupils only yielded a small increase in trust and had smaller effects than constricting pupils (the latter of which were not included in the current study, which could have dampened the effect of dilating pupils as the contrast with static pupils is smaller; e.g. Kret et al., 2015; Kret & De Dreu, 2017). An alternative explanation is tied to our most recent study (Procházková et al, in prep), which shows that perceived pupil size impacts trust mainly subconsciously, possibly through a direct subcortical pathway (Tamietto and De Gelder, 2010). Since tVNS targets subcortical structures (Frangos et al., 2015; Frangos and Komisaruk, 2017; Roosevelt et al., 2006), this result may also offer a potential insight into why tVNS induced pupil contingent trust and control conditions did not. Nevertheless, more research is needed to validate these interpretations.

Finally, we did not find the pupil mimicry-trust linkage in any of our conditions (tVNS, sham, or luminance). Multiple previous studies have consistently shown that pupil mimicry modulated trust decisions (Kret et al., 2015; Kret & De Dreu, 2017; Procházková et al., 2018; Wehebrink, Koelkebeck, Piest, de Dreu, & Kret, 2018). Several methodological differences may provide an explanation as to why we do not find this effect in the current study. The most obvious difference between the current study and prior research is that we used an invasive intervention. Even though tVNS is not painful, it can cause considerable discomfort, also in the sham condition. Considering that the effects of pupil mimicry on trust are very subtle (Kret et al., 2015; Kret & De Dreu, 2017; Procházková et al., 2018; Wehebrink, Koelkebeck, Piest, de Dreu, & Kret, 2018), it is therefore possible that our manipulations influenced participants mood. Such an intrusion might be particularly detrimental to the pupil mimicry-trust link, which relies on participants' ability to attune to subtle internal signals. This effect could spill over to the luminance condition, which followed one of the two types of stimulations (active/sham). We therefore recommend that future studies interested in the effects of pupil mimicry in human behavior refrain from invasive manipulations. Another difference between the current and prior research is that the main focus here was on sympathetic influences of partners' pupils on trust. We therefore omitted pupil constriction from the research design entirely. In consequence, we were not able to assess the effect of partners' constricting pupil mimicry on trust. Thus, we propose that future studies include three levels of pupillary changes (dilating, static and constricting) as these methodological differences may explain the lack of the pupil mimicry influence on trust found in the current study.

In sum, this experiment yielded three main outcomes. First, we demonstrate that pupil mimicry is a robust phenomenon resilient to manipulation. Second, active as compared to sham stimulation lowered trust in partners with static compared to dilating pupils. Third, tVNS did not affect participants' pupil size, nor the expected pupil mimicry contingent trust relationship. Together these results support the theory that the vagal nerve plays a causal role in the recognition of social signals. However, as we did not observe any effect of tVNS on pupil mimicry or other pupillary responses, we conclude that the behavioral effect induced by tVNS cannot be simply ascribed to activation of the LC-NA system. Instead, we propose that tVNS affects pupil contingent trust via alternative neurological pathway.

Methods

To evaluate the effect of partners' pupils on participants' pupils and trust, we used the same stimuli and trust games as in the Kret's, Fischer's and De Dreu (2015) previous research. Participants' task performance (trusting behavior) and eye-tracking data were measured in two sessions during which subjects played trust games with virtual partners whose pupils changed in size. Each participant played three rounds of the game under three different experimental conditions. Once during active tVNS stimulation where a constant current of 0.5mA was delivered to the vagus nerve, once with the same current intensity and duration, but during earlobe sham stimulation (Figure 1; Control condition). Once without simulation but under global luminance manipulation (Figure 1; Global luminance manipulation). The tVNS and sham conditions were counterbalanced across sessions. Unlike in previous studies (Kret et al., 2015; Kret & De Dreu, 2017; van Breen, De Dreu, & Kret, 2018; Wehebrink et al., 2018), the main focus of this research was on the perception of partners' pupil dilation and not constriction. This is because pupil dilation has been linked to LC activity and sympathetic nervous system activity, but not pupil constriction.

Participants

Fifty-one participants were recruited at Leiden University (age: 18 – 25 (M = 21.16, SD = 1.67) who had no history of neurological or psychiatric disorders. The study consisted of 2 experimental sessions approximately four weeks apart. For the majority of participants (85.7 percent) both sessions were scheduled at the same time of the day or within two hours, reducing potential effects of diurnal cycle fluctuations of norepinephrine (Bleske et al., 1999). One participant failed to show up for the second session for undisclosed reasons and was subsequently excluded from all analyses. To ensure enough observations of sufficient quality, the data of the remaining 50 subjects (25 females) were analyzed. This sample size was based on earlier work investigating pupil mimicry (which included between 40 - 69 participants; e.g., Harrison, Singer, Rotshtein, Dolan, & Critchley, 2006; Kret et al., 2015). In eight runs (of seven participants) data were missing for more than 50% of valid pupil trials, following standard procedures we excluded those subjects' runs from models assessing subjects' pupil analyses (*n* = 42) (e.g. Kret et al., 2015). The participants filled out the Informed Consent form prior to the start of the first session and were debriefed following the second session. Participants were informed that they were free to stop their participation at any moment. No participant quit the study during an experimental session. The experimental procedures were in accordance with the Declaration of Helsinki and approved by the Psychology Research Ethical Committee (PREC15-1113/64) of Leiden University.

Stimuli

To create virtual partners in the trust game, we used the same stimuli as in previous research by Kret, Fischer and De Dreu (2015). In total, 18 pictures of eyes (9 females, 9 males) of Western European descent were selected. Pupil dilation was created by increasing the pupil diameter of a static image using Adobe After Effects. In the pupil dilation condition, pupil size was 5 mm for 1.5 seconds and then gradually started to increase to 7 mm over the course of 1.5 seconds after which it remained at its maximum size (7mm) for another second. In the static pupil size condition, pupil size remained 5 mm over the course of 4 seconds of stimulus presentation time (Kret et al., 2015; Kret & De Dreu, 2017; Procházková et al., 2018; van Breen et al., 2018; Wehebrink et al., 2018). The current study deviates from our previous work in that we did not include a pupil constriction condition.

Trust game

In the current study, participants played a series of Trust Games with virtual partners where they had the role of the trustor (see Figure 2). The trustor is endowed with a certain amount of money which can be shared with the virtual trustee. Participants were instructed to decide what share of their six euros they wished to invest after seeing the other player's eyes. The investment was then multiplied by a factor three and the trustee could return a portion of the money. The participants were told that they would be partnered with different student players whose decisions on reciprocation were recorded in previous sessions (which was the truth). The choices were made using a button box with four buttons (€0, €2, €4, €6). Participants were told that they would not receive any immediate feedback regarding partners' decisions. In total, the game consisted of 18 trials per condition and partner pupil size (3 x Condition, 2 x Pupil size, Total = 118 trials).

Procedure

Before the experiment, participants were instructed not to drink coffee or other caffeinated beverages and be well-rested. On arrival to the laboratory, the tVNS stimulator, heart rate electrodes, and Electromyography (EMG) sensors to measure facial muscle activity were attached to collect physiological data. These physiological measures were collected for purposes of a different question, which is outside the scope of the current paper. At this point, luminance in the room was reduced to a constant minimal level for the remainder of the procedure. Participants were then given questionnaires regarding their demographics, after which they played the Trust Game (Figure 1, for details). Finally, participants were given the Reading the Mind in the Eyes task (Baron-Cohen et al., 2001), lasting around four minutes. This task was not related to the current research question and thus was not analyzed for the purposes of this study. The second session followed the same sequence only with another Trust game played at the end of the session, this time with changing background luminance to manipulate pupil dilation. In total, experimental sessions lasted 40 – 50 minutes. Throughout the game, participants chin was placed on a chinrest. The screen was at 50 cm distance from the face.

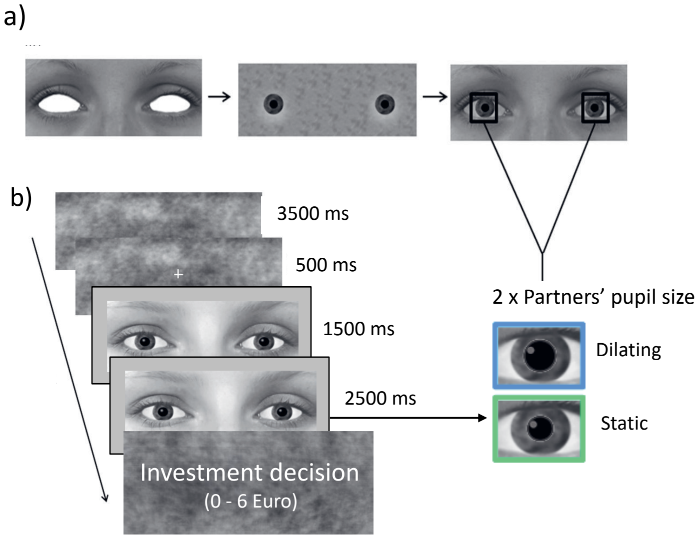

Figure 2: (*a*) To create partner stimuli, we removed the eyes from pictures of the eye regions of faces and then added the same eye white, iris, and pupil to each stimulus. (b) Each trial consisted of the following sequence: (1) a grey screen with scrambled image appears for 3500 milliseconds, (2) a fixation cross appears in the middle of the grey screen for 500 milliseconds, (3) a person's eye area appears on the screen for 4000 milliseconds with a static image for 1500 milliseconds, (4) static or dilating pupils presented for 2500 milliseconds, (5) a screen with four options of the amount to be shared appears.

tVNS

In the experimental condition, the neurostimulation device (CM02, Cerbomed, Erlangen, Germany) was attached to the Cymba conchae part of the left outer ear, under the Inferior crus of antihelix (see Figure 1), an area that has been found to be consistently innervated by the auricle branch of the vagus nerve (ABVN); (Frangos et al., 2015). In the sham condition the tVNS stimulator was attached to the lobule of the auricle of the left ear, which is not innervated by the ABVN (Peuker and Filler, 2002) and has been used in previous research on the effects of tVNS as sham condition (Colzato et al., 2017; Frangos et al., 2015). tVNS has previously been found to be a safe procedure with no known side effects (Kreuzer et al., 2012). tVNS stimulation was intermittent, with 30 seconds of active stimulation followed by 30 seconds break.

Pupil pre-processing

Participants' pupil size was continuously collected with Tobii T120 eye tracker. We interpolated gaps smaller than 250 ms. Trials were excluded if more than 50% of the data within that trial were missing (e.g., because the eye tracker lost the pupil). We also excluded participants that had more than 50% of their pupil data missing per session. We smoothed the data with a 10th-order low-pass Butterworth filter. Preprocessing of pupil size data was conducted using a customized open-source MATLAB script (Kret & Sjak-Shie, 2019). The average pupil size 500 ms before the start of the changes in a partner's pupils (computed per participant and trial) served as a baseline (i.e., 1,000–1,500 ms after stimulus onset) and was subtracted from each sample during the remaining stimulus presentation (1,500–4,000 ms).

Statistical analysis

To investigate whether tVNS and luminance manipulations influenced participants' pupil size, data were analyzed with multilevel models. In the first model, we tested the effect of condition coded as –1 (luminance), 0 (sham), 1 (tVNS) on participants' pupil size (without baseline correction). The multilevel structure was defined by the repeated measures, that is, time (Level 1) nested in trials (Level 2) nested in participants (Level 3). Time (twenty-five 100-ms slots) was included as a repeated factor with a first-order autoregressive covariance structure to control for autocorrelation. Also, we included linear, quadratic, and cubic terms and their interactions with the previously mentioned factors to model the curvilinear relationship between participants' pupil size and time.

According to Aston-Jones and Cohen (2005) and others (Keute et al., 2019) there are two functionally distinct modes of LC activity: tonic activity, leading to a global increase in NE transmission, and phasic activity, leading to an upregulation of NE transmission in response to environmental requirements. While pupil size without baseline correction between individuals can be used to monitor tonic changes of neural gain in individuals, phasic pupil size normalized to baseline are better suited for between-subject comparisons (Eldar et al., 2013). In the second model, we used the same multilevel structure as in the previous model. Condition coded as −1 (luminance), 0 (sham), 1 (tVNS), partner pupil size coded as 0 (static), and 1 (dilating) and partner pupil size × condition were added as predictors of participants' baseline-corrected pupil size. In addition, we included linear, quadratic, and cubic terms and their interactions with the previously mentioned factors to model the curvilinear relationship between participants' pupil size and time.

In the third multilevel model, we predicted participants' trust-related investments. In this model, we included a 2 level structure where different trials (Level 1) were nested within participants (Level 2). Participants' investment decisions (per trial) were used as the target variable and condition, partner pupil size and partner pupil size × condition were used as predictors. Furthermore, since some faces may be perceived as more/less trustworthy, we also included a random effect of stimulus face (9 pictures for males, 9 pictures for females) in the model.

In the final analysis, we tested whether pupil dilation mimicry modulates pupil-contingent trust. We included a 2-level structure where different face stimuli (Level 1) were nested in participants (Level 2). We computed a dilation-mimicry score (per stimulus face: participant's pupil size when partner's pupils dilated minus when partner's pupils were static) and partner-pupil contingent trust (investments in partners with dilating pupils minus investments in partners with static pupils). The multilevel model included the factors condition, dilation mimicry, and condition × dilation mimicry. The dependent variable was partner-pupil contingent trust. Models were implemented in SPSS Version 20. In the Supplemental Material available online, the full model of pupil mimicry in Tables S1, investments are shown in Table S2, and of the link between these two in Table S3.