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# The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans

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ABSTRACT

A critical component of the treatment for anxiety disorders is the extinction of fear via repeated exposure to the feared stimulus. This process is strongly dependent on successful memory formation and consolidation. Stimulation of the vagus nerve enhances memory formation in both animals and humans. The objective of this study was to assess whether transcutaneous stimulation of the vagus nerve (tVNS) can accelerate extinction memory formation and retention in fear conditioned humans. To assess fear conditioning and subsequent fear extinction, we assessed US expectancy ratings, fear potentiated startle responses and phasic heart rate responses. We conducted a randomized controlled trial in thirty-one healthy participants. After fear conditioning participants were randomly assigned to receive tVNS or sham stimulation during the extinction phase. Retention of extinction memory was tested 24 h later. tVNS accelerated explicit fear extinction learning (US expectancy ratings), but did not lead to better retention of extinction memory 24 h later. We did not find a differential physiological conditioning response during the acquisition of fear and thus were unable to assess potential effects of tVNS on the extinction of physiological indices of fear. These findings complement recent studies that suggest vagus nerve stimulation could be a promising tool to improve memory consolidation and fear extinction.


## 1. Introduction

Anxiety disorders are among the most prevalent mental disorders, with a point prevalence of 7.3% and a lifetime prevalence as high as 28.8% (Baxter, Scott, Vos, & Whiteford, 2013; Kessler et al., 2009). A critical component of the treatment of anxiety disorders is the extinction of fear via repeated exposure to the feared stimulus. Although repeated exposure combined with cognitive therapy is the treatment of choice, roughly 22% of patients do not respond to this type of treatment (Stewart & Chambless, 2009). This may be due to the fact that patients with anxiety disorders have more difficulties forming extinction memories (Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Duits et al., 2015; Lissek et al., 2005; Orr et al., 2000). Understanding the neurobiological mechanisms by which full extinction of fear is achieved may improve currently available extinction-based treatments for anxiety disorders, as shown by existing augmentation strategies of exposure therapy using for example MDMA or D-cycloserine (Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015).

Successful extinction of conditioned behavior is strongly dependent on successful memory formation and consolidation. During extinction, a new memory is formed wherein the conditioned stimulus is re-appraised as safe. Critically, fear extinction is not a process of unlearning the conditioned memory or behavior. Instead, a new memory (so called extinction memory) has to be created and consolidated to compete with the conditioned fear memory and reduce conditioned responding (Hermans, Craske, Mineka, & Lovibond, 2006). Patients suffering from anxiety disorders create strong fear memories, and therefore have more difficulties creating and consolidating extinction memories that can contest these fear memories (Lissek et al., 2005).

Most neurobiological studies have focused on the role of the central nervous system in fear extinction and show that increasing central norepinephrine through the use of norepinephrine agonists improves extinction memory (e.g. Berlau & McGaugh, 2006; Lissek,

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Glaubitz, Güntürkün, & Tegenthoff, 2015). In contrast, relatively little is known about the role of the peripheral nervous system. Yet, several studies suggest a critical function of the vagus nerve in memory formation and consolidation (Clark, Smith, Hassert, & Browning, 1998; Hassert, Miyashita, & Williams, 2004; McIntyre, McGaugh, & Williams, 2012). Memory consolidation is often facilitated in arousing circumstances, when excitatory effects of peripheral epinephrine on the vagus nerve lead to the release of norepinephrine in limbic brain structures (for a review, see Grimonprez, Raedt, Baeken, Boon, & Vonck, 2015). Direct stimulation of the vagus nerve during extinction learning may also increase the release of norepinephrine in these learning-relevant brain structures (i.e. hippocampus, amygdala, prefrontal cortex), thereby strengthening the consolidation of extinction memory (Dorr & Debonnel, 2006; Groves & Brown, 2005).

Manipulating vagus nerve activity indeed affects the rate of fear extinction in rats. For instance, cutting the afferent (but not efferent) vagal nerve fibers attenuated extinction learning (Klarer et al., 2014), whereas stimulating the vagus nerve accelerated extinction learning (Peña, Engineer, & McIntyre, 2013; Peña et al., 2014). In humans, chronically low vagal tone may be a risk factor for the onset and maintenance of emotional disorders (Chalmers, Quintana, Abbott, & Kemp, 2014; Thayer & Lane, 2000, 2009; Verkuil, Brosschot, Gebhardt, & Thayer, 2010). Yet, the effects of vagus nerve stimulation on extinction learning have not yet been studied in humans, although positive effects have been found on cognition and memory (Vonck et al., 2014). Furthermore, surgically implanted VN stimulators have been approved by the FDA for treatment-resistant depression since 2005 and are also being investigated for treatment-resistant anxiety disorders (George et al., 2008; Nemeroff et al., 2006). Still, the mechanism of vagus nerve stimulation therapy is not well understood (Grimonprez et al., 2015; Nemeroff et al., 2006).

Using VNS to attenuate fear responses in humans has been relatively understudied because until recently it required surgical implantation of a neurostimulator. However, recent technological developments allow transcutaneous stimulation of the vagus nerve (tVNS) via a vagally innervated part of the outer ear (i.e., the concha; Peuker & Filler, 2002). tVNS has been shown to be a safe method to stimulate this auricular branch of the vagus nerve (Kreuzer et al., 2012). Short periods of tVNS immediately modulate the activation of brain areas related to extinction learning (eg. the hippocampus, amygdala and prefrontal cortex (Frangos, Ellrich, & Komisaruk, 2014; Kraus et al., 2007), and increase performance in memory tasks and other cognitive tasks that are dependent on norepinephrine activity (Jacobs, Riphagen, Razat, Wiese, & Sack, 2015; Sellaro et al., 2015). tVNS is therefore suited to examine the role of the vagus nerve in extinction learning in humans.

The aim of the present study was to test the effects of tVNS on fear-extinction rate in previously fear-conditioned healthy participants. We conducted a randomized controlled trial comparing tVNS versus sham stimulation. Fear learning was operationalized in multiple ways, consistent with the idea that memory formation occurs in different memory systems (Hartley & Phelps, 2010; Soeter & Kindt, 2010). At the explicit level we measured US expectancy ratings, which may be largely dependent on hippocampal activation (Squire, Stark, & Clark, 2004). At the physiological level, we examined the startle blink response and heart rate acceleration, that are not only dependent on the hippocampus, but also on amygdala and prefrontal activation (Marek, Strobel, Bredy, & Sah, 2013). We hypothesized that tVNS would have an effect on both explicit and implicit indices of extinction learning. We also explored whether any effects of tVNS would be maintained the following day by testing the retention and reinstatement of fear and extinction memory.

## 2. Methods

### 2.1. Participants

Thirty-eight participants were recruited from the Leiden University student population (for a breakdown of demographics, see Table 1). Eligible participants were healthy college students between the ages 18 and 25. Participants with epilepsy, bradycardia, cardiac arrhythmia, cardiac diseases, significant head trauma, pregnancy, drug use, neurological or psychiatric disorders were excluded from participating in this study. Participants received either course credits or 12 euro as compensation for participating in the study. The study was approved by the Institutional Ethical Board of Leiden University, Institute of Psychology (CEP #9394209653). All participants gave their written informed consent prior to the start of the experiment.

### 2.2. Stimuli and apparatus

#### 2.2.1. Stimuli

Two geometrical shapes (one blue triangle, one blue square) served as conditioned stimuli (CS; Vervliet, Vansteenwegen, Baeyens, Hermans, & Eelen, 2005). The slides were 400 mm high and 400 mm wide and were presented on a 17-in. CRT monitor in the middle of the screen on a gray background. Conditioned stimuli were assigned as CS+ and CS− in a counterbalanced order. Both CS+ and CS− were presented for 8 s. During the acquisition phase, the CS+ co-terminated with the US in 75% of the trials. The CS− never co-terminated with the US. The US was a 95 dB loud scream presented for 2000 ms, 6 s after CS onset (see Fig. 1). The scream that was used as US was a shorter version of the IADS sound number 275 (Bradley & Lang, 1999; Van Diest, Bradley, Guerra, Van den Bergh, & Lang, 2009). Additionally, a 50 ms, 100 dB burst of white noise was administered to both ears via headphones, 5 s after the onset of every CS presentation and every intertrial interval (ITI). During the ITI, participants were presented with a blank screen. The ITI duration varied randomly between 15 and 25 s.

#### 2.2.2. tVNS and sham stimulation

Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive method of electrically stimulating the afferent auricular branch of the vagus nerve located at the cymba conchae (Kreuzer et al., 2012).

In this study, we used a tVNS instrument that provides electrical stimulation using two titanium electrodes, positioned on top of a silicon earplug, which are connected by a wire to a portable neurostimulator (Nemos®, Cerbomed, Erlangen, Germany). The electrodes deliver 30-s waves of electrical stimulation (0.5 mA,

**Table 1**
Descriptive statistics.

| | tVNS *M* (*SD*) | Sham *M* (*SD*) | *p* |
|---|---|---|---|
| RMSSD | 44.31 (22.57) | 46.52 (41.48) | .88 |
| HR | 76.74 (15.62) | 74.61 (13.23) | .71 |
| Age | 20.72 (1.74) | 22.08 (2.32) | .07 |
| US unpleasantness rating | 68.83 (21.96) | 66.69 (28.59) | .82 |
| PSWQ | 40.06 (8.49) | 43.62 (9.97) | .31 |
| STAI state | 39.94 (6.46) | 38.62 (6.87) | .60 |
| STAI trait | 36.81 (7.41) | 37.23 (7.96) | .89 |
| Positive affect | 61.44 (12.78) | 60.76 (10.76) | .87 |
| Negative affect | 26.85 (14.73) | 24.26 (13.47) | .60 |

*Note*: RMSSD = root mean square of the successive differences between heart rates, HR = heart rate, US = unconditioned stimulus, PSWQ: Penn State Worry Questionnaire, STAI: State Trait Anxiety Inventory.