Transcutaneous Vagus Nerve Stimulation Induced Parasympathetic Activation modulates experimental pain as assessed with the nociceptive withdrawal reflex

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Abstract

The aim of this study was to clarify the effects of transcutaneous vagus nerve stimulation (tVNS) to the left cymba concha on the pain perception using nociceptive withdrawal reflex (NWR), which is known to be associated with chronic pain, and to investigate whether tVNS-induced suppression of the NWR and parasympathetic activation is correlated. We applied either 3.0 mA, 100 Hz tVNS for 120 s in the left cymba concha (tVNS condition) or the left earlobe (Sham condition) for twenty healthy adults. NWR threshold was measured before (Baseline), immediately after (Post 0), 10 min (Post 10) and 30 min after (Post 30) stimulation. The NWR threshold was obtained from biceps femoris muscle by applying electrical stimulation to the sural nerve. During tVNS, electrocardiograph was recorded, and changes in autonomic nervous activity were analyzed. We found that the NWR thresholds at Post 10 and Post 30 increased compared to baseline in the tVNS group (10 min after: $p = 0.048$ 30 min after: $p = 0.037$). In addition, increased parasympathetic activity by tVNS correlated with a greater increase in NWR threshold at Post 10 and Post 30 (Post 10: $p = 0.01$; Post 30: $p = 0.005$). The present results demonstrate the pain-suppressing effect of tVNS as assessed with NWR threshold and suggest that the degree of parasympathetic activation during tVNS may predict the effect of tVNS after its application.

(1)Title

Transcutaneous Vagus Nerve Stimulation Induced Parasympathetic Activation modulates experimental pain as assessed with the nociceptive withdrawal reflex11Table of Abbreviations HRV Heart rate variability LC Locus coeruleus NWR Nociceptive Withdrawal reflex NTS Nucleus tractus solitarius NWR Nociceptive withdrawal reflex

(2)Author's name

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Keywords: Transcutaneous vagus nerve stimulation, vagus afferent, nociceptive withdraw reflex, heart rate variability, LF/HF

Abstract

The aim of this study was to clarify the effects of transcutaneous vagus nerve stimulation (tVNS) to the left cymba concha on the pain perception using nociceptive withdrawal reflex (NWR), which is known to be associated with chronic pain, and to investigate whether tVNS-induced suppression of the NWR and parasympathetic activation is correlated. We applied either 3.0 mA, 100 Hz tVNS for 120 s in the left cymba concha (tVNS condition) or the left earlobe (Sham condition) for twenty healthy adults. NWR threshold was measured before (Baseline), immediately after (Post 0), 10 min (Post 10) and 30 min after (Post 30) stimulation. The NWR threshold was obtained from biceps femoris muscle by applying electrical stimulation to the sural nerve. During tVNS, electrocardiograph was recorded, and changes in autonomic nervous activity were analyzed. We found that the NWR thresholds at Post 10 and Post 30 increased compared to baseline in the tVNS group (10 min after: $p = 0.048$ 30 min after: $p = 0.037$). In addition, increased parasympathetic activity by tVNS correlated with a greater increase in NWR threshold at Post 10 and Post 30 (Post 10: $p = 0.01$; Post 30: $p = 0.005$). The present results demonstrate the pain-suppressing effect of tVNS and suggest that the degree of parasympathetic activation during tVNS may predict the effect of tVNS after its application.

Introduction

Chronic pain is defined as persisting pain that lasts beyond the usual recovery period, which usually lasts 12 weeks. Associated with physical disability, anxiety, depression, and sleep disorders, it has a significant social impact, including decreased quality of life and high medical costs (Geneen *et al.* , 2017). Autonomic nervous system dysfunction which is caused by persistent psychosomatic stress is commonly associated with chronic pain (Hallman *et al.* , 2011). In fact, various reports have drawn associations between chronic pain and autonomic nervous system activity. For instance, sympathetic overexcitation in response to sudden psychosomatic stress or pain induces skeletal muscle tension, which leads to chronic pain; moreover, chronic pain sufferers show increased excessive sympathetic activity in response to sustained contraction tasks and cold stimuli (Passatore & Roatta, 2006).

Transcutaneous vagus nerve stimulation (tVNS) has been widely used to externally modulate autonomic nerve activity and reported to decrease heart rate and increase parasympathetic nerve activity (Clancy *et al.* , 2014; Antonino *et al.* , 2017; De Couck *et al.* , 2017; Badran *et al.* , 2018b). Our previous study revealed that this stimulation increased parasympathetic activity in subjects with high pre stimulus sympathetic activity and that stimulus current intensity and sex differences affected parasympathetic activity induction (Yokota*et al.* , 2022). The background mechanism involves afferent fibers, which account for 80–90% of all vagus nerve fibers, transmitting sensory information from organs in the thoracic and abdominal cavities to the brain. This activity is regulated by the autonomic nervous system, causing changes in efferent control via interneurons in the spinal cord and higher centers (Buschman *et al.* , 2006). The information from afferent fibers inputs to the nucleus tractus solitarius (NTS) in the medulla, then projecting via the locus coeruleus (LC) to various cortical areas such as the sensorimotor cortex, anterior cingulate cortex, and insula (Hachem *et al.* , 2018). Functional magnetic resonance imaging (fMRI) studies have shown that tVNS stimulation of the tragus and cymba conchae increases activity in the NTS and LC as well as the projection areas in the cortex (Frangos*et al.* , 2015; Badran *et al.* , 2018a). As therapy, its efficacy has been reported for a variety of conditions, including refractory epilepsy and drug-resistant depression (Bauer *et al.* , 2016; von Wrede & Surges, 2021), and it shows promise as a treatment against acute and chronic pain (Janner *et al.* , 2018).

In a previous study examining the effect of tVNS at the cervical branch of the vagus nerve on the nociceptive withdrawal reflex (NWR) threshold of the lower extremity, the NWR threshold increased 5 and 30 min after tVNS (De Icco *et al.* , 2018). The NWR is a multi-synaptic spinal reflex (flight reflex) that occurs under strong stimulation of the skin, muscles, or joints of an extremity; it transmits nociceptive information from sensory receptors to interneurons in the spinal cord, causing the flexor muscles on the same side as the

stimulation site to contract and the extensors to relax to escape from the stimulation. The NWR threshold, an objective measure of pain perception, is widely used to evaluate the efficacy of analgesics and to elucidate pain mechanisms (Neziri *et al.* , 2010) and has been associated with chronic pain (Biurrun Manresa *et al.* , 2011; Amiri *et al.* , 2021). However, as stimulation of the cervical branch of the vagus nerve also provides input to the trigeminal nerve, it is unclear whether stimulation confined to the vagus nerve would have a similar effect. Applying tVNS to the cervical branch of the vagus nerve of rats with trigeminal allodynia suppressed increased neurotransmitter secretion associated with trigeminal pain (Oshinsky *et al.* , 2014), suggesting that tVNS to the cervical branch produces combined pain suppression through both the vagus and trigeminal nerves. On the other hand, the cymba conchae, the auricular branches of the vagus nerve, are anatomically innervated only by the afferent fibers of the vagus nerve (Peuker & Filler, 2002), making them ideal for testing responses to vagus nerve stimulation. In addition, the relationship between tVNS-induced pain relief and autonomic nervous activity modulation has not been investigated so far and the underlying mechanism remains unknown.

Therefore, this study aimed to examine the effect of applying tVNS to the cymba concha on NWR thresholds in the lower extremity and to determine their relationship with changes in autonomic nervous activity.

Materials and methods

Participants

Twenty healthy adults (nine women, mean age 21 ± 0.63 years) with no history or family history of epileptic seizures, no neurological, psychiatric, or cardiac diseases, and no history of external ear trauma participated in this study. Additional inclusion criteria were as follows: absence of metal implants in the body, pregnancy, alcohol or illicit drug dependence, and daily medication. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Niigata University of Health and Welfare; all subjects were received sufficient explanations on the procedures and aims of the research and provided written informed consent before participation.

Transcutaneous vagus nerve stimulation

A battery driven stimulation device (NEMOS, Cerbmed, Germany) was used for tVNS. After wiping with an alcohol-soaked cotton, two hemispheric titanium stimulating electrodes were fitted to the left cymba concha. In addition, saline-soaked sponges were attached to the electrodes to ensure optimal conductivity. The cymba conchae were used as a stimulation site , the earlobe, reported to have minimal vagal innervation (Peuker *et al* ., 2002), was used as stimulation site in Sham condition. In this condition, in case of insufficient electrode fixation, the electrode was fixed to the skin with surgical tape (Janner*et al.* , 2018) (Fig. 1).

Nociceptive withdrawal reflex threshold

The NWR for the left lower extremity was used to assess the pain threshold. The short head of left biceps femoris muscle was wiped with an alcohol-soaked cotton and an Ag/AgCl surface electrode was placed on the middle of the muscle at an interelectrode distance of 2 cm. The skin over the sural nerve caudal to the lateral malleolus, on which a stimulating electrode was placed, was wiped with an alcohol-soaked cotton beforehand. The ground electrode was affixed by wrapping it around the ipsilateral proximal left lower leg. Five consecutive 1 ms short waves at 200 Hz were randomly applied to the sural nerve at 8–12 s intervals using an electrical stimulator (SEN-7203, Nihon Koden, Japan), so avoid that the subject became aware of the time of stimulus application; the muscle activity derived from the knee flexion withdrawing from the electrical stimulation was recorded with an electromyograph (Synergy, Medelec, UK). First, the sensory threshold to electrical stimulation of the sural nerve was measured; then, the stimulation increased in steps of 0.5 mA. The NWR threshold was the point at which a reflex amplitude >20 μV and [?]10 ms was recorded in the 70–200 ms post stimulation time window [?]3 subsequent times after applying five consecutive 1 ms short 200 Hz waves to the sural nerve. The EMG signals were amplified by an amplifier (A-DL-720140,4 ASSIST), converted to A/D, and stored on a personal computer using Power lab (AD Instruments, Colorado Springs, CO, USA) for offline analysis with a sampling frequency of 1 kHz and a bandpass filter from 3 to

3000 Hz (Rhudy & France, 2007; Jensen *et al.* , 2015). To prevent movement of the stimulation site during measurements, the stimulating electrode was placed in close contact with the sural nerve and fixed with surgical tape during sensory threshold detection.

Experimental procedures

The participants were told to avoid alcohol and caffeine consumption 12 h to have breakfast [?]2 h before the experiment. Each participant entered the laboratory after emptying his/her bladder and laid on his/her stomach for attachment of the Ag/AgCL electrodes on the short head of the left biceps femoris muscle. All experiments were conducted between 9:00 a.m. and 12:00 p.m. in a quiet, dimly lit room with a room temperature of 22–24. The participant was seated in a resting position on a reclining chair with armrests, an ECG electrode and the stimulating electrode of an electrical stimulator were attached, and he/she was instructed to remain seated for 20 mins to reduce the autonomic nervous activity. The tVNS device was set at a square wave with a stimulus frequency of 100 Hz, a stimulus intensity of 3.0 mA, and a stimulus duration of 250 μs, the stimulation protocol used in our previous study to effectively enhance parasympathetic activity (Yokota *et al* , 2022). First, participants were stimulated with tVNS to experience the sensations which would take place during the experiment. Then, the sensory threshold of the sural nerve was determined. After 3 min of rest, the baseline NWR threshold was measured. After another 5 min of rest, 2 min of tVNS or Sham stimulation were delivered, and the NWR threshold was measured immediately (Post 0), 10 min (Post 10), and 30 min after tVNS (Post 30), respectively. In addition, the ECG waveform was recorded during the whole procedure (Fig. 2). The trial order in the tVNS and Sham conditions was randomized across subjects, with a 7-day interval between conditions for the same subject.

Electrocardiogram

Two electrocardiographs, Bio Amp (AD Instruments) and LRR-05 (Arm Electronics), were used to measure ECG data because of their different analysis characteristics. The cathode was placed just above the right clavicle, the anode just above the left 10th rib, and the ground electrode below the left 12th rib, respectively; the three point induction method was used. ECG signals were amplified by an amplifier (A-DL-720140,4 ASSIST), converted to A/D, and stored on a personal computer using Power lab (AD Instruments, Colorado Springs, CO, USA) for offline analysis with a sampling frequency of 1 kHz and a bandpass filter from 0.5 to 35 Hz. The monitor was placed out of the subjects' sight to avoid influencing their autonomic nervous system activity and monitored in real time for safety.

Data and statistical analyses

For data analysis, the NWR threshold was calculated by Labchart8 (AD Instruments, Colorado Springs, CO, USA). Similarly, the heart rate was extracted from each R wave of the ECG by Labchart 8; heart rate variability was analyzed by the maximum entropy method using MemCalc/Bonary Light (GMS Corporation). Power spectral analysis was performed by calculating area under the low frequency (LF) (0.04–0.15 Hz) band, a component indicating sympathetic and parasympathetic activity, and the high frequency (HF) (0.15–0.40 Hz) band, a component indicating parasympathetic activity alone. LF/HF was then calculated to determine the balance of autonomic activity. The higher the LF/HF value, the more dominant the sympathetic activity; conversely, the lower the value, the more dominant the parasympathetic activity. The LF/HF value at 1 min before NWR threshold measurement was defined as baseline; the analysis was divided into five intervals: LF/HF from the start to the end of tVNS, LF/HF at 1 min before the end of NWR threshold measurement at Post 0, LF/HF at 1 min immediately before the end of NWR threshold measurement at Post 10, and LF/HF at 1 min immediately before the end of NWR threshold measurement at Post 30.

Statistical software (SPSS; IBM) was used for statistical processing; after testing for normality, a repeated measures two-way ANOVA was performed for stimuli (tVNS condition/Sham condition) and time factors for changes in NWR thresholds over time, and the Bonferroni method was used for post-hoc analysis. The Spearman's rank correlation coefficient was used to analyze the relation between changes in heart rate and LF/HF during tVNS to changes in NWR threshold at Post 0, 10, and 30. The significance level for all tests was set at 5%.

Results

Effects of tVNS on NWR threshold

A repeated measures two-way ANOVA on the change in NWR thresholds revealed an interaction between stimuli and time factors ($F = 4.671$, $p = 0.005$). The post-hoc analysis showed an increased NWR threshold in the tVNS condition at 10 and 30 min compared to the Baseline NWR threshold (10 min: $t = -2.96$, $p = 0.008$ 30 min: $t = -3.076$, $p = 0.006$). In contrast, in the sham condition, there was no significant difference in any comparison ($p > 0.05$; Fig. 3).

Correlation between LF/HF changes and NWR threshold

A significant negative correlation was found between the amount of LF/HF changes during tVNS and the amount of NWR threshold changes at Post 10 and Post 30, indicating that subjects whose LF/HF decreased significantly during tVNS, that is, those whose parasympathetic activity increased during tVNS stimulation, tended to show larger increases in NWR threshold at Post 10 and Post 30 (Post 10: $\rho = -0.561$, $p = 0.01$; Post 30: $\rho = -0.704$, $p = 0.005$). On the other hand, no correlation was found between LF/HF change and NWR threshold at Post 0 in the tVNS condition (Fig. 4) neither between LF/HF and NWR threshold changes at all time points in the Sham condition. No association between the heart rate and NWR threshold was observed.

Adverse events

None of the subjects showed excessive heart rate reduction, headache, pain at the stimulation site, or redness during or after tVNS stimulation.

Discussion

This study aimed to clarify the effect of tVNS applied to the auricular branch of the vagus nerve, cymba concha, on the NWR thresholds in the lower limb and to verify the relationship between changes in pain perception and autonomous activity modulation. The results showed a significant increase in NWR thresholds in the tVNS condition compared to baseline at 10 and 30 min post stimulation, supporting the results of a previous study applying tVNS on the cervical branch (De Icco *et al.* , 2018). Furthermore, significantly decreased LF/HF during tVNS –increased stimulation parasympathetic activity– increased the likelihood of larger pain suppression at 10 and 30 min after stimulation.

Pain is considered to be expressed by an extensive subcortical network of regions in the primary and secondary somatosensory cortex, insula, anterior cingulate cortex, prefrontal cortex, thalamus, and brainstem nuclei (Apkarian *et al.* , 2005; Tracey & Mantyh, 2007; Apkarian*et al.* , 2011).Although NWR has been associated with chronic pain, it is also an experimental acute pain experience and known to increase activity in pain related areas such as those above (Apkarian*et al.* , 2005). tVNS was shown to suppress the activity of these pain related areas in response to painful stimuli (Janner *et al.* , 2018; Lerman *et al.* , 2019). Similarly, in the present study, the tVNS suppressed activity in cortical areas associated with pain perception caused by nociceptive stimulation of the sural nerve, which may have resulted in increased NWR thresholds. This could be explained because stimulation of vagal afferent fibers inhibits the conduction of nociceptive stimuli by activating the NTS, a pain relay nucleus (Napadow*et al.* , 2012), and modulating the excitability of nociceptive circuits including those at the spinal level and higher centers (Napadow*et al.* , 2012; Busch *et al.* , 2013). Moreover, high intensity tVNS, which is perceivable but does not reach the pain threshold, has a pain suppressive effect (Chakravarthy *et al.* , 2015); the stimulus intensity of 3.0 mA used in this study, which is just about below the pain threshold, was suggested to be effective in suppressing pain.

It is also possible that the descending pain inhibitory system was activated by tVNS. This system originates in the periaqueductal gray (PAG) of the midbrain and descends to the dorsal horn of the spinal cord to inhibit the transmission of pain information. The PAG comprises nerve fibers in the rostroventromedial medulla (RVM), which includes the raphe nucleus, and in the dorsolateral pontomesencephalic tegmentum (DLPT), which includes the LC. In a previous study using 100 Hz tVNS during the expiratory phase, fMRI

indicated significant activity in the PAG, LC, and raphe nucleus (Sclocco *et al.* , 2020), suggesting that the 100 Hz tVNS used in the present study also increased activity in these regions. Furthermore, afferent inputs from the NTS, LC, and the raphe nucleus have inhibitory effects on dorsal horn neurons in the spinal cord (Ren *et al.* , 1990) and activation of vagal afferent fibers has been reported to suppress nociceptive neurons in the spinal cord (Sator-Katzenschlager & Michalek-Sauberer, 2007; Kaniusas *et al.* , 2019). Therefore, it is possible that tVNS affected nuclei at the brainstem level and activated the descending pain inhibition system, which suppressed pain processing in spinal dorsal horn neurons, thus increasing the NWR threshold.

The increase in NWR thresholds did not occur immediately after tVNS, it was delayed; similar results were observed in the VNS-induced increase in NWR thresholds using tVNS in the cervical branch (De Icco *et al.* , 2018). Plastic changes in the brain can be immediate, such as synaptic activity synchronization, or relatively slow and long lasting, involving a wide range of neural circuits, suggesting that tVNS associated plasticity may also be long lasting, and related to the release of neurotransmitters and analgesic hormones (Sandkühler, 2000; Oleson, 2002; Beekwilder & Beems, 2010). In a previous study using fMRI, tVNS produced sustained activity in cortical areas involved in pain processing for >10 min (Frangos *et al.* , 2015); moreover, insular cortical activity in response to pain was detected 11 to 17 min after tVNS (Lerman *et al.* , 2019). Therefore, the short term tVNS used in this study may have induced long term plastic changes in the brain.

Regarding the association between modulation of pain perception and changes in autonomic nervous activity, we believe that this is the first study which revealed the pain suppression capacity due to tVNS depends on the amount of decrement in subject's LF/HF during tVNS. Sympathetic nervous activity has been associated with activation of pain processing brain regions in response to pain (Mobascher *et al.* , 2009). Further, studies using fMRI have revealed that pain mediated activation of brain regions involved in pain processing, including secondary somatosensory cortex, insula, anterior cingulate cortex, prefrontal cortex, thalamus, and midbrain was correlated with sympathetic nerve activity (Maihöfner *et al.* , 2011; Seifert *et al.* , 2013; Lerman *et al.* , 2019). Therefore, it is possible that tVNS suppressed the activity of brain regions involved in pain processing, something intrinsically associated with increased sympathetic nervous activity, which may have resulted pain suppression. Furthermore, as LF/HF correlated with changes during tVNS, LF/HF could be used as an effective biomarker to predict tVNS' pain suppressing effects.

This study has some limitations. First, we could not measure subcortical and spinal level changes induced by tVNS, so the actual inhibitory mechanism implicated remains unknown. In addition, we only measured the NWR threshold until 30 min after tVNS; thus, the extent of the pain suppression effect resulting from a 2 min tVNS application remains unknown. Therefore, further research is needed to validate the present results.

Conclusions

The present results show that tVNS with a 250 μs square wave at a stimulus frequency of 100 Hz and a stimulus current intensity of 3.0 mA, increased the NWR threshold at 10 and 30 min after stimulation. Furthermore, the increase in NWR threshold at 10 and 30 min was greater in subjects showing a significant LF/HF reduction during tVNS. This suggests that LF/HF may correlate with capacity of tVNS' pain suppressing effects.

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Declarations of interests

All authors report no conflict of interest.

Author Contributions

Conceptualization, H.Y. and H.O.; methodology, N.O.; software, K.S.; validation, S.M., S.K. and R.H.; formal analysis, N.O.; investigation, H.Y.; resources, C.S.; data curation, H.Y. and Y.K.; writing—original draft preparation, H.Y.; writing—review and editing, H.Y.; visualization, S.K.; supervision, H.O.; project administration, H.O.; funding acquisition, H.Y. and H.O. All authors have read and agreed to the published version of the manuscript.

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Figure legends

Figure 1. Stimulation site for tVNS (Left) and sham (Right) conditions.

The stimulation site was the left cymba concha (Fig. 1: Left) in the tVNS condition and the left earlobe (Fig. 1: Right) in the Sham condition. In the Sham condition, the electrode was fixed with tape as necessary to ensure appropriate contact with the skin.

Figure 2. Experimental protocol.

First, after detecing the sensory threshold and after 3 min of rest, the Baseline NWR threshold was measured. After another 5 min of rest, tVNS/sham stimulation was performed for 2 min, and NWR thresholds were measured immediately after tVNS (Post 0), 10 min later (Post 10), and 30 min later (Post 30), respectively.

Figure 3. Effects of tVNS on NWR threshold.

Repeated measures two-way ANOVA revealed an interaction between stimulus and time factor ($F = 4.671$, $p = 0.005$). Moreover, t-test results with Bonferroni correction showed an increased NWR threshold in the tVNS condition at Post 10 and Post 30 compared to the Baseline NWR threshold (10 min: $t = -2.96$, $p = 0.008$; 30 min: $t = -3.076$, $p = 0.006$). There was no significant difference in the Sham condition ($p > 0.05$).

Figure 4. Correlation between LF/HF differences during tVNS and NWR thresholds.

A significant negative correlation was found between LF/HF changes during tVNS and the NWR threshold change at Post 10 and Post 30, indicating that subjects whose LF/HF significantly decreased during tVNS, that is, those whose parasympathetic activity increased during tVNS stimulation, tended to have larger increases in NWR threshold at Post 10 and Post 30. No such correlation was observed at Post 0 ($p > 0.05$).