TITLE

TRANSCUTANEOUS VAGUS NERVE STIMULATION PREVENTS THE DEVELOPMENT OF, AND REVERSES ESTABLISHED, OESOPHAGEAL PAIN HYPERSENSITIVITY

SHORT TITLE/RUNNING HEAD

VAGUS NERVE STIMULATION AND OESOPHAGEAL PAIN HYPERSENSITIVITY

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AUTHOR CONTRIBUTIONS

Adam D Farmer, Ahmed Albusoda & Gehanjali Amarasinghe: acquisition of data; manuscript preparation; statistical analysis; critical revision of the manuscript for important intellectual content.

James K Ruffle, Heather Fitzke, Ruqaya Idrees, Ron Fried: acquisition of data; critical revision of the manuscript for important intellectual content.

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ABSTRACT

BACKGROUND: The vagus nerve exerts an anti-nociceptive effect in the viscera.

AIMS/OBJECTIVES: We investigated whether transcutaneous vagal nerve stimulation (t-VNS) prevents the development and/or reverses established visceral hypersensitivity in a validated model of acid-induced oesophageal pain.

METHODS: Before and after a 30-minute infusion of 0.15M hydrochloric acid into the distal oesophagus, pain thresholds to electrical stimulation were determined in the proximal non-acid exposed oesophagus. Validated sympathetic (cardiac sympathetic index) and parasympathetic (cardiac vagal tone (CVT)) nervous system measures were recorded. In study 1, 15 healthy participants were randomized in a blinded crossover design to receive either t-VNS or sham for 30 minutes during acid infusion. In study 2, 18 different healthy participants were randomized in a blinded crossover design to receive either t-VNS or sham, for 30 minutes after acid infusion.

RESULTS: *Study 1*: t-VNS increased CVT (31.6% ± 58.7 vs. -9.6 ± 20.6, p=0.02) in comparison to sham with no effect on cardiac sympathetic index. The development of acid-induced oesophageal hypersensitivity was prevented with t-VNS in comparison to sham (15.5 mA per unit time (95% CI 4.9 - 26.2), p=0.004). *Study 2:* t-VNS increased CVT (26.3% ± 32.7 vs. 3 ± 27.1, p=0.03) in comparison to sham with no effect on cardiac sympathetic index. t-VNS reversed established acid-induced oesophageal hypersensitivity in comparison to sham (17.3mA /unit time (95% CI 9.8 - 24.7), p=0.0001).

CONCLUSIONS: t-VNS prevents the development of, and reverses established, acid induced oesophageal hypersensitivity. These results have therapeutic implications for the management of visceral pain hypersensitivity.

INTRODUCTION

Functional gastrointestinal disorders (FGID) are common and are associated with marked personal and societal costs [1]. Visceral pain hypersensitivity is an important cause of chronic abdominal pain in FGID [2]. Current analgesic drugs are of limited efficacy, with side effects frequently limiting their tolerability and adherence [3]. The management of visceral pain is often sub-optimal and represents an unmet clinical need. Therefore, there is a requirement to develop novel interventions.

The autonomic nervous system (ANS) is a bidirectional brain body interface that ingrates the internal milieu with the external environment, principally serving to maintain homeostasis. The ANS is traditionally considered to be composed of two opposing branches referred to as the sympathetic (SNS) and parasympathetic nervous systems (PNS). The major neural substrate of the PNS is the vagus nerve which exerts an anti-nociceptive and anti-inflammatory effect within the viscera [4,5]. Given that the SNS and PNS are largely antithetic in nature, it has been proposed that a balance between the two is crucial for normal pain perception. Imbalance between the SNS and PNS has been implicated in the pathophysiology of a number of GI disorders including inflammatory bowel disease (IBD) and the FGID [3,6]. Therefore, restoration of this imbalance has therapeutic interest.

Previously, we have developed and validated a human model of oesophageal pain hypersensitivity, where following acid infusion into the distal oesophagus, pain tolerance thresholds (PTT) to electrical stimulation in the non-acid exposed proximal oesophagus are reduced due to central sensitization [7]. Using this model, we have shown that physiologically increasing parasympathetic vagal tone, using deep slow paced breathing, prevents the development of acid induced oesophageal pain hypersensitivity [8].

The auricular branch of the vagus nerve innervates the concha of the ear and is located directly under the skin, making it a suitable target for transcutaneous electrical stimulation. Transcutaneous vagus nerves stimulation (t-VNS) can be achieved using an earplug-like electrode which sits on the cymba conchae of the outer ear and a handheld battery-powered electrical stimulator. Brain imaging studies

in humans, and tract-tracing studies in animals, have shown that the auricular branch of the vagus nerve projects to the nucleus tractus solitarius in the brainstem which is the primary relay centre for vagal afferents [9]. T-VNS has been demonstrated to induce similar patterns of cerebral activation to surgically implanted VNS [10]. In the current study, we tested the hypothesis that t-VNS can prevent the development, and reverse established, acid induced oesophageal pain hypersensitivity in healthy participants.

MATERIALS AND METHODS

Study population and study design

Healthy participants aged 18-60 years, who had no past medical history and were not currently taking any prescribed or over the counter medications, took part in the study. All participants were non-smokers and were asked to avoid caffeine and alcohol for 24 hours prior to the study and had never previously been studied with this model of acid perfusion. All females of child-bearing potential were studied in the follicular stage of their menstrual cycle. The validated Hospital Anxiety and Depression Scale was used to screen for sub-clinical anxiety and depression [11]. Written informed consent was obtained from each participant. All protocols were approved by the Queen Mary University of London City Research Ethics Committee, UK (*reference QMREC 2016/26 and QMREC 2014/5)* and were undertaken according to the Declaration of Helsinki. Both studies were blinded, randomised, sham controlled, cross over trials comparing t-VNS vs sham using a validated model of acid induced oesophageal hypersensitivity. In study 1, t-VNS/sham was applied during oesophageal acidification to evaluate whether the intervention would prevent the development of acid induced oesophageal pain hypersensitivity. In study 2, t-VNS/sham was applied after the development of acid induced oesophageal pain hypersensitivity to evaluate whether it could be reversed.

Randomisation and allocation concealment

Participants were randomly assigned (1:1) in blocks of three, using a code generated at www.randomization.com, to t-VNS at the first study visit followed by sham t-VNS at the second or vice versa. Allocation was concealed from participants and those conducting the analysis of the results. In

order to mitigate potential effect of participants performing an internet search to establish which intervention was t-VNS, the participant information sheet explained that we were testing the stimulation of two different nerve on oesophageal sensitivity to acid infusion.

Main Measurements

Oesophageal pain tolerance thresholds

Following identification of the location of the lower oesophageal sphincter (LOS) using high resolution oesophageal manometry (Manoscan ESO, Medtronic, Watford, UK), sensory testing was undertaken 18cm proximal to the LOS using a pair of silver-silver chloride bipolar ring electrodes (inter-electrode distance 1cm) mounted 16cm proximal to the tip of a 3mm diameter catheter containing a distal infusion port (Unisensor, Gaeltec, Isle of Skye, UK). Electrodes were connected to an electrical stimulator (Model DS7, Digitimer Ltd, Welwyn Garden City, UK). Electrical stimuli consisting of square wave pulses of 500 milliseconds (ms) duration were delivered at a frequency of 0.5Hz, with intensities varying between 0 and 80mA. The intensity of stimulation was increased in 2mA increments [8,12-17]. Participants were asked to report both their sensory threshold for the stimulus (visual analogue scale (VAS) of 1 out of 10) and their PTT, i.e. when they could not tolerate any further increase (VAS of 7 out of 10). Stimulation was discontinued at the PTT. Oesophageal pH was measured continuously in the proximal (site of electrical stimulation) and distal (site of acidification) oesophagus using a twin-channel pH catheter and recording box (Synectics Medical, Enfield, UK).

Psychological factors

At baseline, state and trait anxiety was assessed using the validated Spielberger Anxiety Inventory, as anxiety can modulate PTT [18,19].

Autonomic nervous system

Mixed measures: blood pressure and heart rate - Blood pressure (BP) was continuously non-invasively measured using a validated photoplethysmographic technique (Portapres, Finpres Medical Systems, Amsterdam, Netherlands) [20]. Electrocardiographic (ECG) electrodes (Ambu Blue Sensor P, Ballerup, Denmark) were placed in right and left sub-clavicular areas and cardiac apex. The ECG was acquired at 5kHz using a biosignals acquisition system (Neuroscope, Medifit Instruments, Essex, UK). Heart rate

(HR) was derived from intervals between successive R waves. ANS parameters were recorded according to internationally agreed recommendations [21]. *Parasympathetic nervous system measures: cardiac vagal tone* - The Neuroscope derives a real-time index of parasympathetic nervous system activity, known as cardiac vagal tone (CVT). CVT is measured on a validated linear vagal scale (LVS), where 0 represents full atropinisation [22]. CVT is described in elsewhere [23], but in contrast to power spectral analysis of heart rate variability, it is validated for time epochs of less than 5 minutes [22]. *Sympathetic nervous system measures: cardiac sympathetic index* - RR interval data was extracted and manually reviewed and edited to remove any missed, or extra beats, as per accepted recommendations as these can result in significant artefacts [21]. In 2 out of 33 participants, the data needed to be edited due to movement artefact, which resulted in a short period (<10 seconds) of R-R intervals of <250ms. Following this, the RR data was reformatted and entered into the Cardiac Metric program (CMet, University of Arizona, Arizona, USA) to derive the validated Toichi's cardiac sympathetic index (CSI) [24]. CSI is a ratio of R-R intervals and therefore has no units.

Main exposures

Oesophageal acid infusion

After being warmed to body temperature, 0.15M hydrochloric acid (HCl) (Stockport Pharmaceuticals, Stepping Hill Hospital, Stockport, UK) was infused into the distal oesophagus, 3cm proximal to the LOS at a rate of 8 mL per minute for 30 minutes via an infusion pump (KDS Scientific 100, Linton Instrumentation, Pulgrave, Norfolk, UK) [8,12-17].

Main interventions

Vagus nerve or sham stimulation

The t-VNS device (NEMOS system, CerboMed, Erlangen, Germany) was positioned in the participants' left ear ensuring that the stimulating electrodes made good contact with the cymba concha to ensure optimal stimulation. If the skin contact is sub-optimal, the system alarms thereby allowing repositioning. The t-VNS device produces rectangular pulses with a pulse width of 0.1ms at a frequency of 25 Hz. The device was then switched on and the intensity of stimulation was gradually increased in 0.1mA increments until a tingling sensation was achieved but not to a level that was

uncomfortable or caused pain. This intensity was then used for the rest of the intervention period. The t-VNS device stimulates for a period of 30 seconds followed by 30 seconds rest. Sham t-VNS was achieved in a similar manner by inverting the VNS device such that the electrode was placed on the earlobe of the left external ear, *see Figure 1,* with the intensity of stimulation increased in an identical fashion to achieve a light tingling sensation.

Outcome measures

Three measurements of PTT were taken in the proximal oesophagus and the mean value derived. Measurements were taken prior to acid infusion (T0), then 60 minutes (T60), 90 minutes (T90) and 120 minutes (T120) after completion of the acid infusion. As previously defined by Sharma *et al.* [12], sensitizers to the oesophageal acidification were defined as having a post acid infusion reduction in PTT of ≥6 mA at T60. Non-sensitizers were defined as having an increase or a reduction of <6 mA in oesophageal PTT, at T60. The primary outcome was the differences in PTT between t-VNS and sham stimulation over the time periods after stimulation. Secondary outcome measures included the effect of t-VNS on autonomic and anxiety measures as well as safety aspects.

Study protocols

All participants were studied in the morning (from 0900-1200hrs) in a temperature controlled (20-22°C), quiet laboratory. All experiments were conducted with participants resting on an examination couch at 45°, with their legs supported, having fasted for a minimum of 6 hours. The catheter was introduced into the oesophagus trans-nasally without local anaesthetic until the infusion port and stimulating electrodes were 3 and 18cm proximal to the LOS respectively. Participants were allowed to rest for at least 10 minutes prior to undertaking any further interventions [25]. During all study procedures, autonomic measures and blood pressure was measured at baseline and continuously thereafter.

Study 1 - The effect of vagus nerve stimulation on the development of oesophageal pain hypersensitivity

Prior to acid infusion, baseline PTT (i.e. T0) was measured. Acid was then infused into the distal oesophagus for 30 minutes, during which participants were randomised to receive either t-VNS or sham VNS for the duration of the acid infusion, i.e. 30 minutes. PTTs were then measured at T60, T90 and T120 minutes. Participants who received sham VNS at the first visit who did not sensitize were excluded from the study at that point and no further visits were undertaken as it would not be possible to demonstrate any effect of t-VNS or sham VNS. Participants were then restudied after a period of 2 weeks, during which they were crossed over to receive the VNS intervention to which they had not been exposed during the first visit. Participants who received sham VNS at the second visit who did not sensitize were excluded from the subsequent study analysis for the aforementioned reason. We have previous utilised this enriched pragmatic design in order to improve recruitment, participant retention and study efficiency [8].

Study 2 - The effect of vagus nerve stimulation on reversing established oesophageal pain hypersensitivity

As in study 1, prior to acid infusion, baseline PTT (i.e. T0) was measured following which acid was infused into the distal oesophagus for 30 minutes. PTTs were measured at T60, T90 and T120. At T60 (i.e. after sensitization had occurred), participants were randomised to receive either t-VNS or sham VNS for 30 minutes. Participants were then restudied after a period of 2 weeks, during which they were crossed over to receive the VNS intervention to which they had not been exposed during the first visit. Participants who did not sensitize were excluded for the same reasons as stated above.

Data analysis

As determined by visual inspection of histograms and Shapiro-Wilk testing, results are presented as mean (with standard deviation (SD)), medians and inter-quartile ranges dependent on their distribution. The changes in PTT were analysed using linear mixed effects regression models with maximum restricted likelihood (fixed effects: time, intervention (i.e. t-VNS/sham t-VNS); random effect = subject) with T0 PTTs in Study 1 and T60 in Study 2 accounted for in the model as zero to yield a regression coefficient for t-VNS intervention effect (with 95% confidence interval (CI)). As data were

paired, additional analyses were performed with paired t-tests and linear regression as appropriate. The investigator analysing the study results was blinded to the treatment allocation. *P*<0.05 was considered to represent statistical significance and the analyses were undertaken using proprietary software (Stata V13.0, Stata Corporation, Texas, USA).

RESULTS

Study 1 - The effect of vagus nerve stimulation on autonomic tone and pain thresholds

Demographics

34 participants (18 males, mean age 29 years, range 19-48) were recruited. 19 participants (10 males, mean age 24 years, range 21-40) were classified as non-sensitizers (9 participants after the first and 10 after the second visit), *see supplementary material*. This was an expected rate of non-sensitization based on our previous work, thus leaving 15 participants (9 males, mean age 31 years, range 21-48).

Vagus nerve stimulation increases cardiac vagal tone

Relative to baseline, t-VNS resulted in an increase in CVT in comparison to sham VNS (Δ CVT 31.6% ± 58.7 vs. -9.6% ± 20.6, *p*=0.016). Relative to baseline, t-VNS had no effect of CSI in comparison to sham (Δ CSI -5.8% ± 17.7 vs. 7.6% ± 8.7, *p*=0.3).

Vagus nerve stimulation prevents the development of oesophageal hypersensitivity

In all participants, during oesophageal acidification, pH fell to <2.0 in the distal oesophagus but remained >6.0 in the proximal oesophagus. The most common symptoms reported during the acid infusion was a mild discomfort/warm feeling in the lower chest (6/15, 40%) and nausea (3/15, 20%). Absolute PTT data at T0 and after acid infusion (T60, T90, T120) are shown in *Table 1*. There were no differences in absolute values of PTT at T0 in participants undergoing t-VNS vs. sham (mean (SD): 37.5 mA (16.6) vs. 33.6mA (11.4), *p*=0.45). t-VNS prevented the development of proximal oesophageal acid-induced hypersensitivity, *see Figure 2*. Mixed effects regression showed a coefficient of effect for t-VNS of 15.5 mA per unit time (95% CI 4.9 - 26.2), *p*=0.004. There was no relationship between state or trait anxiety and T0 thresholds, nor the degree of acid sensitization at subsequent time points or changes in autonomic measures.

Study 2 - The effect of vagus nerve stimulation on reversing established oesophageal pain hypersensitivity

Demographics

25 participants (12 males, mean age 26.4 years, range 19-41) were recruited. 7 participants (4 males, mean age 27.7 years, range 19-36) were excluded with 5 classified as non-sensitizers with 2 participants being unable to tolerate naso-oesophageal intubation, *see supplementary material*. This was an expected rate of non-sensitization based on our previous work thus leaving, 18 participants (8 males, mean age 26 years, range 19-41).

Vagus nerve stimulation increases cardiac vagal tone

Relative to baseline, t-VNS resulted in an increase in CVT in comparison to sham VNS (Δ CVT 26.3% ± 32.7 vs. 3 ± 27.1, p=0.03). Relative to baseline, t-VNS had no effect of CSI in comparison to sham (Δ CSI -10.9% ± 37.7 vs. 11.5 ± 79.7, p=0.3).

Vagus nerve stimulation reverses established oesophageal hypersensitivity

During acid infusion, pH fell to <2.0 in the distal oesophagus of all participants but remained >6.0 in the proximal unexposed oesophagus. The most common symptom reported with acid infusion was nausea (4/18, 22.2%). Absolute threshold data at (T0) and after acid infusion (T60, T90, T120) are shown in *Table 2*. There were no differences in absolute values of PT at T0 or T60 in participants receiving t-VNS or sham VNS (T0 mean (SD) t-VNS 38.7mA (12.6) vs. sham t-VNS 37.3mA (15.7), p=0.69, T60 t-VNS 28.7mA (11)) vs. sham t-VNS 27.2mA (11.2), p=0.55). Relative to the T60 time-point, there was an increase in PTT with t-VNS at T90 of 3mA (95% CI 1 - 5.1) in comparison to sham t-VNS of 0.7 mA (95% CI -1 – 2.3). Similarly, at T120, there was an increase in PTT with t-VNS of 3.8mA (95% CI 1.5 - 6.1) in comparison to sham t-VNS, 1.3mA (95% CI -0.4 – 3). Mixed effects regression showed a significant effect for t-VNS (coefficient 17.3mA /unit time (95% CI 9.8 - 24.7), p=0.0001), *see Figure 3*.

DISCUSSION

Our results provide evidence that t-VNS prevents the development, as well as reverses established, acid induced oesophageal pain hypersensitivity by increasing parasympathetic tone. Although there remains considerable uncertainty, the effect that we observed could be potentially mediated by vagal

modulation of nociplastic pain including the inhibition of inflammation, the SNS and the pain neuromatrix – all of which are factors that contribute to central sensitisation.

Inflammation exerts a pivotal role in pain perception with glial, immune cells and proinflammatory cytokines implicated in chronic pain states [26]. In addition to its primary function of regulating heart rate, respiratory patterns and digestion, the vagus nerve has been demonstrated to exert an important role in the modulation of both the central and peripheral anti-inflammatory response with a significant body of research demonstrating an anti-inflammatory pathway mediated by acetylcholine and/or noradrenaline, referred to as the cholinergic anti-inflammatory pathway [27,28]. In the context of t-VNS, a number of studies have shown short term stimulation exerts an anti-inflammatory effect [29]. Following acid induced oesophageal cell injury, there is an influx of inflammatory mediators whose function is to repair squamous epithelium although can cause direct injury through alterations in neuromuscular function of oesophageal smooth muscle [30]. In animal models, this deleterious effect of acid-induced oesophageal inflammation can be ameliorated with ketotifen, a non-competitive H1-antihistamine and mast cell stabilizer [31]. Moreover, acid induced acute oesophagitis is worsened by destruction of the dorsal motor nucleus of the vagus, the efferent source of vagal tone, and in combination with our data suggest that the cholinergic anti-inflammatory pathway may represent a therapeutic target [32] .

Previous functional neuroimaging studies have demonstrated that t-VNS modulates areas associated with central pain neuromatrix such as the thalamus, orbitofrontal cortex, cerebellum, hypothalamus, medulla and the limbic system [33]. For instance, VNS has been shown to result in insular and cortical activation, areas that have been shown to be important in mediating acid induced oesophageal pain in health participants and in patients with gastro-oesophageal reflux disease [34-36]. We have also recently illustrated how higher resting parasympathetic tone conveys greater network connectivity in a number of subcortical regions implicated in descending analgesia, including the anterior insula, amygdala and hypothalamus, suggesting a prospective neural mechanism for t-VNS induced anti-nociception [37].

Oesophageal hyperalgesia that is observed in the non-acid exposed proximal oesophagus occurs due to central sensitization, reflecting enhanced nociception. This is the results of three broad mechanisms namely, temporal summation, increased activation of nociceptive facilitatory pathways and/or impairment of descending pain inhibitory pathways. Dysfunction within the descending pathways may particularly promote and maintain central sensitization [38]. Within the brainstem, primary afferent vagal fibres terminate in the nucleus tractus solitarius, which is also the origin of descending inhibitory pathways which form a spinal-bulbo-spinal anti-nociceptive circuit [39]. The central analgesic effect of VNS has been proposed to increase such descending pain modulatory pathways [40].

It was interesting to observe that anxiety levels were not associated with pain thresholds, as the former can influence ANS function [41], although we have reported similar findings in a previous larger study using this model [8]. It is plausible to suggest that our participants were less prone to anxiety given that we actively screened for subclinical anxiety prior to entry into the study. Nevertheless, that is not to say that this model is not influenced by psychiatric state. Using this model, albeit in a smaller number of healthy participants, Sharma et al. demonstrated that acid induced oesophageal hypersensitivity could be increased using an experimental paradigm that induced anxiety [12].

Our findings have potential therapeutic implications. Heartburn and chest pain are common symptoms in functional oesophageal disorders, such as reflux hypersensitivity syndrome [42]. Although proton pump inhibitors (PPIs) are the gold standard for the treatment of gastro-oesophageal reflux disease, a substantial proportion of such patients fail to respond [43]. Amongst PPI non-responders, three-quarters of patients will have a FGID [44], disorders which are characterized by heartburn and chest pain [56]. These symptoms are mediated, in part, by oesophageal hypersensitivity, which is frequently challenging to manage effectively [45]. Non-pharmacological interventions are increasingly being sought to treat chronic pain disorders. Coupled with the data from our study, t-VNS could represent an attractive non-invasive neuromodulatory intervention that warrants further study in this

group. This is particularly salient given that we have demonstrated that t-VNS can reverse established oesophageal hypersensitivity.

Our study is subject to limitations. Firstly, both our studies were not traditional crossover designs, in that sensitization was defined at the sham t-VNS visit in study 1 and at either visit in study 2, with participants excluded on that basis. Nevertheless, we have previously shown that sensitization status is temporally stable and the design used in this study represents a pragmatic approach to ensuring maximal recruitment and retention and one that we have used previously in studies of physiological parasympathetic modulation [8]. Within any cross-over design there is potential for a carryover effect although we attempted to ameliorate this by using an interval of at least two weeks between study visits, as we have used in our previous studies. As with most studies of neurostimulation, achieving adequate blinding can be challenging due to the proximity of other neuronal structures. However, brain imaging studies have demonstrated that t-VNS sham stimulation causes somatosensory activation but does not activate the central autonomic network, providing evidence from a mechanistic point of view that it is a true sham [46]. The t-VNS device that was used has a fixed stimulation frequency of 25Hz. Within animal models, vagal stimulation is frequency dependant although this remains unclear in humans and warrants further study [46]. Whether the visceral analgesic effect of t-VNS occurs in response to pain induced by other modalities, such as mechanical or thermal stimulation, is unclear. However, Busch *et al*. demonstrated that t-VNS was associated with an analgesic effect into response to somatic mechanical and thermal pain in comparison sham t-VNS in the absence of any effect on non-noxious somatosensory processing [47]. Finally, we did not perform a study to ascertain whether the analgesic effect of t-VNS could be antagonised using a vagolytic agent, such as atropine. However, in our previous study we demonstrated that atropine reversed the analgesic effect of deep breathing (a method for physiologically increasing vagal tone), which was indexed by a concomitant withdrawal of CVT [8]. Thus, it seems plausible to suggest that given we demonstrated a rise in CVT in both of the current studies, that the effect of t-VNS is mediated by the PNS.

In conclusion, we report the first human studies demonstrating that t-VNS prevents, and reverses established, acid induced oesophageal pain hypersensitivity. The mechanism by which this occurs remains to be fully elucidated but is likely to be mediated by the PNS considering its pivotal role in modulating central sensitisation. Further studies are now warranted to ascertain whether t-VNS is efficacious in managing visceral pain in a clinical setting, and particularly pain that is associated with central sensitization, such as non-erosive reflux disease or reflux hypersensitivity syndrome.

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TABLES

TABLE 1

a. Pain tolerance thresholds in study 1 – t-VNS during oesophageal acidification				
	T0	T60	T90	T120
Pain thresholds: mean (SD) mA	37.5 (16.6)	39.1 (14.9)	36.2 (15)	36.7 (15.7)
b. Pain tolerance thresholds in study 1 – sham t-VNS during oesophageal acidification				
Pain thresholds: mean (SD) mA	33.6 (11.4)	25.2 (6.9)	25.1 (8.9)	28.7 (9.2)

Table 1: Absolute values for proximal oesophageal PTT before (T0) and after (T60, T90 and T120) acid infusion with (a) t-VNS and (b) sham t-VNS delivered during acid infusion.

TABLE 2

a. Pain tolerance thresholds in study 2 – t-VNS after oesophageal acidification				
	T0	T60	T90	T120
Pain thresholds: mean (SD) mA	38.7 (12.6)	28.7 (11.0)	32.1 (16.5)	34.5 (20.7)
b. Pain tolerance thresholds in study 2 – sham t-VNS after oesophageal acidification				
Pain thresholds: mean (SD) mA	37.3 (15.7)	27.2 (11.2)	30.3 (12.1)	32.9 (13.9)

Table 2: Absolute values for proximal oesophageal PTT before (T0) and after (T60, T90 and T120) acid infusion with (a) t-VNS and (b) sham t-VNS delivered after acid infusion.

FIGURE LEGENDS

Figure 1 – The t-VNS device in situ. *A) Active stimulation with the device located in the cymba concha and tragus and B) sham VNS where the outer aspect of the earlobe is stimulated.*

Figure 2 - The effect of t-VNS (■) and sham t-VNS (•) on the development of oesophageal pain hypersensitivity, derived from the paired change in pain thresholds (mean ± standard error of the mean), in the proximal oesophagus at T60, T90 and T120, with mixed effects regression showing a coefficient of effect for t-VNS of 15.5 mA per unit time (95% CI 4.9-26.2), p=0.004.

Figure 3 - The effect of t-VNS (■) and sham t-VNS (•) on the reversing established oesophageal pain hypersensitivity, derived from the paired change in pain thresholds (mean ± standard error of the mean), in the proximal oesophagus at T60, T90 and T120, with mixed effects regression showing a coefficient of 17.3mA /unit time (95% CI 9.8 - 24.7), p=0.0001.