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Research Submission

A Randomized Sham-Controlled Cross-Over Study on the Short-Term Effect of Non-Invasive Cervical Vagus Nerve Stimulation on Spinal and Supraspinal Nociception in Healthy Subjects

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Objective.—The aim of the present study was to test the effects of vagus nerve stimulation (VNS) on the descending pain inhibition, quantified by the nociceptive flexor (RIII) reflex and the conditioned pain modulation (CPM) paradigm, and on supraspinal nociceptive responses, assessed by pain intensity and unpleasantness ratings and late somatosensory evoked potentials (SEPs), in healthy subjects.

Background.—Non-invasive vagus nerve stimulation (nVNS) showed promising effects on headache and pain treatment. Underlying mechanisms are only incompletely understood but may include the activation of the descending pain inhibitory system and/or the modification of emotional responses to pain.

Methods.—Twenty-seven adult, healthy, and pain-free subjects participated in this double-blind cross-over study conducted at a university research center. They received 4 minutes of cervical nVNS or sham stimulation in randomized order. RIII reflexes, pain ratings, and SEPs were assessed before, during, and 5, 15, 30, and 60 minutes after nVNS/sham stimulation, followed by CPM testing. The primary outcome was the nVNS effect on the RIII reflex size. Three subjects were excluded after the preparatory session (before randomization), 1 subject was excluded after outlier analysis, leaving 23 for analysis.

Results.—RIII reflex areas were 917.1 ± 563.8 µV × ms (mean ± SD) before, 952.4 ± 467.4 µV × ms during and 929.2 ± 484.0 µV × ms immediately after nVNS and 858.4 ± 489.2 µV × ms before, 913.9 ± 539.7 µV × ms during and 862.4 ± 476.0 µV × ms after sham stimulation, revealing no differences between the immediate effects of nVNS and sham stimulation ($F_{[3,66]} = 0.67$, $P = .574$). There also were no effects of nVNS over sham on RIII reflex areas up to 60 minutes after nVNS ($F_{[1.7,37.4]} = 1.29$, $P = .283$). Similarly, there was no statistically significant effect of nVNS on pain intensity ratings and thresholds, RIII reflex thresholds, late SEP amplitudes, and the CPM effect, compared to sham. Pain unpleasantness ratings statistically significantly decreased from 4.4 ± 2.4 (NRS 0-10) to 4.1 ± 2.5 during nVNS compared to sham stimulation ($F_{[1,22]} = 8.74$, $P = .007$), but there were no longer lasting effects (5-60 minutes after stimulation).

Conclusions.—The present study does not support an acute effect of nVNS on descending pain inhibition, pain intensity perception or supraspinal nociception in healthy adults. However, there was a small effect on pain unpleasantness during nVNS, suggesting that nVNS may preferentially act on affective, not somatosensory pain components.

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Key words: non-invasive vagus nerve stimulation, descending pain inhibition, mood, conditioned pain modulation, nociceptive flexor reflex

Abbreviations: BDI Beck Depression Inventory, CPM conditioned pain modulation, NRS Numerical Rating Scale, nVNS non-invasive vagus nerve stimulation, PANAS positive and negative affect schedule, PCS Pain Catastrophizing Scale, RIII reflex nociceptive flexor reflex, SEPs late somatosensory evoked potentials, STAI State-Trait Anxiety Inventory

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INTRODUCTION

Recent studies have shown promising effects of non-invasive vagus nerve stimulation (nVNS) in acute and preventive treatment of migraine and cluster headache[1-5] and also in the treatment of other types of pain, eg, fibromyalgia.[6] Consistent with the clinical effects on headache and migraine, VNS has been shown to reduce trigeminal nociception[7,8] and cortical spreading depression.[9] However, the exact mechanism by which VNS modulates nociception and pain perception is only incompletely understood. Animal research has suggested the involvement of descending pathways. As reviewed in Randich (1992),[10] VNS can inhibit spinally mediated tail-flick responses and responses of nociceptive dorsal horn neurons in rats. Lesion and local anesthetic injection studies suggest that this effect of VNS is mediated via the nucleus tractus solitarii and areas involved in the descending pain inhibitory system such as the locus coeruleus and raphe nuclei.[10] From these areas, neurons descend to the spinal and trigeminal dorsal horn, where they inhibit nociceptive transmission, resulting in reduced nociceptive input to the brain.[11] VNS-induced activation of the locus coeruleus and raphe nuclei is also seen in human functional MRI.[12,13] In summary, 1 possibility of how VNS may reduce pain is by activating descending pain inhibitory systems. In addition, supraspinal effects may also play a role. Curiously, while reported VNS effects in clinical pain are mostly analgesic, effects on human experimental pain perception are mixed,[14-16] which might in part be due to differences in stimulation parameters. Bidirectional effects of VNS on pain intensity (increased by improved attention vs reduced by positive effects on mood) have been forwarded as an alternative explanation for inconsistent results, and it has been recommended that pain unpleasantness and mood are measured in addition to pain intensity to better characterize the effect of VNS.[7]

Based on the above considerations, we hypothesized that VNS (1) activates the descending pain inhibition and (2) reduces supraspinal nociception, possibly with a differential effect on pain unpleasantness vs pain intensity ratings.

The spinally mediated nociceptive flexor reflex (RIII reflex) is considered a measure of human spinal nociception,[17,18] and activation of descending pain inhibitory pathways can be detected by the reduction of the reflex size.[17,19] The CPM paradigm is an alternative measure, specifically assessing the "pain inhibits pain" aspect of pain inhibition, which is thought to include the activation of descending pain inhibitory pathways in humans.[20] It assesses the effect of a noxious conditioning stimulus (eg, a cold water bath) on the pain perception of a heterotopic noxious test stimulus (eg, a heat pain stimulus). Late somatosensory evoked potentials (SEPs) reflect nociceptive, but in part also non-nociceptive activity in various brain regions[21,22] and can be used to estimate supraspinal nociception beyond pain ratings.[23]

Therefore, to test the above hypotheses, we assessed the effect of cervical nVNS in healthy volunteers on (1) descending pain inhibition, as quantified by the RIII reflex and CPM paradigm and on (2) supraspinal nociceptive responses, as quantified by pain intensity and

Conflict of Interest: EL is an employee of electroCore, Inc, and receives stock ownership. The other authors declare no conflict of interest.

This work has been presented in the abstract form at the Congress of the German Pain Society in Mannheim, Germany, in 2018.

pain unpleasantness ratings and by late SEPs. In addition, mood was assessed before and after VNS.

METHODS

Participants.—The study was conducted in accordance with the Declaration of Helsinki and all procedures were approved by the local ethics committee (project number 17-464, approved on 10/AUG/2017) of the Ludwig-Maximilians-University Munich. Experiments were performed between August 2017 and April 2018 at the University Hospital in Munich, Germany. A total of 27 participants were recruited by advertisements at the University Hospital. All participants signed an informed consent form. They had to meet the following criteria: (1) ≥ 18 years old; (2) adequate knowledge of German; (3) no psychiatric, neurological or internal diseases, including contraindications for performing a cold pressor test (immersion of the hand in painfully cold water, used as conditioning stimulus within the CPM paradigm) such as Raynaud's syndrome, coronary heart disease, or hypertension[24]; (4) no frequent or chronic pain like migraine, tension headache or chronic back pain > 5 days a month; (5) no contraindications for use of the gammaCore® device, including any active implant (eg, pacemakers or cochlear implants), diagnosed stenosis of the carotid arteries, history of cervical vagotomy, and cervical stents or metal implants; (6) no alcohol or substance abuse and no regular use of drugs except for oral contraceptives and substitution of thyroid hormones; (7) not pregnant or breast-feeding; (8) no current or past use of nVNS.

Sample size: Given the unknown effect size of nVNS on descending inhibition, the sample size was based on our previous studies, successfully using RIII reflexes to detect the influence of various interventions on spinal nociception in samples of 14-22 subjects,[25,26] so we aimed at including 22 subjects.

Participant disposition: Of the 27 initially recruited subjects, 3 were excluded after the preparatory session because of large early, non-nociceptive (RII) reflex components interfering with RIII area quantification, because the electrical stimulus used to evoke the RIII reflex was too painful or the RIII reflex was too small (RIII area < 200 μV × ms). One subject was excluded after statistical outlier analysis (see section 2.9), leaving 23 subjects for analysis. [25,26]

Overview of the Procedure.—The study design was randomized, double-blind, sham-controlled, and cross-over. It consisted of 1 preparatory and 2 experimental sessions. During the preparatory session, participants completed the questionnaires and were familiarized with the electrical stimulation of the sural nerve, pain intensity, and pain unpleasantness ratings, and the CPM test. A total of 24 subjects participated in the 2 experimental sessions and were randomly (1:1) assigned to receive nVNS or sham stimulation first using the RAND() function in Microsoft Office Excel. The randomization sequence was generated by RR prior to the start of subject recruitment and was concealed from the other investigators, including LA who enrolled the subjects and performed the experiments. Subjects were assigned to the respective random sequence based on the order of enrollment. All experiments were performed by LA. Except for the type of stimulation, the 2 experimental sessions were identical and performed at least 7 days apart to avoid cross-over effects (12.4 ± 2.2 days).

In the clinical research, nVNS statistically significantly suppresses migraine pain at 30 and 60 minutes after stimulation.[5] Therefore, we assessed nVNS effects during and up to 60 minutes after stimulation. An outline of the experimental sessions is shown in Figure 1. To allow for reflex stabilization, experimental sessions started with the assessment of RIII and pain thresholds, followed by an 8-minutes run of suprathreshold electrical sural nerve stimulation (48 stimuli, applied every 8-12 seconds with an intensity ~170% of RIII threshold, intensity was kept constant within the session, stabilization phase not shown in Figure 1). Then, at baseline, participants completed the positive and negative affect schedule (PANAS) and RIII and pain thresholds were assessed again. A 2-minutes run of suprathreshold electrical stimulation (12 stimuli) was performed and RIII areas and SEPs were obtained in response to each stimulus. Pain intensity and unpleasantness ratings were obtained at the end of the 2-minutes block, rating the average of the preceding 5 stimuli. This was followed by the intervention run, a 10-minutes run of suprathreshold electrical stimulation (60 stimuli), consisting of 2 cof reflex stabilization (not further analyzed), 2 minutes before nVNS/sham stimulation ("Pre"), 4 minutes during nVNS/sham

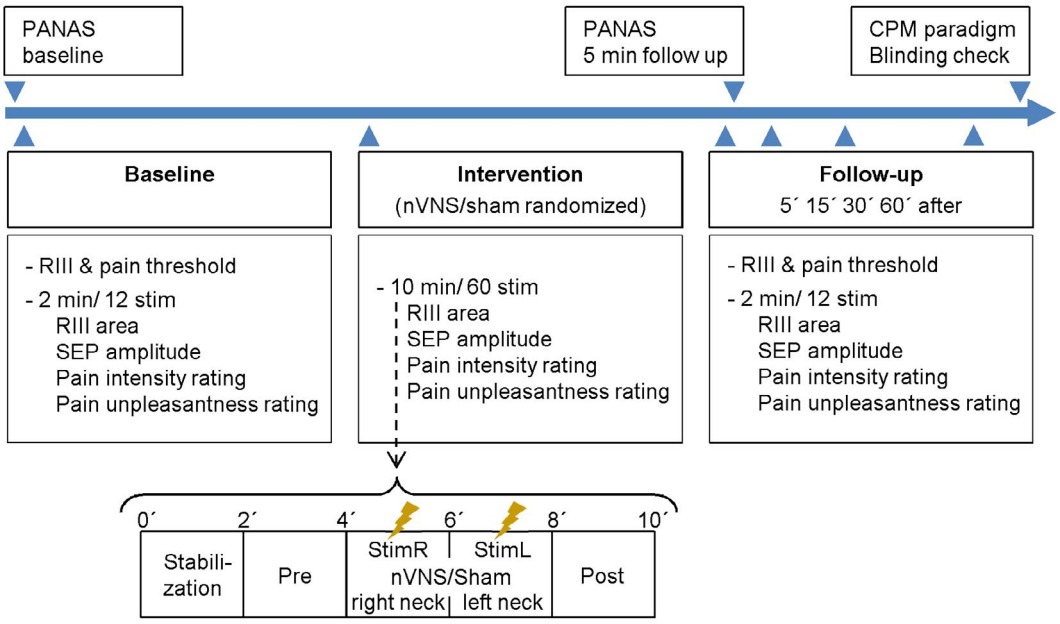

Fig. 1.—Outline of the experimental sessions. The study was randomized, double-blind, sham-controlled, and cross-over. Two experimental sessions were conducted on separate days. Subjects were randomly assigned to receive nVNS or sham stimulation in the first/second session. At baseline, participants completed the positive and negative affect schedule (PANAS). Then, RIII reflex and pain thresholds were obtained. Next, RIII reflex areas, somatosensory evoked potential (SEP) amplitudes, and pain intensity and unpleasantness ratings were obtained in response to 12 stimuli of suprathreshold electrical stimulation (intensity ~170% of RIII threshold). The intervention (nVNS/sham) was performed during a 10 minutes run of suprathreshold electrical sural nerve stimulation (60 stimulations). RIII areas, SEPs, pain intensity, and unpleasantness ratings were assessed during a stabilization block (not analyzed), and immediately before (pre), during, and after (post) the intervention. nVNS/sham stimulation was applied for a total of 4 minutes (StimR, 2 minutes on the right side of the neck, and StimL, 2 minutes on the left side of the neck). Five minutes after nVNS/sham stimulation, participants again rated the PANAS. During follow-up, RIII and pain thresholds were assessed followed by 2 minutes runs of suprathreshold electrical stimulations at 5, 15, 30, and 60 minutes after nVNS/sham stimulation. Finally, the Conditioned Pain Modulation (CPM) paradigm and blinding check were conducted. [Color figure can be viewed at wileyonlinelibrary.com]

stimulation, which was applied first on the right side of the neck ("StimR," 2 min), then on the left ("StimL," 2 min), followed by 2 minutes follow-up ("Post"). Like before, RIII areas and SEPs were assessed in response to each stimulus, and pain intensity and unpleasantness ratings were obtained after each 2-minutes block. After 5 minutes nVNS/sham stimulation participants answered the PANAS again. At each of the follow-up time points (5, 15, 30, and 60 minutes after nVNS/sham stimulation), RIII and pain thresholds were assessed followed by a 2-minutes run of suprathreshold stimulation (12 stimuli) to quantify RIII areas, SEP amplitudes, pain intensity, and unpleasantness ratings. Finally, the Conditioned Pain Modulation (CPM) paradigm and blinding check were performed. The study was conducted according to the original protocol.

The study was stopped after reaching a targeted sample size of 22. When subject 22 finished his last experimental session, there were 2 more subjects who already had started with the experiments, so these measurements were finished, too.

Questionnaires.—As cognitive and affective factors (eg, depression and anxiety) are known to influence pain perception,[27,28] German versions of the Pain Catastrophizing Scale (PCS),[29,30] the Beck Depression Inventory (BDI),[31,32] and the State-Trait Anxiety Inventory (STAI)[33,34] were obtained and used to screen for potential clinically relevant levels of catastrophizing (cut-off > 30[30]) depressive symptoms (cut-off > 13[35]) during the preparatory session. There are no established cut-offs for anxiety as measured by the German version of the STAI.

To quantify the effects of nVNS on mood, participants answered the German version[36] of the PANAS.[37] The PANAS is a self-report questionnaire consisting of 20 items (10 for positive and 10 for negative affect). Each item is rated on a 5-point scale from 1 (not at all) to 5 (very much) and the final positive and negative affect scores are formed by averaging item scores. It is known to be sensitive to detect short-term fluctuations in mood.[37]

RIII Reflex and SEP Recording.—During the experiment, participants were sitting in a reclining chair with the right knee flexed at ~150°. Stimulation and recording of the RIII reflex and SEPs were performed with a Keypoint Portable EMG System (Natus, Planegg, Germany). The RIII reflex was evoked and recorded following established techniques.[18,25] For stimulation and recording, skin sites were abraded with electrode gel and cleaned with 70% alcohol. A bipolar stimulation electrode (distance between the electrodes was 23 mm) was placed at the retro-malleolar space over the sural nerve of the right lower limb. Each electrical stimulus consisted of a train of 5 impulses of 1 ms duration separated by 4 ms (= 200 Hz). The electromyographic response was recorded using Ag/AgCl surface electrodes placed on the ipsilateral short head of the biceps femoris muscle ~4-5 cm apart from each other. A ground electrode was fixed at the knee. Impedance was kept below 2 kΩ.

To record SEPs in response to painful sural nerve stimulation, standard EEG electrodes were placed at the vertex (Cz) with reference to the forehead (Fpz) as described previously.[25] Impedance was kept below 1 kΩ. Participants were asked to close their eyes, avoid eye movements, and relax their head, neck, and face muscles. After the preparatory session and the stabilization run, where participants were reminded to relax and avoid eye movements, if artifacts were evident in the SEP traces, satisfactory SEP recordings were obtained throughout.

Electromyographic responses and EEG signals were amplified (up to 10,000 times) and band-pass filtered (20-1000 Hz for RIII recording; 0.5 to 500 Hz for SEP recording). RIII and SEP traces were saved for offline analysis from 90 ms before to 410 ms after stimulation.

Quantification of RIII and Pain Thresholds.—RIII and pain thresholds were assessed using procedures as described elsewhere.[38,39] Briefly, participants received electrical stimuli at the sural nerve separated by irregular intervals of 5 to 10 seconds to avoid stimulus habituation. Starting from 2 mA, stimulus intensity was increased in 2 mA steps until the RIII reflex appeared. An RIII reflex was detected if the mean EMG response during the interval from 90 to 150 ms post-stimulation was at least 1.5 times the size of the standard deviation of the baseline period (65 to 5 ms before stimulation). Then, stimulus intensity was decreased and increased in 0.5 mA steps until the reflex was no longer detected or appeared again in response to 2 successive stimuli. The RIII threshold was defined as the average of the 4 intensities when the reflex disappeared for the first time, appeared for the second time, disappeared for the second time, and finally appeared again.[38] The same procedure was used to quantify the pain threshold, using the appearance and disappearance of the pain sensation as a marker.

Quantification of RIII Areas, SEP Amplitudes, and Pain Intensity and Unpleasantness Ratings.—For suprathreshold stimulation, participants received a series of electrical stimuli with an intensity of ~170% of RIII threshold at pseudorandomized intervals of 8-12 seconds to avoid stimulus habituation. To determine the RIII reflex area, RIII traces were baseline adjusted (baseline: 65 to 5 ms before stimulation), rectified, integrated within the analysis window (90 to 150 ms after stimulation) and corrected for baseline activity by subtracting the rectified integrated baseline area. RIII reflex areas were averaged over 3 consecutive stimuli for illustration and over 2-minutes blocks (12 stimuli) for analysis. SEP traces were rejected when the amplitude exceeded 100 μV.[22] In addition, every SEP trace was visually inspected for artifacts. These procedures resulted in the rejection of 0-2 traces per 2-minutes block (each block consisted of 12 traces). Next, SEP traces were baseline adjusted (65 to 5 ms before stimulation) and averaged within each 2-minutes block. Averaged SEP traces consistently showed 4 components: (1) a positive peak around 45 ms (=P45),[40] (2) a negativity around 75-100 ms, likely corresponding to N100; (3) a negativity around 120 ms (=N120); (4) a broad positive

wave around 260 ms (=P260).[21,22] In accordance with our previous procedure,[25,41] we defined analysis windows from 35-50 ms after stimulation to pick up the P45 peak, 70-100 ms for the N100, 100-150 ms for the N120, and from 280-350 ms for the P260 and measured mean amplitudes within those windows.

At the end of each 2-minutes block (after 12 stimuli), subjects rated the average pain intensity and pain unpleasantness of the electrical sural nerve stimulation over the last 5 stimuli on the Numerical Rating Scale (NRS) from 0 to 10 (0 = no pain/unpleasantness, 10 = strongest pain/unpleasantness imaginable). Participants were asked to focus their pain intensity rating on the magnitude of pain, without taking into account emotional responses to pain such as aversion or fear, while focusing on the emotional aspects when giving the pain unpleasantness rating.

nVNS and Sham Devices.—There are different methods and devices to stimulate the vagus nerve in humans, ranging from implanted stimulators at the neck to non-invasive stimulation at the neck or at the vagal innervation area at the outer ear.[5,42,43] For the purpose of the present study (to investigate the physiological mechanisms underlying possible analgetic effects of VNS), we decided to use cervical nVNS with the GammaCore® device (Electrocore, Basking Ridge, USA) because several recent clinical studies demonstrate its efficacy in headache treatment.[1,2,5] The nVNS and sham devices (both provided by GammaCore®) looked identical and were marked with "A" and "B." Both, the experimenter (LA) and participants were blinded to the type of stimulation. Whereas the nVNS device produced a proprietary low-voltage (maximum 24 V) electrical signal consisting of 5 impulses each of 0.2 ms duration (5000 Hz), administered at 25 Hz, the sham device provided a low-frequency (0.1 Hz) biphasic electrical signal, designed by Electrocore to provide a sensation of stimulation without actually stimulating the vagus nerve. These correspond to the devices and stimulation parameters used in clinical studies.[4,5] For nVNS/sham stimulation, the experimenter placed the devices' 2 stainless steel contact areas at the trigonum caroticum over the carotid pulse. To improve contact, a high conductivity contact gel (Parker Laboratories) was used. According to the procedure in previous clinical and basic research studies, stimulus intensity was adjusted until the stimulation was perceived as strong but not unpleasant or painful.[4,5,44] The exact stimulation intensity needed varies individually as a function of neck geometry and was not recorded in this or the previous studies. Stimulation was applied for a total of 4 minutes (2 minutes on the right side of the neck, 2 minutes on the left), corresponding to the procedure used in the acute treatment of migraine.[5]

CPM Paradigm.—The CPM paradigm was conducted as an alternative measure of pain inhibition, including descending pain inhibition.[20,45,46] Two identical painful "test" stimuli (contact heat for 30s) were applied at left volar forearm once before and once during a painful "conditioning" stimulus (cold water bath for 60 seconds). During the preparatory session, heat and cold temperatures were tailored individually, targeting a pain of about 6 on the NRS (0-10) for the heat stimulus (resulting in an average temperature of 46.0 ± 1.5°C) and of at least 3 (after 30 seconds of application) for the cold water bath (resulting in an average temperature of 8.8 ± 2.9°C). These predetermined temperatures were used during both experimental sessions. Heat stimuli were applied using the 3 × 3 cm ATS Thermode of the Pathway Pain & Sensory Evaluation System (Medoc, Israel), using a baseline of 32°C, up and down ramps of 2°C per second and a time at target temperature of 30 seconds. Participants rated pain intensity on the NRS (0-10) every 10 seconds and the average of the 3 ratings was calculated. After a break of at least 5 minutes, participants put their open right hand up to the wrist into a styrofoam box filled with cold water at the target temperature for 60 seconds. After the first 30 seconds of cold stimulation, the "test" (heat) stimulus was initiated and participants again rated heat pain intensity every 10 seconds. The CPM effect was quantified as the difference in the average heat pain ratings obtained before and during the cold water bath. A negative CPM effect indicated the activation of pain inhibition by the "conditioning" stimulus.

Statistical Analyses.—For statistical analyses, the Statistical Package of Social Sciences, version 22 for Windows (IBM®, Armonk, NY, USA) was used. Descriptive statistics are mean ± standard deviation unless indicated otherwise. Two-sided tests were used throughout and statistical significance was assumed at

$P < .05$. Statistical testing for outliers was performed for all variables.

A 2-way repeated-measures analysis of variance (ANOVA) with the factors device (nVNS/sham) and time (Pre/StimR/StimL/Post) was performed to test for immediate effects of nVNS on RIII areas (primary outcome, hypothesis: nVNS induces a larger reduction of RIII areas compared to sham stimulation (superiority testing)) and on SEP amplitudes (only Pre and Post), pain intensity, and unpleasantness ratings (secondary outcomes) during the 10-minutes suprathreshold sural nerve stimulation run.

To evaluate effects of nVNS on RIII areas, SEP amplitudes, pain intensity, unpleasantness ratings, RIII and pain thresholds up to 60 minutes after stimulation (secondary outcomes), 2-way repeated-measures ANOVA with the factors device (nVNS/sham) and time (baseline/5′/15′/30′/60 minutes follow-up) was conducted. The CPM effect on the test stimulus pain intensity rating was assessed using a 2-way repeated-measures ANOVA with the factors device (nVNS/sham) and conditioning (before/during cold water bath). To test for changes in PANAS scores, 2-way repeated-measures ANOVAs with the factors device (nVNS/sham) and time (baseline/5 minutes post) were conducted for both positive and negative affect scores. Violations of sphericity were corrected using Greenhouse-Geisser adjustment. Effect sizes were quantified by partial eta squared. Significant differences were further analyzed using Bonferroni adjusted targeted post hoc 2-way repeated-measures ANOVAs or Bonferroni adjusted t tests for paired samples. The number of comparisons that Bonferroni correction was applied for is indicated in the text. 95% confidence intervals (CI) are reported for pairwise comparisons. McNemar Test was used for the blinding check.

nVNS or sham stimulation was applied in a randomized order as detailed above. Including the order of nVNS/sham stimulation in the ANOVA did not reveal significant main effects of order or significant interactions with the order for any of these variables (see Supporting Tables S6 and S7), so for the ease of interpretation, we report the results without this additional factor.

Table 1.—Characteristics of the study population (n = 23)

	Mean	Standard deviation	Range
Age (y)	27.1	1.3	18-45
% Female	60.9	-	-
PCS	13.9	9.6	0-28
BDI	3.7	3.6	0-13
STAI state	33.9	7.8	24-52
STAI trait	33.5	7.6	21-54

No participant had questionnaire scores above the clinically relevant cut-off values (see Methods).
BDI = Beck Depression Inventory; PCS = Pain Catastrophizing Scale; STAI = State-Trait Anxiety Inventory.

A total of 24 subjects participated in nVNS and sham stimulation in a randomized order on 2 separate days as shown in Figure 1. Box plot analysis was conducted for all outcome variables and revealed extreme values (exceeding the triple interquartile range of the sample) for change in RIII area at 30 and 60 minutes after stimulation in 1 subject. On visual inspection, this was due to a strong and continuous upward trend of the RIII reflex size starting 15 minutes after stimulation, that was not seen in any other subject or in the other experiments of the same subject. We judged this most likely to be due to a technical problem with stimulation (eg, shifting of the stimulation electrode) and excluded this subject. Thus, the final sample consisted of 23 participants.

RESULTS

Data from 23 subjects were included in the analysis. Age, sex, and results of the PCS, BDI, and STAI questionnaires are shown in Table 1. Mean sural nerve stimulation intensity was 14.2 ± 3.9 mA during the nVNS session and 14.1 ± 4.1 mA for the sham session ($t_{[22]} = -0.21$, $P = .827$, mean difference: −0.2 mA (95%CI: −1.6; 1.3). None of the participants spontaneously reported any adverse event of the nVNS or sham stimulation.

Please note that within this section, "significant" always means "statistically significant."

Immediate Effects of nVNS.—The immediate effects of nVNS/sham stimulation were assessed during a 10-minutes experimental run performed immedi-

ately before/during/after nVNS/sham as illustrated in Figure 1. Results are shown in Figure 2 and Supporting Tables S1 and S3. In summary, there was no immediate effect of nVNS vs sham stimulation on RIII reflex areas, SEP amplitudes, and pain intensity ratings. However, unpleasantness ratings decreased significantly during nVNS stimulation compared to the immediately pre/post-stimulation blocks and compared to sham stimulation.

In more detail, ANOVA on *RIII reflex areas* showed no main effects for device ($F_{[1,22]} = 0.21$, $P = .649$) or time ($F_{[2.1,46.4]} = 1.87$, $P = .164$), and no significant interaction between both ($F_{[3,66]} = 0.67$, $P = .574$). *SEPs* were available only for the pre and post-blocks because of VNS/sham stimulation artifacts. ANOVA revealed no significant interaction between device and time for any of the 4 SEP components ($F_{[1,22]} = 0.11$, $P = .75$ (P45), $F_{[1,22]} = 0.00$, $P = .977$ (N100), $F_{[1,22]} = 0.24$, $P = .630$ (N120), and $F_{[1,22]} = 0.00$, $P = .96$ (P260)). There were also no significant main effects of device or time (see Supporting Table S3). For *pain intensity ratings*, there was no main effect of time ($F_{[2.1,46.5]} = 3.14$, $P = .050$) or device ($F_{[1,22]} = 0.10$, $P = .751$) and no interaction ($F_{[1.9,42.7]} = 0.55$, $P = .576$). For *pain unpleasantness ratings*, ANOVA revealed no main effect of device ($F_{[1,22]} = 0.77$, $P = .390$), but a significant main effect of time ($F_{[1.9,41.9]} = 4.10$, $P = .025$, $\eta^2_p = 0.157$), and a significant interaction effect between device and time ($F_{[3,66]} = 3.06$, $P = .034$,

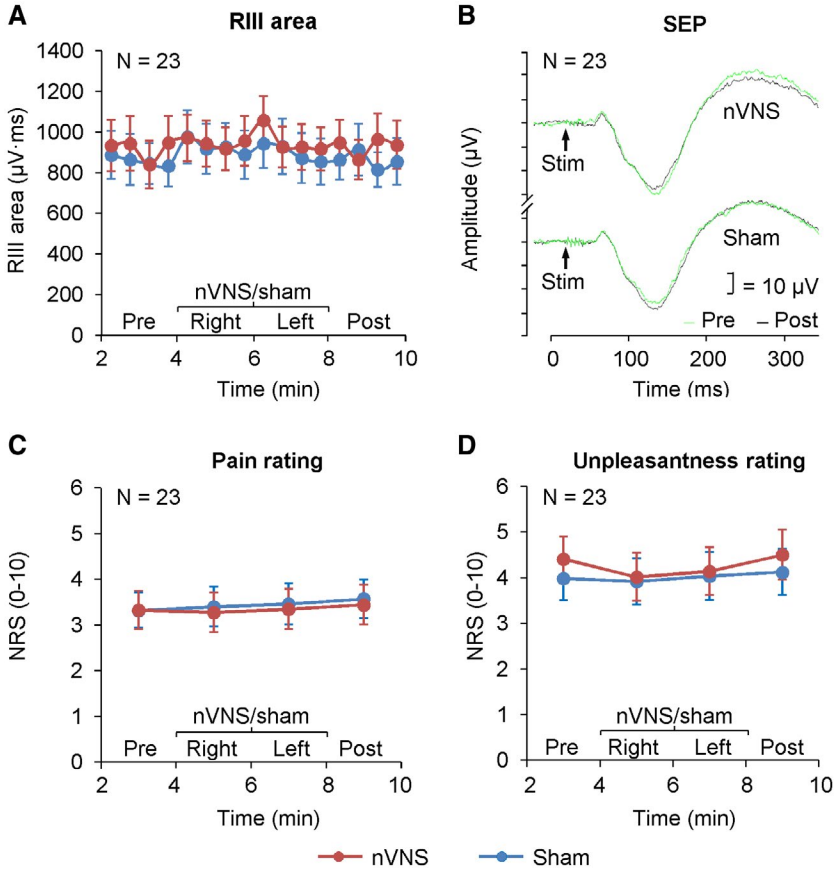

Fig. 2.—Immediate effects of nVNS/sham. RIII reflex areas (A), SEP amplitudes (B), pain intensity (C), and unpleasantness ratings (D) in response to sural nerve stimulation before, during, and directly after nVNS or sham stimulation are illustrated. For RIII reflex areas, each data point illustrates a 30 seconds epoch, of 3 reflexes. SEP traces were averaged over 2 minutes and are available only during "Pre" (green) and "Post" (black) intervention blocks. Pain intensity and unpleasantness ratings were obtained every 2 minutes, as an average rating of the preceding 5 stimuli. Values are mean ± SEM. Statistical analyses revealed a statistically significant reduction of unpleasantness ratings during nVNS compared to sham stimulation, but no nVNS effects on the other parameters (see Results). [Color figure can be viewed at wileyonlinelibrary.com]

η^2_p = 0.122). A post hoc test comparing the 2 nVNS (respectively sham) stimulation blocks combined with the pre and post-blocks combined showed that unpleasantness ratings were significantly lower during nVNS/sham stimulation than immediately before/after stimulation ($F_{[1,22]}$ = 6.82, P = .016, η^2_p = 0.237). Further subordinate testing showed a significant decrease of unpleasantness ratings during nVNS ($F_{[1,22]}$ = 12.16, P = .004, η^2_p = 0.356, mean difference: −0.4 (NRS 0-10) (95%CI: −0.6; −0.2), but not during sham stimulation ($F_{[1,22]}$ = 0.68, P = .838, mean difference: −0.1 (NRS 0-10) (95%CI: −0.3; 0.1); corrected for 2 comparisons). Unpleasantness ratings in the nVNS session were 4.5 ± 2.5 before/after and 4.1 ± 2.5 during stimulation. In the sham session, they were 4.1 ± 2.3 before/after and 4.0 ± 2.5 during stimulation.

Effect of nVNS up to 60Minutes After Stimulation.— Effects of nVNS/sham stimulation were assessed during 2-minutes blocks at the baseline and 5, 15, 30, and 60 minutes after stimulation (Fig. 1). Results are shown in Figure 3 and in Supporting Tables S2, S4, and S5. In summary, there was no effect of nVNS vs sham stimulation on RIII reflex areas, SEPs, RIII and pain thresholds and pain intensity and unpleasantness ratings up to 60 minutes after stimulation compared to

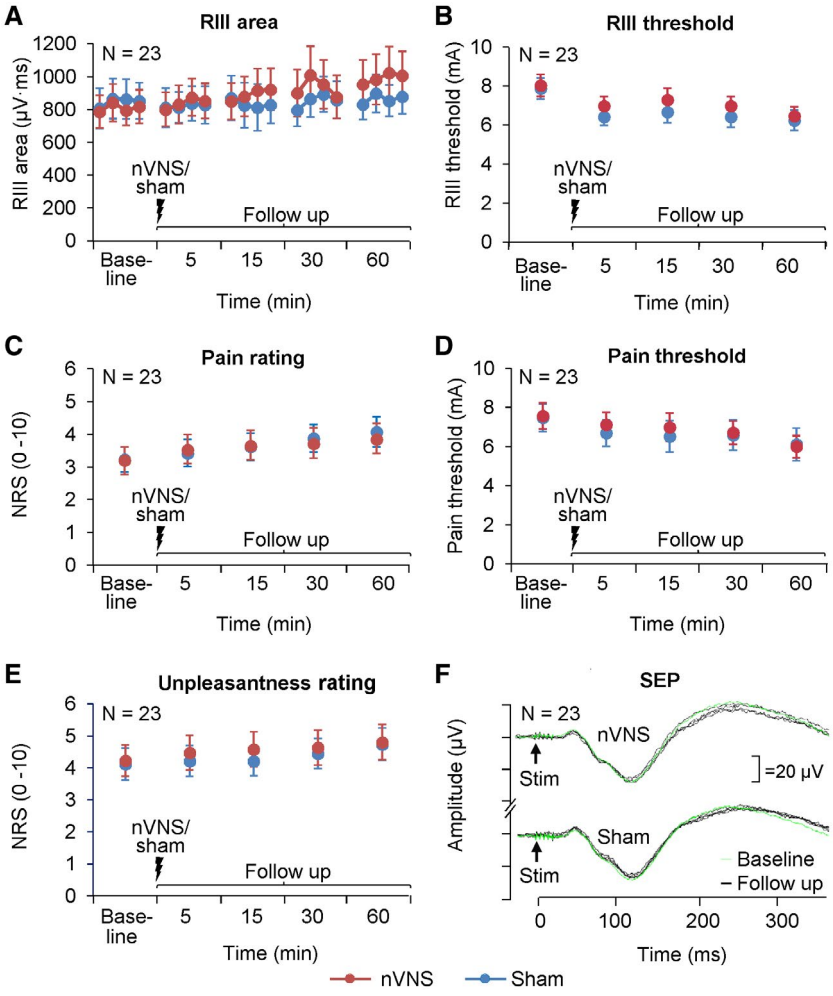

Fig. 3.—Effect of nVNS/sham stimulation up to 60 minutes after intervention. RIII reflex areas (A) and thresholds (B), pain intensity ratings (C) and thresholds (D), pain unpleasantness ratings (E), and SEP amplitudes (F) in response to sural nerve stimulation are illustrated at baseline and 5, 15, 30, and 60 minutes after nVNS/sham stimulation. Baseline SEP traces are marked in green, traces up to 60 minutes after nVNS/sham stimulation in black. Values are mean ± SEM. Statistical analyses revealed no significant differences between nVNS and sham stimulation (see Results). [Color figure can be viewed at wileyonlinelibrary.com]

baseline. Pain intensity and unpleasantness ratings increased over time, while RIII reflex and pain thresholds decreased over time.

In detail, for *RIII reflex areas*, there was no significant main effect of device ($F_{[1,22]} = 0.25$, $P = .619$) or time ($F_{[2.0,44.5]} = 2.70$, $P = .078$), and no significant interaction ($F_{[1.7,37.4]} = 1.29$, $P = .283$). For *SEP amplitudes* there was no significant interaction between device and time for P45 ($F_{[4,88]} = 1.91$, $P = .115$), N100 ($F_{[4,88]} = 0.83$, $P = .512$), N120 ($F_{[4,88]} = 2.30$, $P = .065$), and P260 ($F_{[4,88]} = 1.15$, $P = .340$) and no main effects of device or time (Supporting Table S4). For both *RIII and pain thresholds*, ANOVA revealed a significant effect of time ($F_{[4,88]} = 22.08$, $P < .0001$, $\eta^2_p = 0.501$ (RIII); $F_{[2.5,54.5]} = 17.66$, $P < .0001$, $\eta^2_p = 0.445$ (pain)) but no main effect of device ($F_{[1,22]} = 0.87$, $P = .362$ (RIII); $F_{[1,22]} = 0.23$, $P = .639$ (pain)) and no interaction between device and time ($F_{[2.6,58.0]} = 0.84$, $P = .467$ (RIII); $F_{[4,88]} = 1.10$, $P = .363$ (pain)). Post hoc tests comparing each of the 4 time points to baseline (corrected for 4 comparisons) showed that both RIII and pain thresholds decreased significantly over time (all $P < .005$, see Supporting Table S5). ANOVA on *pain intensity ratings* showed a significant main effect of time ($F_{[1.5,33.8]} = 9.35$, $P = .001$, $\eta^2_p = 0.298$), but no main effect of device ($F_{[1,22]} = 0.04$, $P = .843$) and no significant interaction ($F_{[4,88]} = 2.28$, $P = .067$). Post hoc t-tests revealed that pain ratings significantly increased over time when compared to baseline ($P < .05$ for all time points, see Supporting Table S5). For *pain unpleasantness*, ANOVA revealed a significant main effect of time ($F_{[2.6,56.9]} = 8.57$, $P < .0001$, $\eta^2_p = 0.280$), but no significant main effect of device ($F_{[1,22]} = 0.41$, $P = .529$) or interaction ($F_{[4,88]} = 0.77$, $P = .546$). Post hoc t-tests showed significantly higher unpleasantness ratings at 60 minutes compared to baseline ($t_{[22]} = 4.14$, $P < .001$, mean difference: 0.6 (NRS 0-10) (95%CI: 0.3; 0.9) corrected for 4 comparisons), but not at the other time points (see Supporting Table S5).

Effect of nVNS on CPM.—The CPM test was performed at the end of each experiment (~70 minutes after nVNS/sham stimulation). Shortly, there was a significant CPM effect, which was not significantly affected by nVNS vs sham stimulation. Average test pain ratings before conditioning stimulation were 4.8 ± 1.8 (nVNS session) and 5.3 ± 2.1 (sham session). Average test pain ratings during conditioning stimulation were 3.8 ± 1.9 (nVNS) and 4.1 ± 1.9 (sham). ANOVA revealed a significant main effect of conditioning ($F_{[1,22]} = 18.21$, $P < .001$, $\eta^2_p = 0.453$, mean difference: −1.1 (NRS 0-10) (95%CI: –2.0; –0.6), ie, a significant CPM effect amounting to a total of −20.3 ± 25.7% after nVNS and −18.1 ± 34.4% after sham stimulation. No significant interaction effect ($F_{[1,22]} = 1.09$, $P = .307$) and significant main effect of device ($F_{[1,22]} = 2.12$, $P = .159$, mean difference: −0.4 (NRS 0-10) (95%CI: −0.9; 0.2) were found.

Effects of nVNS on PANAS Ratings.—The PANAS was assessed at baseline and 5 minutes after nVNS/sham stimulation (Supporting Table S2). In summary, there was a small but a significant decrease of positive affect over time, independent of nVNS or sham stimulation. ANOVA showed a significant effect of time on positive affect ($F_{[1,22]} = 8.23$, $P = .009$, $\eta^2_p = 0.272$, mean difference: –0.2 (PANAS scale: 1-5) (95%CI: −0.3; −0.04) but not negative affect ($F_{[1,22]} = 0.20$, $P = .657$, mean difference: 0.0 (95%CI: 0.0; 0.1). Participants' positive affect score decreased over time from 2.6 ± 0.6 (baseline) to 2.4 ± 0.7 (5 minutes). There was no significant interaction between device and time on subjects´ positive ($F_{[1,22]} = 0.17$, $P = .685$) or negative affect score ($F_{[1,22]} = 0.01$, $P = .913$) and no significant main effect of device ($F_{[1,22]} = 0.72$, $P = .404$, mean difference: 0.1 (95%CI: −0.1; 0.3) (positive affect); $F_{[1,22]} = 0.14$, $P = .716$, mean difference: 0.0 (95%CI: −0.2; 0.1) (negative affect)).

Blinding Check.—At the end of each experimental session, participants indicated whether they believed that they had received nVNS or sham stimulation. After the sham and nVNS session, 43.5% and 60.9% of the subjects believed to have received true nVNS stimulation, respectively, which was not significantly different (McNemar chi-square = 1.143, $P = .285$).

DISCUSSION

The main results of the present study are as follows. No activation of the descending pain inhibition could be demonstrated during and up to an hour after nVNS, as shown by a lack of effect of nVNS on the RIII reflex and the CPM effect. nVNS also did not affect supraspinal nociceptive responses as quantified by pain intensity ratings and late SEPs. However, there

was a small but statistically significant reduction of pain unpleasantness ratings during nVNS compared to sham stimulation.

Effects of nVNS on Descending Pain Inhibition (RIII Reflex and CPM Paradigm).—nVNS had no effect on RIII reflex areas during and up to an hour after nVNS, or on RIII thresholds up to an hour after nVNS. In contrast, De Icco et al[44] found a statistically significant increase of RIII reflex thresholds 5 and 30 minutes after nVNS compared to stimulation at the wrist in 10 healthy participants. Similar to our results, they also did not find an effect on RIII reflex areas. Differences in the present study may include using stimulation at the wrist as control instead of sham stimulation at the neck. Although the sham stimulation at the neck has been explicitly designed to not activate the vagus nerve and has successfully been used in several clinical studies,[3-5] it has recently been discussed if it is indeed completely inactive as effects on EEG[47] and the trigemino-autonomic reflex[48] has been reported. However, in our study, neither nVNS nor sham demonstrated a statistically significant antinociceptive effect compared to baseline.

Although the RIII reflex is a well-established marker of human spinal nociception[17,18] that is sensitive for the detection of changes in the descending pain modulation, eg, by stimulation of the periaqueductal gray[23] and cognitive strategies,[25,39] it also has its limitations. These include a little contribution of C-fiber (vs Aδ-fiber) mediated nociception and the influence of interneuron and motor components.[17,18,49] Therefore, we also investigated the effect of nVNS on the CPM effect, another paradigm used to assess pain inhibition, including descending pain inhibition.[20] Consistent with the RIII reflex results, there was no effect of nVNS on CPM. This is similar to previous studies using different stimulation parameters and investigating other cohorts (eg, chronic pain patients), that also found no effect of VNS on CPM.[16,50]

When looking at the animal literature in more detail, cervical VNS effects on spinal nociception are mixed. While the inhibition of the tail-flick response predominates at strong VNS, facilitation is seen with less intense VNS. At the single dorsal horn neuron level, some neurons are consistently inhibited or consistently facilitated by VNS, while many show a biphasic response, depending on VNS intensity.[10] The present results suggest that at the clinically used nVNS stimulation parameters, there is no net effect on descending pain modulatory pathways, at least not during and after a single short (4 minutes) bout of stimulation.

Effects of nVNS on Pain Intensity and Unpleasantness Ratings.—There was no direct analgesic effect of nVNS as measured by pain intensity ratings and pain thresholds, both obtained in response to the electrical stimulation used to evoke the RIII reflex. Similarly, previous studies using auricular VNS also found no nVNS induced changes in heat pain thresholds,[15] electrical pain thresholds,[14] electrical pain intensity ratings,[44] or pain evoked by laser stimulation.[47] However, there is also evidence for pro-[51] and anti-nociceptive effects of VNS[52,53] in experimental pain testing.

These heterogeneous results have been hypothesized to be due to contrary effects of nVNS on different psychological factors. Frangos et al[12] proposed that nVNS increases attention toward pain and therefore may increase pain intensity ratings, while at the same time enhancing mood, thereby reducing the reaction to pain. Consistently, there has been evidence from human fMRI studies that nVNS reduced the activity of the medial pain system without altering pain thresholds.[15] Positive effects of nVNS on mood and anxiety have also been reported.[16,54] Indeed, we found that subjects estimated electrical stimulation of the sural nerve as less unpleasant during nVNS than immediately before/after stimulation. Although this effect was small, it was not found during the sham session. However, there were no longer lasting effects of nVNS on pain unpleasantness (5-60 minutes after stimulation). The PANAS, conducted at baseline and 5 minutes after stimulation failed to detect positive changes in subjects´ mood. This could argue for an immediate and short-lived effect of nVNS on pain unpleasantness independent from mood, but given the small size of the effect, this interpretation remains speculative and needs to be tested in further studies.

Effects of nVNS on SEPs.—Late SEPs have nociceptive and non-nociceptive contributions.[21,22] Consistent with previous work,[22,25] we identified 4 SEP components: (1) the P45, likely generated in primary somatosensory cortex,[40] (2) the N100, that originates around the primary somatosensory cortex and

spreads to parietal association areas, (3) the N120, generated in the parietal operculum and insula, and (4) the P260, likely generated in multiple areas, including the anterior cingulate cortex, inferior parietal cortex, and supplementary somatosensory association areas.[21,22]

In the present study, we did not find an effect of nVNS on any of these SEP components. To the best of our knowledge, this is the first study to investigate the effect of VNS on late SEPs. However, a previous study detected a statistically significant effect of nVNS over sham on laser-evoked P2 potentials (corresponding to the P260 component described above) during stimulation, which was short-lived (almost back to baseline 2 minutes after stimulation).[47] In our hands, artifacts of the nVNS/sham stimulation prevented the analysis of SEPs during stimulation, so that a direct comparison with the previous study was not possible. A previous functional imaging study has shown the activation of several cortical regions, including prefrontal, anterior insular, and anterior cingulate cortex during and up to 10 minutes after nVNS.[12] While these are regions contributing to late SEP components (see above), it must be considered that SEPs represent responses of these regions to nociceptive (and also non-nociceptive) stimulation, not spontaneous activity. It should also be noted that the montage used in the present study (vertex vs frontal reference) was chosen to detect pain intensity reducing effects of nVNS and therefore to increase the contributions of afferent nociceptive areas (primary somatosensory cortex, operculum, insula), while reducing the contribution of areas involved in pain modulation (anterior cingulate cortex, prefrontal cortex).

Strengths and Limitations.—Important strengths include the rigorous sham-controlled, double-blind cross-over design, and the use of a number of different measures to assess descending pain inhibition (RIII reflex areas and thresholds, CPM effect), pain (pain intensity ratings and thresholds), supraspinal nociceptive responses (SEPs), and affective components (pain unpleasantness and mood).

Limitations include the following. Firstly, we did not directly corroborate the successful stimulation of the vagus nerve. As nVNS mainly stimulates afferent vagal fibers,[55] heart rates are not useful to confirm successful nVNS.[16,56] However, previous results strongly suggest that cervical nVNS indeed activates vagal afferents. For example, cervical nVNS statistically significantly activates vagal afferent projection areas, including the nucleus tractus solitarii[12] and, somatosensory potentials evoked by cervical nVNS (vagal SEPs) are comparable to those evoked by invasive and auricular VNS.[57]

Secondly, there are some possible limitations concerning the RIII reflex recording. Although we used irregular stimulation intervals > 1 second (between 5 and 12 seconds), which have previously been described to minimize RIII reflex habituation,[58,59] we cannot completely exclude that the relatively large number of suprathreshold stimuli used during the course of the experiment may have induced some habituation, possibly reducing the ability of nVNS to induce further reflex reduction. However, RIII reflexes were stable over time and of medium size, allowing modulation in both directions. An additional concern may be that electrical sural nerve stimulation might have interfered with the action of nVNS on descending pathways, as both were simultaneously applied to allow the assessment of immediate nVNS effects. Both points are further differences to the previous study of de Icco (2018)[44] which might have contributed to the divergent results. Moreover, similar protocols have successfully demonstrated the effect of psychological interventions on the reflex size, proving that the RIII reflex can be modulated.[26,43] In addition, although we measured the nVNS effect on RIII reflex thresholds and on suprathreshold RIII reflex sizes, we did not assess RIII temporal summation thresholds, which would have allowed quantifying the nVNS effect on the spinal windup phenomenon. This would be an important follow-up project. Thirdly, although most of the positive clinical results have been obtained in headache, we used a paradigm assessing the activation of descending pain inhibition as assessed by the measures of somatic (not trigeminal) nociception and pain perception. However, descending pain inhibitory pathways are thought to lack somatotopic organization, affecting the entire body. Fourthly, to avoid interference with the experimental procedure (RIII reflex and SEP recording), pain and unpleasantness

ratings were measured at the end of every 2-minutes block as an average of the preceding 5 stimuli, entailing a risk of recall bias. Moreover, we studied healthy young participants, whereas clinical studies providing evidence for an analgesic nVNS effect investigated chronic pain patients. The larger effect of VNS in clinical vs healthy populations might be related to autonomic dysfunction in chronic pain and headache disorders.[60,61] In addition, the present study only investigated the short term (up to 1 hour) effects of a single short (2 × 2 minutes) bout of nVNS. In clinical studies, both short-term effects of a single stimulation bout and long-term effects of repeated stimulation have been reported.[1-5] Clearly, further studies are needed to clarify the relation of nVNS effects on experimental pain in healthy volunteers to those on clinical pain in patients. Moreover, from the different methods and devices available for VNS in humans (invasive and non-invasive stimulation at the neck, stimulation of the vagal innervation area at the outer ear[42,43]), we investigated only the effect of cervical nVNS. It is not clear if the results can be generalized to other forms of VNS. Finally, as a general rule, the imprecision of the results due to measurement error (eg, random error) has always to be considered.

CONCLUSIONS

The present results do not support an acute effect of nVNS on descending pain inhibition, pain intensity perception or supraspinal nociception in healthy adults. However, there was a small effect on pain unpleasantness during nVNS, suggesting that nVNS may preferentially act on affective, not somatosensory pain components in the present setting. The present study adds to a body of literature showing little or inconsistent effects of VNS on experimental pain in healthy subjects, while clinical studies have been encouraging regarding both acute and preventive effects in migraine and cluster headache. Maybe this means that VNS specifically targets nociceptive mechanisms of clinical pain, different from those that can be investigated by experimental pain testing.

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REFERENCES

1. Gaul C, Diener HC, Silver N, et al. Non-invasive vagus nerve stimulation for PREVention and Acute treatment of chronic cluster headache (PREVA): A randomised controlled study. *Cephalalgia*. 2016; 36:534-546.
2. Goadsby PJ, de Coo IF, Silver N, et al. Non-invasive vagus nerve stimulation for the acute treatment of episodic and chronic cluster headache: A randomized, double-blind, sham-controlled ACT2 study. *Cephalalgia*. 2018;38:959-969.
3. Silberstein SD, Calhoun AH, Lipton RB, et al. Chronic migraine headache prevention with noninvasive vagus nerve stimulation: The EVENT study. *Neurology*. 2016;87:529-538.
4. Silberstein SD, Mechtler LL, Kudrow DB, et al. Non-invasive vagus nerve stimulation for the acute treatment of cluster headache: Findings from the randomized, double-blind, sham-controlled ACT1 study. *Headache*. 2016;56:1317-1332.
5. Tassorelli C, Grazzi L, de Tommaso M, et al. Noninvasive vagus nerve stimulation as acute therapy for migraine: The randomized PRESTO study. *Neurology*. 2018;91:e364-e373.

6. Lange G, Janal MN, Maniker A, et al. Safety and efficacy of vagus nerve stimulation in fibromyalgia: A phase I/II proof of concept trial. *Pain Med*. 2011;12:1406-1413.
7. Frangos E, Richards EA, Bushnell MC. Do the psychological effects of vagus nerve stimulation partially mediate vagal pain modulation? *Neurobiol Pain*. 2017;1:37-45.
8. Akerman S, Simon B, Romero-Reyes M. Vagus nerve stimulation suppresses acute noxious activation of trigeminocervical neurons in animal models of primary headache. *Neurobiol Dis*. 2017;102:96-104.
9. Chen SP, Ay I, de Morais AL, et al. Vagus nerve stimulation inhibits cortical spreading depression. *Pain*. 2016;157:797-805.
10. Randich A, Gebhart GF. Vagal afferent modulation of nociception. *Brain Res Brain Res Rev*. 1992;17:77-99.
11. Basbaum AI, Fields HL. Endogenous pain control mechanisms: Review and hypothesis. *Ann Neurol*. 1978;4:451-462.
12. Frangos E, Komisaruk BR. Access to vagal projections via cutaneous electrical stimulation of the neck: fMRI evidence in healthy humans. *Brain Stimul*. 2017;10:19-27.
13. Yakunina N, Kim SS, Nam EC. Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation*. 2017;20:290-300.
14. Laqua R, Leutzow B, Wendt M, Usichenko T. Transcutaneous vagal nerve stimulation may elicit anti- and pro-nociceptive effects under experimentally-induced pain – A crossover placebo-controlled investigation. *Auton Neurosci*. 2014;185:120-122.
15. Usichenko T, Laqua R, Leutzow B, Lotze M. Preliminary findings of cerebral responses on transcutaneous vagal nerve stimulation on experimental heat pain. *Brain Imaging Behav*. 2017;11:30-37.
16. Napadow V, Edwards RR, Cahalan CM, et al. Evoked pain analgesia in chronic pelvic pain patients using respiratory-gated auricular vagal afferent nerve stimulation. *Pain Med*. 2012;13:777-789.
17. Sandrini G, Serrao M, Rossi P, Romaniello A, Cruccu G, Willer JC. The lower limb flexion reflex in humans. *Prog Neurogibol*. 2005;77:353-395.
18. Skljarevski V, Ramadan NM. The nociceptive flexion reflex in humans – Review article. *Pain*. 2002;96:3-8.
19. Willer JC, Boureau F, Albe-Fessard D. Supraspinal influences on nociceptive flexion reflex and pain sensation in man. *Brain Res*. 1979;179:61-68.
20. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y. Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. *Pain*. 2012;153:1193-1198.
21. Dowman R. SEP topographies elicited by innocuous and noxious sural nerve stimulation. I. Identification of stable periods and individual differences. *Electroencephalogr Clin Neurophysiol*. 1994;92:291-302.
22. Dowman R, Darcey T, Barkan H, Thadani V, Roberts D. Human intracranially-recorded cortical responses evoked by painful electrical stimulation of the sural nerve. *NeuroImage*. 2007;34:743-763.
23. Fields HL, Basbaum AI. Brainstem control of spinal pain-transmission neurons. *Annu Rev Physiol*. 1978;40:217-248.
24. Hirsh AT, George SZ, Bialosky JE, Robinson ME. Fear of pain, pain catastrophizing, and acute pain perception: Relative prediction and timing of assessment. *J Pain*. 2008;9:806-812.
25. Ruscheweyh R, Baumler M, Feller M, Krafft S, Sommer J, Straube A. Learned control over spinal nociception reduces supraspinal nociception as quantified by late somatosensory evoked potentials. *Pain*. 2015;156:2505-2513.
26. Ruscheweyh R, Kreusch A, Albers C, Sommer J, Marziniak M. The effect of distraction strategies on pain perception and the nociceptive flexor reflex (RIII reflex). *Pain*. 2011;152:2662-2671.
27. Asmundson GJ, Katz J. Understanding the co-occurrence of anxiety disorders and chronic pain: State-of-the-art. *Depress Anxiety*. 2009;26:888-901.
28. Dickens C, McGowan L, Dale S. Impact of depression on experimental pain perception: A systematic review of the literature with meta-analysis. *Psychosom Med*. 2003;65:369-375.
29. Meyer K, Sprott H, Mannion AF. Cross-cultural adaptation, reliability, and validity of the German version of the Pain Catastrophizing Scale. *J Psychosom Res*. 2008;64:469-478.
30. Sullivan MJ, Bishop SR, Pivik J. The pain catastrophizing scale: Development and validation. *Psychol. Assess*. 1995;7:524-532.
31. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561-571.
32. Hautzinger M, Keller F, Kühner C. *Das Beck Depressionsinventar II. Deutsche Bearbeitung und Handbuch zum BDI II*. Frankfurt: Harcourt Test Services; 2006.

33. Laux L, Glanzmann P, Schaffner P, Spielberger CD. *State-Trait-Angstinventar (STAI), Theoretische Grundlagen und Handanweisungen*. Weinheim: Beltz; 1981.
34. Spielberger CD, Gorsuch R, Lushene RL. *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press; 1970.
35. Kuhner C, Burger C, Keller F, Hautzinger M. [Reliability and validity of the Revised Beck Depression Inventory (BDI-II). Results from German samples]. *Nervenarzt*. 2007;78:651-656.
36. Krohne HW, Egloff B, Kohlmann C-W, Tausch A. Untersuchungen mit einer deutschen Version der «Positive and Negative Affect Schedule» (PANAS). *Diagnostica*. 1996;42:139-156.
37. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: The PANAS scales. *J Pers Soc Psychol*. 1988;54:1063-1070.
38. France CR, Rhudy JL, McGlone S. Using normalized EMG to define the nociceptive flexion reflex (NFR) threshold: Further evaluation of standardized NFR scoring criteria. *Pain*. 2009;145:211-218.
39. Rhudy JL, France CR. Defining the nociceptive flexion reflex (NFR) threshold in human participants: A comparison of different scoring criteria. *Pain*. 2007;128:244-253.
40. Allison T, McCarthy G, Luby M, Puce A, Spencer DD. Localization of functional regions of human mesial cortex by somatosensory evoked potential recording and by cortical stimulation. *Electroencephalogr Clin Neurophysiol*. 1996;100:126-140.
41. Krafft S, Gohmann HD, Sommer J, Straube A, Ruscheweyh R. Learned control over spinal nociception in patients with chronic back pain. *Eur J Pain*. 2017;21:1538-1549.
42. Mauskop A. Vagus nerve stimulation relieves chronic refractory migraine and cluster headaches. *Cephalalgia*. 2005;25:82-86.
43. Straube A, Ellrich J, Eren O, Blum B, Ruscheweyh R. Treatment of chronic migraine with transcutaneous stimulation of the auricular branch of the vagal nerve (auricular t-VNS): A randomized, monocentric clinical trial. *J Headache Pain*. 2015;16:543.
44. De Icco R, Martinelli D, Bitetto V, et al. Peripheral vagal nerve stimulation modulates the nociceptive withdrawal reflex in healthy subjects: A randomized, cross-over, sham-controlled study. *Cephalalgia*. 2018;38:1658-1664.
45. Yarnitsky D, Crispel Y, Eisenberg E, et al. Prediction of chronic post-operative pain: Pre-operative DNIC testing identifies patients at risk. *Pain*. 2008;138: 22-28.
46. Granot M, Weissman-Fogel I, Crispel Y, et al. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: Do conditioning stimulus painfulness, gender and personality variables matter? *Pain*. 2008;136: 142-149.
47. Vecchio E, Bassez I, Ricci K, Tassorelli C, Liebler E, de Tommaso M. Effect of non-invasive vagus nerve stimulation on resting-state electroencephalography and laser-evoked potentials in migraine patients: Mechanistic insights. *Front Hum Neurosci*. 2018;12:366.
48. Schroeder CF, Möller M, May A. nVNS sham stimulation significantly affects the trigeminal-autonomic reflex: A single-blind, randomized, controlled study. *Neurology*. 2019;93:e518-e521.
49. Schouenborg J, Weng HR, Kalliomaki J, Holmberg H. A survey of spinal dorsal horn neurones encoding the spatial organization of withdrawal reflexes in the rat. *Exp Brain Res*. 1995;106:19-27.
50. Frokjaer JB, Bergmann S, Brock C, et al. Modulation of vagal tone enhances gastroduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol Motil*. 2016;28:592-598.
51. Borckardt JJ, Kozel FA, Anderson B, Walker A, George MS. Vagus nerve stimulation affects pain perception in depressed adults. *Pain Res Manag*. 2005;10:9-14.
52. Kirchner A, Stefan H, Bastian K, Birklein F. Vagus nerve stimulation suppresses pain but has limited effects on neurogenic inflammation in humans. *Eur J Pain*. 2006;10:449-455.
53. Busch V, Zeman F, Heckel A, Menne F, Ellrich J, Eichhammer P. The effect of transcutaneous vagus nerve stimulation on pain perception–an experimental study. *Brain Stimul*. 2013;6:202-209.
54. Kraus T, Hosl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *J Neural Transm (Vienna)*. 2007;114:1485-1493.
55. Mourdoukoutas AP, Truong DQ, Adair DK, Simon BJ, Bikson M. High-resolution multi-scale computational model for non-invasive cervical vagus nerve stimulation. *Neuromodulation*. 2018;21:261-268.

56. Oshinsky ML, Murphy AL, Hekierski H Jr, Cooper M, Simon BJ. Noninvasive vagus nerve stimulation as treatment for trigeminal allodynia. *Pain*. 2014;155:1037-1042.
57. Nonis R, D'Ostilio K, Schoenen J, Magis D. Evidence of activation of vagal afferents by non-invasive vagus nerve stimulation: An electrophysiological study in healthy volunteers. *Cephalalgia*. 2017;37:1285-1293.
58. Arendt-Nielsen L, Brennum J, Sindrup S, Bak P. Electrophysiological and psychophysical quantification of temporal summation in the human nociceptive system. *Eur J Appl Physiol*. 1994;68:266-273.
59. Dimitrijevic MR, Faganel J, Gregoric M, Nathan PW, Trontelj JK. Habituation: Effects of regular and stochastic stimulation. *J Neurol Neurosurg Psychiatry*. 1972;35:234-242.
60. Shechter A, Stewart WF, Silberstein SD, Lipton RB. Migraine and autonomic nervous system function: A population-based, case-control study. *Neurology*. 2002;58:422-427.
61. Tracy LM, Ioannou L, Baker KS, Gibson SJ, Georgiou-Karistianis N, Giummarra MJ. Meta-analytic evidence for decreased heart rate variability in chronic pain implicating parasympathetic nervous system dysregulation. *Pain*. 2016;157:7-29.

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