Noninvasive vagus nerve stimulation and the trigeminal autonomic reflex
An fMRI study

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Abstract

Objective
The trigeminal autonomic reflex is a physiologic reflex that plays a crucial role in primary headache and particularly in trigeminal autonomic cephalalgias, such as cluster headache. Previous studies have shown that this reflex can be modulated by the vagus nerve, leading to an inhibition of the parasympathetic output of the reflex in healthy participants. The aim of the present study was to characterize neural correlates of the modulatory effect of noninvasive vagus nerve stimulation (nVNS) on the trigeminal autonomic reflex.

Methods
Twenty-one healthy participants were included in a 2-day, randomized, single-blind, within-subject design. The reflex was activated inside the MRI scanner using kinetic oscillation stimulation placed in the left nostril, resulting in an increase in lacrimation. After the first fMRI session, the participants received either sham vagus nerve stimulation or nVNS outside the scanner and underwent a subsequent fMRI session.

Results
nVNS prompted an increase in activation of the left pontine nucleus and a decreased activation of the right parahippocampal gyrus. Psychophysiologic interaction analyses revealed an increased functional connectivity between the left pontine nucleus and the right hypothalamus and a decreased functional connectivity between the right parahippocampal gyrus and the bilateral spinal trigeminal nuclei (sTN).

Conclusions
These findings indicate a complex network involved in the modulatory effect of nVNS including the hypothalamus, the sTN, the pontine nucleus, and the parahippocampal gyrus.
The trigeminal autonomic reflex is a physiologic reflex that has important protective functions. However, its activation also plays a crucial role in a variety of primary headache syndromes, such as cluster headache and other trigeminal autonomic cephalalgias. The major components of this reflex and the activation mechanism have been described previously. It has been shown that the trigeminal autonomic reflex can be modulated by noninvasive vagus nerve stimulation (nVNS). The reflex is inhibited bilaterally by nVNS when applied to the left cervical part of the vagus nerve, leading to a significant decrease in lacrimation. The inhibition of the reflex might explain the efficiency of nVNS in cluster headache and partly in migraine, as previously suggested in clinical studies.

Findings from rodent studies suggest a modulatory effect via the nucleus of the solitary tract (NTS), bilaterally innervated by the left cervical vagus nerve. Another plausible mechanism might be the top-down modulation via the hypothalamus that has been shown to play an important role in trigeminal and autonomic processing. However, the precise anatomical structures involved in nVNS modulation of the trigeminal autonomic reflex in humans remain to be investigated.

Using functional imaging, we focused on the thalamic-hypothalamic-brainstem network and were specifically interested in unraveling whether the modulation by nVNS is mediated by the NTS, by the hypothalamus, or by additional structures as part of a more complex modulatory network.

### Glossary

- **ASL** = arterial spin labelling;
- **CBF** = cerebral blood flow;
- **GLM** = general linear model;
- **KOS** = kinetic oscillation stimulation;
- **MNI** = Montreal Neurologic Institute;
- **NTS** = nucleus of the solitary tract;
- **nVNS** = noninvasive vagus nerve stimulation;
- **PPI** = psychophysiologic interaction;
- **sTN** = spinal trigeminal nuclei.

### Methods

#### Standard protocol approvals, registrations, and patient consents

Prior to the start of the experimental procedure, the participants gave written informed consent. The study was approved by the local ethics committee (Arztekammer Hamburg PV 5522) and conducted in accordance with the Declaration of Helsinki.

#### Participants and experimental design

Participants were recruited via local Internet platforms and were interviewed by trained research assistants. We excluded...
participants who had any primary headache disorder, any neurologic or psychiatric disorder, infections of the upper airways, recent surgery of the head or face or deviation of the nasal septum, pregnancy, breastfeeding, implanted metallic or electrical devices, cervical vagotomy, or any cardiovascular disorder. Primary headache was excluded by a specific headache questionnaire and the general psychological condition was assessed using the patient health questionnaire in German.

 Altogether 25 healthy participants were included in our study. For technical reasons (artifacts, 3 participants) and due to missing data (1 participant), 4 participants were excluded, leaving a sample of 21 participants (age 25.5 ± 3.3 years; 11 women, 10 men) for the final statistical imaging analyses.

 The study was performed in a 2-day, randomized, single-blind, within-subject design. On the 2 study days, either sham stimulation or nVNS with the gammaCore stimulation device (electroCore, Basking Ridge, NJ) was applied. Each appointment was separated by a minimum of 3 days. For nVNS, the stimulation device was placed over the left cervical portion of the vagus nerve. The sham stimulation was performed by placing the same nVNS stimulation device over the trapezius muscle on the posterior neck.4

 The trigeminal autonomic reflex was activated by kinetic oscillation stimulation (KOS) (Chordate Medical AB, Kista, Sweden).13 Prior to the MRI session, a balloon catheter with an inflatable tip was moistened with paraffin and placed into the participant’s left nostril. To prevent the catheter from moving during the MRI measurements, the catheter was attached to the nose with adhesive tape and was attached to the head coil inside the scanner.

 On each study day, participants first participated in 2 runs of KOS inside the scanner during fMRI acquisition (termed PRE), followed by stimulation (sham or nVNS) outside the scanner room and afterwards participated in 2 additional runs of the KOS (termed POST). For the intranasal KOS, the balloon was inflated and oscillated, induced by the KOS device, which was located outside of the scanner room, connected to the catheter by an 8-m Teflon tube.13 Within each MRI session, the balloon was inflated and vibrated 22 times for a duration of 8 seconds, followed by a break of 52 seconds (jittered between 5 and 25 seconds). During the resting period (jittered onset 8–10 s after the end of stimulation), the participants were asked whether the intranasal stimulation was perceived as painful. If affirmed, they were asked to rate the pain intensity on a visual analogue scale between 0 (no pain) and 100 (worst imaginable pain) via button press inside the scanner. Unpleasantness was also assessed after every stimulation, independent from pain. The intertrial interval was thereby 52 seconds on average, leading to a total duration of 1 minute per run. The experimental design is sketched in figure 1.

 MRI acquisition and preprocessing

 Images were recorded using a Siemens PRISMA 3T MRI system (Siemens; Erlangen, Germany). For anatomical registration, we recorded a high-resolution magnetization-prepared rapid gradient echo sequence image (voxel size 1 mm,3 repetition time 2.3 seconds, echo time 2.98 ms, flip angle 9°, field of view 256 mm, 240 axial slices gap 50%) of each participant with a 64-channel head coil at the first day of the study. These images were normalized to Montreal Neurologic Institute (MNI) space on templates provided by Blaiotta and colleagues14 using a nonlinear image registration algorithm15 implemented in SPM12 (Wellcome Trust Center for Neuroimaging, London, UK). This procedure provides a transformation matrix for each participant to warp all other images from participant space to MNI space. Warped anatomical images were averaged and served as a template for all presented results. A gray and white matter mask was calculated by segmenting the averaged and warped anatomical images.

 Arterial spin labeling (ASL) was recorded to exclude general changes of cerebral blood flow (CBF) affecting fMRI. As ASL is relatively time-consuming, we decided to cover the midbrain and brainstem in 2 separate runs. The coverage of the 2 image stacks is sketched in figure e-1 (links.lww.com/WNL/B37). Each ASL sequence used pulsed ASL recorded with 91
repetitions in 15 slices with a repetition time of 2.5 seconds (echo time 12 ms, 90° flip angle, bolus duration 1,800 ms, inversion time 700 ms, PICORE Q2T perfusion mode, voxel size $2 \times 2 \times 5$ mm [slice thickness]).

For analyses, relative CBF maps calculated by the scanner software were coregistered to the anatomical image, warped to MNI space using the transformation calculated on the anatomical image, and smoothed with a 12-mm isotropic Gaussian kernel. On group level, we calculated a paired $t$ test for both stacks separately to compare sham and vagus stimulation and present the F-contrast to evaluate alterations in both directions. Results are presented for low statistical thresholds of $p < 0.001$ (uncorrected) as we hypothesized only a minor or no effect for ASL.

During the actual experiment, we recorded for each run 235 functional images with an echoplanar imaging sequence optimized for blood oxygenation level–dependent brainstem imaging. This technique was developed further for the present study to cover a large volume (in z direction) by using simultaneous acquisition of 2 slices in parallel. The covered volume reached from the top of the thalamus to C3 and was recorded with 72 slices resulting in a slice thickness of 2 mm while the in plane resolution was $1.3 \times 1.3$ mm$^2$ (repetition time 2.93 seconds, echo time 33 ms, flip angle 80°, GRAPPA acceleration mode, field of view readout 215 mm, phase partial Fourier 7/8, flow rephasing) and no gap between slices. Simultaneously, we recorded pulse and breathing (Expression; Philipps, Best, the Netherlands) to correct for movements not intercepted by the realignment, function and used them as regressors in the GLM. To further correct for artifacts induced by movement of the CBF, we extracted the average time course of the 4th ventricle by setting a $4$ mm$^3$ sphere in its center and including the averaged resulting time course as a further regressor. All results were masked by a gray and white matter mask resulting from a segmentation of the group’s average anatomical image.

**Data availability**

All patients who participated in this study gave written informed consent. However, this consent did not include a provision stating that individual raw data can be made freely accessible to the public. Therefore, in accordance with the German data protection act §4, the underlying raw data cannot be made accessible to the public. Researchers meeting the criteria for access to confidential data may access the data upon request, involving the documentation of data access.

**Statistics**

**Main effect of KOS**

The overall effect of the KOS was estimated with a $t$ test on stimulations from all runs using a voxel-wise family-wise error corrected $p$ value of $p < 0.05$ as statistical threshold and a minimal cluster extent of 20 voxels ($20$ mm$^3$).

**Effect of nVNS**

To estimate significant differences between sham and nVNS, we calculated a flexible factorial analysis of variance with the between-subject factor SUBJECT and the within-subject factors TIME (levels: PRE and POST) and STIMULATION (levels: SHAM and nVNS) in each voxel comparing the effects of sham and nVNS on KOS. Interaction between TIME and STIMULATION were modeled by 4 regressors as automatically calculated by the SPM12 toolbox. We then looked for positive and negative interactions of TIME $\times$ STIMULATION, that is, $(nVNS_{POST} - nVNS_{PRE}) > (SHAM_{POST} - SHAM_{PRE})$ and $(nVNS_{POST} - nVNS_{PRE}) < (SHAM_{POST} - SHAM_{PRE})$. To further exclude an effect of sequence, for example, which stimulation (sham or nVNS) was run first, we included the order of sequence as a covariate. This is reasonable since the sequence was slightly unbalanced after exclusion of participants (13 participants received SHAM first, 8 nVNS). Resulting voxels were threshold by $p < 0.0005$ and a minimum cluster extent of 20 voxels.

**Functional connectivity**

To estimate the functional connectivity changes caused by nVNS, we ran psychophysiological interaction (PPI) analyses. Regions of interest were extracted from the previous analyses and used as a seed region, setting aforementioned interaction as contrast. The statistical threshold for this analysis was set to $p < 0.0005$ uncorrected with a minimum cluster extent of 20 voxels. Small volume corrections were calculated, using peak voxels from previous publications, for the right pontine nucleus ($9; -21; -25; 4$ mm), the right parahippocampal gyrus ($33; -18; 10$ mm), and the right temporal pole ($50; -48; 24$ mm).
the right hypothalamus (8; 1; −11; 4 mm), and the left spinal trigeminal nucleus (−4, −45, −53; 4 mm).\textsuperscript{16}

Results

Behavior
We did not find any statistical difference in pain ratings or unpleasantness ratings between the nVNS and sham.

Main effect of KOS
KOS activated the insular cortices bilaterally close to previous published coordinates\textsuperscript{13} and 2 clusters in the left cerebellum (ipsilateral to the KOS) (figure 2).

CBF changes induced by vagus nerve stimulation
ASL showed no difference in CBF comparing sham and nVNS at a threshold of \( p < 0.001 \) (uncorrected) for both measured volumes (see figure e-1, links.lww.com/WNL/B37, for the coverage of the volumes).

Effect of vagus nerve stimulation
The interaction of STIMULATION \( \times \) TIME showed an increase in the left pontine nucleus and a decrease in 2 clusters of the right parahippocampal gyrus (figure 3 and table 1).

Alterations in functional connectivity induced by nVNS
Enhanced functional connectivity changes of the pontine nucleus induced by nVNS could be observed in 14 clusters located in the right hypothalamus, bilateral thalamus, cerebellar vermis, and the left temporal lobe. Furthermore, a small portion of the left posterior cingulate gyrus and the left putamen showed increased connectivity with the pontine nucleus. No decreased functional connectivity changes were observed at the chosen statistical threshold taking the pontine nucleus as a seed region (figure 4A and table 2).

Functional connectivity from the right parahippocampal gyrus to the bilateral spinal trigeminal nuclei (sTN), bilateral precuneus, left temporal lobe, right cerebellum, and bilateral...
cerebellar vermis decreased after nVNS (figure 4B and table 2). No significant results were observed for increased connectivity.

**Discussion**

With the present study, we mainly aimed at understanding how the modulatory effect of nVNS is mediated. Based on our previous findings, we suggested a top-down modulation via the hypothalamus, a modulatory pathway directly acting via the NTS, or possibly a more complex modulatory pathway.4 Our main finding is an increased activation of the left pontine nucleus following nVNS while the trigeminal autonomic reflex is triggered. The pontine nuclei have been shown to be involved in the trigeminal autonomic reflex activation in our previous study, regardless whether the trigeminal intranasal stimulus is perceived as painful or nonpainful.13 The findings of these 2 studies indicate that the pontine nuclei might play a more important role in the modulation of the reflex than previously thought. The pontine nuclei are involved in the integration of information from the cerebral cortex forwarded via mossy fibers to the cerebellum.22,23 In addition to information received from various cortical regions, the pontine nuclei receive input from the trigeminal nuclei, previously described in rats.24,25 Interestingly, our PPI analysis revealed an increased functional connectivity between the left pontine nucleus and the right hypothalamus. The hypothalamus is known to play an important role in cluster headache,26,27 migraine,21,28 and also during activation of the trigeminal autonomic reflex by mechanical intranasal stimulation.13 nVNS increases this functional connectivity, which may contribute to its effect in the treatment of cluster headache.6 These results support the assumption that nVNS facilitates a top-down modulation mediated by the hypothalamus, inhibiting the trigeminal autonomic reflex bilaterally4 (figure 4).

The second finding is a decreased activation of the right parahippocampal gyrus after cervical nVNS. A decreased

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**Table 1** Interaction STIMULATION × TIME at a statistical threshold of $p < 0.0005$, uncorrected and a minimum cluster extent of 20 voxels (20 mm$^3$)

<table>
<thead>
<tr>
<th>Cluster size (1 mm$^3$/voxel)</th>
<th>t Value</th>
<th>MNI coordinates</th>
<th>Region</th>
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</thead>
<tbody>
<tr>
<td>$(n\text{VNS}<em>{\text{POST}} - n\text{VNS}</em>{\text{PRE}}) &gt; (\text{SHAM}<em>{\text{POST}} - \text{SHAM}</em>{\text{PRE}})$</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>37</td>
<td>4.19</td>
<td>−9 −19 −22</td>
<td>L pontine nucleus$^{a,b}$</td>
</tr>
<tr>
<td>$(n\text{VNS}<em>{\text{POST}} - n\text{VNS}</em>{\text{PRE}}) &lt; (\text{SHAM}<em>{\text{POST}} - \text{SHAM}</em>{\text{PRE}})$</td>
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<tr>
<td>60</td>
<td>4.29</td>
<td>42 −18 −19</td>
<td>R parahippocampal gyrus$^{a,b}$</td>
</tr>
<tr>
<td>34</td>
<td>4.22</td>
<td>44 −33 −13</td>
<td>R parahippocampal gyrus</td>
</tr>
</tbody>
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Abbreviations: MNI = Montreal Neurologic Institute; nVNS = noninvasive vagus nerve stimulation. For the small volume correction, a statistical threshold of $p < 0.05$ was applied.

$^a$ Used as seed in psychophysiologic interaction analysis.

$^b$ Significant small volume correction.

Figure 4 Changes in functional connectivity induced by noninvasive vagus nerve stimulation

(A) Increased functional connectivity with the pontine nucleus. (B) Decreased functional connectivity with the temporal lobe.
activation of the parahippocampal gyrus has been previously reported following invasive 29–31 and noninvasive vagus nerve stimulation32 in refractory epilepsy. The hippocampus and the parahippocampal gyrus have been linked to pain modulation,33 receiving input from SII via the insular cortex.34 More to the point, our PPI analysis revealed a decreased functional connectivity between the parahippocampal gyrus and the sTN on both sides following nVNS (figures 4 and 5). Given that nVNS inhibits the trigeminal autonomic reflex,4,35 an inhibitory modulation of the sTN by the parahippocampal gyrus seems a plausible mechanism. This inhibitory effect might originate in the NTS, which has been shown to project to hippocampal and parahippocampal areas.36 The bilateral NTS in turn receives input from the left cervical vagus.10 Our imaging results suggest a possible inhibition of the trigeminal autonomic reflex by the parahippocampal gyrus, possibly downregulated by the NTS. The downregulation of the bilateral sTN might lead to decreased firing in the superior salivatory nucleus, bilaterally attenuating the parasympathetic outflow, resulting in decreased lacrimation.

Table 2 Changes in functional connectivity as calculated by a psychophysiologic interaction setting the interaction STIMULATION × TIME as contrast

<table>
<thead>
<tr>
<th>Cluster size (1 mm³/voxel)</th>
<th>t value</th>
<th>MNI coordinates</th>
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<tr>
<td>Seed in pontine nucleus for the interaction STIMULATION × TIME showing an increase in functional connectivity</td>
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<tr>
<td>52</td>
<td>6.47</td>
<td>23</td>
<td>−14 −4</td>
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<tr>
<td>84</td>
<td>5.82</td>
<td>−20</td>
<td>−20 −5</td>
</tr>
<tr>
<td>35</td>
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<td>5</td>
<td>2 −7</td>
</tr>
<tr>
<td>63</td>
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<td>−50 6</td>
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<td>71</td>
<td>4.69</td>
<td>−28</td>
<td>−1 −8</td>
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<tr>
<td>24</td>
<td>4.47</td>
<td>−13</td>
<td>−32 0</td>
</tr>
<tr>
<td>46</td>
<td>4.32</td>
<td>−50</td>
<td>−62 −10</td>
</tr>
</tbody>
</table>

| Seed in parahippocampal gyrus for the interaction STIMULATION × TIME showing a decrease in functional connectivity |         |                 |                |
| 164                       | 7.49    | −6              | −48 −52        | L sTN*         |
| 100                       | 7.08    | −11             | −62 12         | L precuneus    |
| 439                       | 5.83    | 7               | −50 −53        | R sTN          |
| 52                        | 5.38    | −52             | −21 −6         | L superior temporal gyrus |
| 90                        | 4.82    | 8               | −62 −46        | R cerebellum   |
| 32                        | 4.73    | 4               | −58 12         | R precuneus    |
| 50                        | 4.52    | 2               | −72 −42        | Both cerebellar vermis |
| 31                        | 4.19    | 9               | −72 −40        | R cerebellum   |

Abbreviations: MNI = Montreal Neurologic Institute; sTN = spinal trigeminal nucleus.
Results are presented for a statistical threshold of p < 0.0005 (uncorrected) and a minimum cluster extent of 20 voxels (20 mm³). For the small volume correction, a statistical threshold of p < 0.05 was applied.
* Significant small volume correction.
Taken together, our findings suggest a more complex modulatory network by nVNS acting on the trigeminal autonomic reflex. This network includes the left pontine nucleus as well as the right parahippocampal gyrus. We were able to show that the pontine nucleus in turn has a modulatory effect on the hypothalamus and the parahippocampal gyrus has an inhibitory effect on the bilateral sTN. The hypothalamus has been suggested to be a top-down modulator of the trigeminal autonomic reflex. The parahippocampal gyrus, on the other hand, is innervated by the NTS, which might modulate the neural activation in the parahippocampal gyrus. The results of the present study contribute to the understanding of the modulatory effect underlying nVNS that has been shown to be effective in cluster headache and partly in migraine.

**Author contributions**

M.M.: data acquisition and analysis, drafting and writing of the manuscript. J.M.: data analysis, drafting and writing of the manuscript. C.F.S.: data acquisition, drafting and writing of the manuscript. A.M.: drafting of the study, data analysis, drafting and writing of the manuscript.

**Study funding**

This work was supported by the German Research Foundation SFB936/AS to A.M. and by an unrestricted scientific grant to the University Clinic Hamburg Eppendorf by Electrocore. The funding sources did not influence study conduction in any way.

**Disclosure**

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

**Publication history**

Received by Neurology July 17, 2019. Accepted in final form September 18, 2019.

**References**

Noninvasive vagus nerve stimulation and the trigeminal autonomic reflex: An fMRI study
Maike Möller, Jan Mehnert, Celina F. Schroeder, et al.
Neurology published online February 6, 2020
DOI 10.1212/WNL.0000000000008865

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