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A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI

Funktionelle Magnetresonanztomographie zeigt Aktivierungen des Hirnstamms und weiterer zerebraler Strukturen unter transkutaner Vagusnervstimulation

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Abstract

Background: Left cervical vagus nerve stimulation (VNS) using the implanted NeuroCybernetic Prosthesis (NCP®) can reduce epileptic seizures and has recently been shown to give promising results for treating therapy-resistant depression. To address a disadvantage of this state-of-the-art VNS device, the use of an alternative transcutaneous electrical nerve stimulation technique, designed for muscular stimulation, was studied. Functional magnetic resonance imaging (MRI) has been used to test non-invasively access nerve structures associated with the vagus nerve system. The results and their impact are unsatisfying due to missing brainstem activations. These activations, however, are mandatory for reasoning, higher subcortical and cortical activations of vagus nerve structures. The objective of this study was to test a new parameter setting and a novel device for performing specific (well-controlled) transcutaneous VNS (tVNS) at the inner side of the tragus. This paper shows the feasibility of these and their potential for brainstem and cerebral activations as measured by blood oxygenation level dependent functional MRI (BOLD fMRI).
Materials and methods: In total, four healthy male adults were scanned inside a 1.5-Tesla MR scanner while undergoing tVNS at the left tragus. We ensured that our newly developed tVNS stimulator was adapted to be an MR-safe stimulation device. In the experiment, cortical and brainstem representations during tVNS were compared to a baseline.
Results: A positive BOLD response was detected during stimulation in brain areas associated with higher order relay nuclei of vagal afferent pathways, respectively the left locus coeruleus, the thalamus (left ≫ right), the left prefrontal cortex, the right and the left postcentral gyrus, the left posterior cingulated gyrus and the left insula. Deactivations were found in the right nucleus accumbens and the right cerebellar hemisphere.
Conclusion: The method and device are feasible and appropriate for accessing cerebral vagus nerve structures, respectively. As functional patterns share features with fMRI BOLD, the effects previously studied with the NCP® are discussed and new possibilities of tVNS are hypothesised.

Keywords: blood oxygenation level dependent functional magnetic resonance imaging (BOLD fMRI); pinna; transcutaneous vagus nerve stimulation (tVNS); vagus nerve stimulation.

Zusammenfassung

Hintergrund: Die Vagusnervstimulation am linken Halsstrang mit einer implantierten neurokybernetischen Prothese hat sich als suffizient bei der Behandlung von epileptischen Episoden erwiesen und zeigt zudem vielversprechende Behandlungsansätze für therapieresistente Depressionen. Aufgrund zahlreicher Nachteile einer derzeit alleinig verfügbaren implantologischen Lösung wurden in der letzten Zeit Studien zur Verwendung der transkutanen elektrischen Nervenstimulation, die hauptsächlich der Muskelstimulation dient, als nicht-invasiver Zugang zu Hirnstamm- und Kortexstrukturen über afferente Vagusnervfasern am menschlichen Ohr unter Verwendung der funktionellen Magnetresonanztomographie durchgeführt. Die Resultate und Folgerungen dieser Studien sind aber fragwürdig, da keine Aktivierungen des Hirnstamms ermittelt wurden. Diese allerdings sind für eine Aktivierung höherer Zentren unbedingt notwendig. Aufgabe der hier vorliegenden Arbeit war es daher, eine Methode und ein Gerät zur transkutanen Vagusnervstimulation (tVNS) an der Innenseite des Tragus zu entwickeln, welche auch eine solche Hirnstammaktiverung ermöglichen, und diese unter Zuhilfenahme des BOLD-Effekts (blood oxygenation level dependent functional magnetic resonance imaging, BOLD fMRI) basierend auf der funktionellen Magnetresonanztomographie zu studieren.

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Material und Methode: Vier männliche und gesunde Probanden wurden während tVNS an der linken Ohrmuschel in einem 1,5-Tesla-MR-Scanner untersucht. Hierfür wurde eine speziell konstruierte MR-taugliche Stimulationseinheit entwickelt und verwendet. In diesem Experiment wurden die BOLD-Effekte in Phasen während der Stimulation mit Ruhephasen ohne Stimulation verglichen.
Ergebnisse: Positive BOLD-Effekte während der Stimulation traten insbesondere in Hirnarealen auf, welche Relais höherer Ordnung für vagale Afferenzen darstellen, wie dem Locus coeruleus, dem Thalamus (links ≫ rechts), dem linken präfrontalen Kortex, dem rechten und linken Gyrus postcentralis, dem linken hinteren Gyrus cinguli und der linken Inselregion. Deaktivierungen wurden im rechten Nucleus accumbens und der rechten Kleinhirnhemisphäre sichtbar.
Schlussfolgerung: Vorgeschlagene Methode und Gerät können zerebrale Strukturen des Vagusnervsystems, insbesondere auch Hirnstammareale, aktivieren. Die Aktivierungsmuster unter tVNS ähneln jenen der zerebralen regionalen Blutflussänderungen aus früheren Studien unter Verwendung der NCP. Daher scheinen neue diagnostische und therapeutische Ansätze zumindest theoretisch möglich zu sein.

Schlüsselwörter: blood oxygenation level dependent functional magnetic resonance imaging (BOLD fMRI); Ohrmuschel; transkutane Vagusnervstimulation (tVNS); Vagusnervstimulation.

Introduction

Current state-of-the-art vagus nerve stimulation (VNS) comprises an implantable device, called the Neuro-Cybernetic prosthesis (NCP®) (Cyberonics Inc., Houston, TX, USA) [33]. It has shown to have beneficial clinical effects in treating epilepsy [3, 36] and recently promising results in treating patients with therapy-refractory depression [20, 22, 28, 29]. At present, VNS requires a surgical intervention for implanting the stimulation unit in the chest and connecting an electrode array wrapped around the left cervical vagus nerve branch in the neck. The array delivers electrical current with variable parameters, such as amplitude, pulse width, frequency and on-time/off-time ratio, to the nerve with the objective of beneficially influencing brain areas involved in the onset of epilepsy or depression. Although promising, the current implantable device has numerous disadvantages, namely high costs, the requirement of a clinical infrastructure for surgical intervention, surgical risks, an irreversible surgical procedure, a risk of nerve injuries and voice alterations when the device is activated. Furthermore, long-term effects of this method are not known. This in mind, a non-invasive method to stimulate the vagus nerve would have substantial benefits for neurological and neuropsychiatric patients. In 2000, Ventureyra [35] proposed a non-invasive concept by stimulating the Ramsey Hunt zone in the pinna, a delimitated skin area supplied by the ganglion geniculi of the nervus intermedius. It was suggested that stimuli could reach the vagus nerve and the nucleus of the solitary tract via the intermediary nerve of Wrisberg, a branch of the facial nerve. Fallgatter et al. [6, 7] and Polak et al. [25] further investigated the possibility of stimulating cutaneous representations of the vagus nerve in the external auditory canal including the inner side of the tragus using established techniques of early acoustically evoked potentials for use as a diagnostic tool for Alzheimer's disease and Parkinson's disease.

The assumption that direct transcutaneous VNS (tVNS) at the external ear is possible is reasoned from interpreting functional anatomy. The auricular branch of the vagus nerve (ramus auricularis nervi vagi) transverses the canaliculus mastoideus and the fissura petrotympanica and is distributed to the inner side of the pinna and the external auditory canal. Irritations of this nerve are mainly responsible for vegetative reactions, such as cough reflex and nausea [12, 32].

The functional neurobiology of how VNS – invasive or non-invasive – works is poorly understood. Several groups have used positron emission tomography [4, 8, 10, 11, 14], single photon emission computed tomography [27, 34, 37, 39] or blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI) [2, 16, 17, 21, 23, 24, 31] to study VNS brain effects using the implantable NCP®. The literature appears inconsistent due to questionable methodologies, findings and conclusions. Overall, however, VNS causes acute and long-term changes in brain areas ascribed to the vagus nerve system and involved in the onset of neuropsychiatric disorders.

To date, only one fMRI study has been performed to assess acute effects when undergoing tVNS in the human external canal [13]. This experiment was performed using a device designed and approved for the stimulation of neuromuscular tissue. The characteristics of this stimulation were a pulse width of 20 μs and a frequency of 8 Hz. Unfortunately, no information on the chosen shape of impulse (whether symmetric or asymmetric) was reported. The main difference between neuromuscular stimulation and neural tissue stimulation, however, are the parameters of the output signal.

Our interpretation of the psychophysical research and literature shows that tVNS at the inner side of the tragus (parasympathetic stimulation) requires even more specific stimulation parameters. As an example, Lomarev et al. [17] have reported that VNS at 20 Hz resulted in significant brain activations, while at 5 Hz stimulation no significant difference was reached compared to baseline. The authors concluded that the activity of VNS is frequency-dependent in favour towards 20 Hz. Current literature supports these results [38]. Low-frequency stimulation between 0.5 and 10 Hz activates the sympathetic system, whilst 20–25 Hz is more suitable for parasympathetic nerve activation. Changing the pulse width towards 200–500 μs is therefore reasonable. Last but not least, we believe that using devices suitable for neuromuscular stimulation rather leads to unpleasant skin sensations that again will activate pain pathways and the sympathetic system.

The objective of this study was to assess the effects of new parameter settings provided by a novel tVNS device using BOLD fMRI. This study contributes to the

field, because it is both a feasibility and mode-of-action study of tVNS in healthy subjects.

Materials and methods

Subjects

A total of four healthy male volunteers (aged 26–32 years) took part in this study. The participants had a mean age of 30 (±2.7) years. Ethics committee approval was obtained and granted from the Freiburg Ethics Commission International, Germany (code 06/1441). Written informed consent was obtained from all volunteers. An itemised questionnaire was used to gain general anamnesis information. No volunteer ever had a clinical diagnosis of epilepsy, depression or neurofunctional disorders or had undergone associated therapy treatment in the past or indeed during the study.

In a training session, all subjects were familiarised with the stimulation procedure. All subjects reported that they were used to the stimulus amplitude after a few seconds and so the current was lifted stepwise up to a level where a constant sensation was reached. The stimulation was always kept below a pain threshold (between 4 and 8 mA). The stimulation algorithm (see section below) was performed as in the later fMRI session. For this procedure, the subjects lay horizontally on an examination couch. Blood pressure and heart rate were recorded continuously during the training session (using PowerLab® 4/25T and Chart™ Software, AD Instruments, Spechbach, Germany). Laser Doppler flowmetry was performed at the finger pad of the left index to measure peripheral skin blood flow (OxyFlo XP Probe, Oxford Optronix Ltd., Oxford, UK).

Stimulation procedure

tVNS was performed at the inner side of the left tragus (Figure 1A) using a stand-alone electrical nerve stimulator connected with carbon fibre wires to an acrylic electrode array housing a sterling silver stimulation electrode and a reference electrode (Figure 1B). The centre-to-centre distance of the surface electrodes was approximately 8 mm. The array was attached to the skin with an adhesive tape and ear canals were sealed by ear protection. All components of the electrode array and the connecting wires to the stimulator were manufactured without the use of any ferromagnetic components inside the scanner's magnetic field. The connecting wires were placed on the subject's chest along the longitudinal body axis. The tVNS stimulator was placed outside the scanner room (Figure 1C). The stimulus was a monophasic-modified rectangle impulse (Figure 1D) with a pulse width of 250 μs. Electrical current amplitude was varied individually between 4 and 8 mA. Individual adjustment of stimulation intensity was performed additionally before the

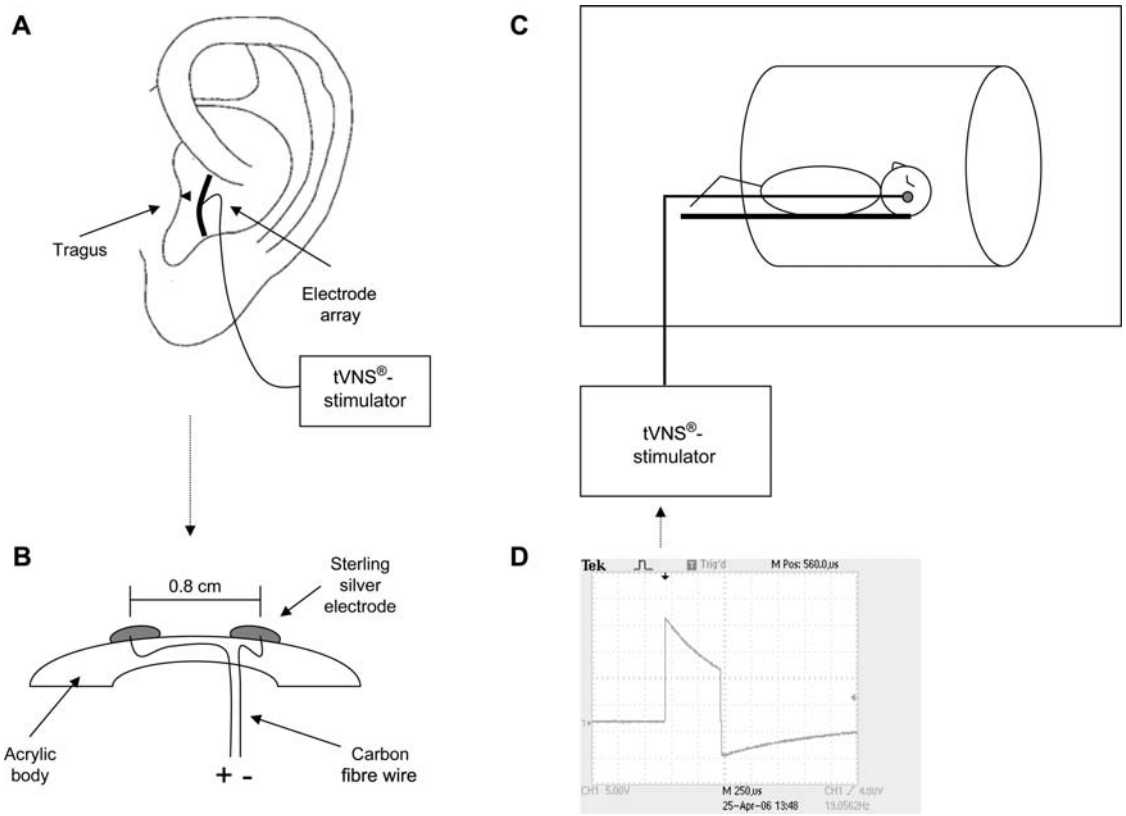

Figure 1 Method and tVNS stimulator design.
(A) Application site of the electrode. The stimulation electrode is placed at the tragus of the left pinna. (B) The bipolar stimulation electrode is made of silver and placed on an acrylic body for a comfortable fit in the pinna. (C) For fMRI stimulation, the tVNS stimulator is placed outside the scanner room and connected to the electrodes by an MR-compatible cable. (D) The stimulus was a modified monophasic rectangle impulse with a pulse width of 250 μs. The amplitude varied between 4 and 8 mA.

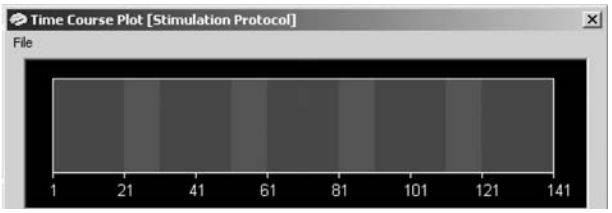

Figure 2 fMRI stimulation and BrainVoyager® QX evaluation protocol.
Dark grey areas represent the baseline in which no stimulus was applied. Each baseline is 100 s followed by a stimulation period of 50 s in light grey colour. The fMRI experiment lasted 700 s in total.

scan. Stimulation frequency was kept at 25 Hz, which is known to activate parasympathetic nerve fibres [36].

Experimental protocol

Functional MRI sessions were performed using the following protocol: the experiment lasted 700 s and was started with a baseline lasting 100 s. This was followed by a first stimulation period of 50 s and a break/baseline of 100 s. Four alternating stimulation and baseline sequences were performed according to the scheme depicted in Figure 2.

Magnetic resonance imaging

Functional MRI was performed with a 1.5-Tesla Avanto MRI scanner (Siemens Medical Solutions, Erlangen, Germany) at the Institute of Imaging and Therapy, Erlangen, Germany. The head of the subject was fixed in a head coil by rubber pads and both ears were plugged. A magnetisation prepared rapid gradient echo (MPRAGE) sequence was recorded consisting of 176 sagittal slices of 1-mm thickness and an inplane resolution of 256×256 pixel matrix (field of view: 224×224 mm^2). Functional T2* weighted images were obtained using an echo planar imaging technique consisting of 36 axial slices (TR = 110 ms, TE = 60 ms, flip angle = 90°, slice time of 5000 ms per block of 36 slices, slice thickness of 3 mm, field of view 224×224 mm^2, 64×64 pixel). Possible head movements of the subject were corrected using the motion correction function of the SYNGO® scanner software (Siemens Medical Solutions).

Data analysis and statistics

Psychophysical data were analysed with SCOPE™ and CHART™ (AD Instruments, Spechbach, Germany). Functional MRI post-processing was performed using BrainVoyager® QX (BrainInnovations, Maastricht, The Netherlands) with motion correction, temporal high-pass filter and linear trend removal. A general linear model (GLM) for multistudies was used to detect activated brain areas. For displaying the activated clusters at different brain sites, the functional images were co-registered with the three-dimensional (3D) MPRAGE dataset using the routines according to the BrainVoyager® QX. Resulting transformations were merged to an overlay 3D activation map. Regions of interest (RoI) were identified (Figure 3) based on a printed human brain atlas [19] and compared to fMRI data sets. In the case of overlays, clusters were included in subsequent statistical analysis.

Results

Advance information

General anamnesis was without pathological findings. High-resolution T1-weighted structural images did not show obvious brain abnormalities or pathologies. There were no adverse effects during the training session and the fMRI experiment. Chosen tVNS parameters ensured that there were no cough reflexes as occasionally reported in the literature as a result of vagus nerve irritations [12].

Psychophysics

The evaluation of the psychophysiological parameters blood pressure, heart rate and laser Doppler flow showed no significant changes during training session (t-test). After the stimulation, all subjects reported a relaxed yet focussed condition.

Cortical and subcortical activations

The main results are presented in Figure 4 and Table 1. In the tVNS_BASELINE comparison, significant areas of activation were detected in the brainstem, more precisely the left locus coeruleus, the thalamus (left ≫ right), the left prefrontal cortex, right and left postcentral gyrus, the

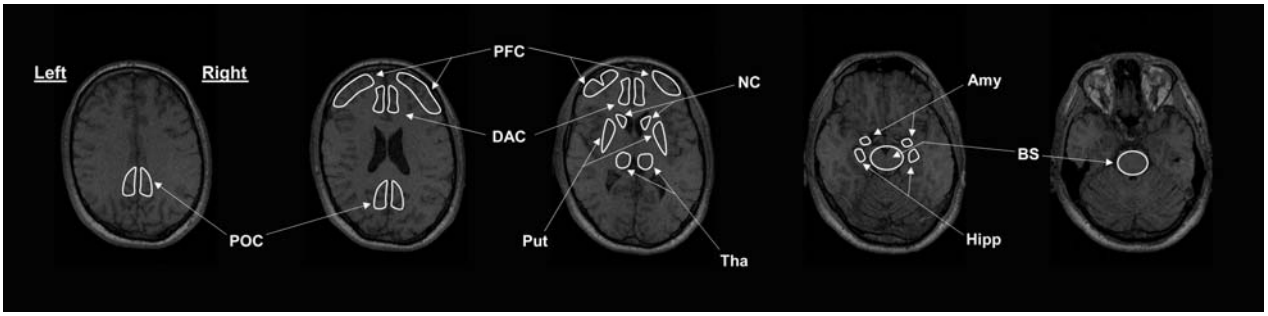

Figure 3 Regions of interest (RoI) for the evaluation of fMRI data.
Areas of interests were BS: brainstem, Tha: thalamus, PFC: prefrontal cortex, POC: postcentral gyrus, NC: nucleus caudatus, Amy: amygdala, Hipp: hippocampus, Put: putamen, DAC: anterior cingulum.

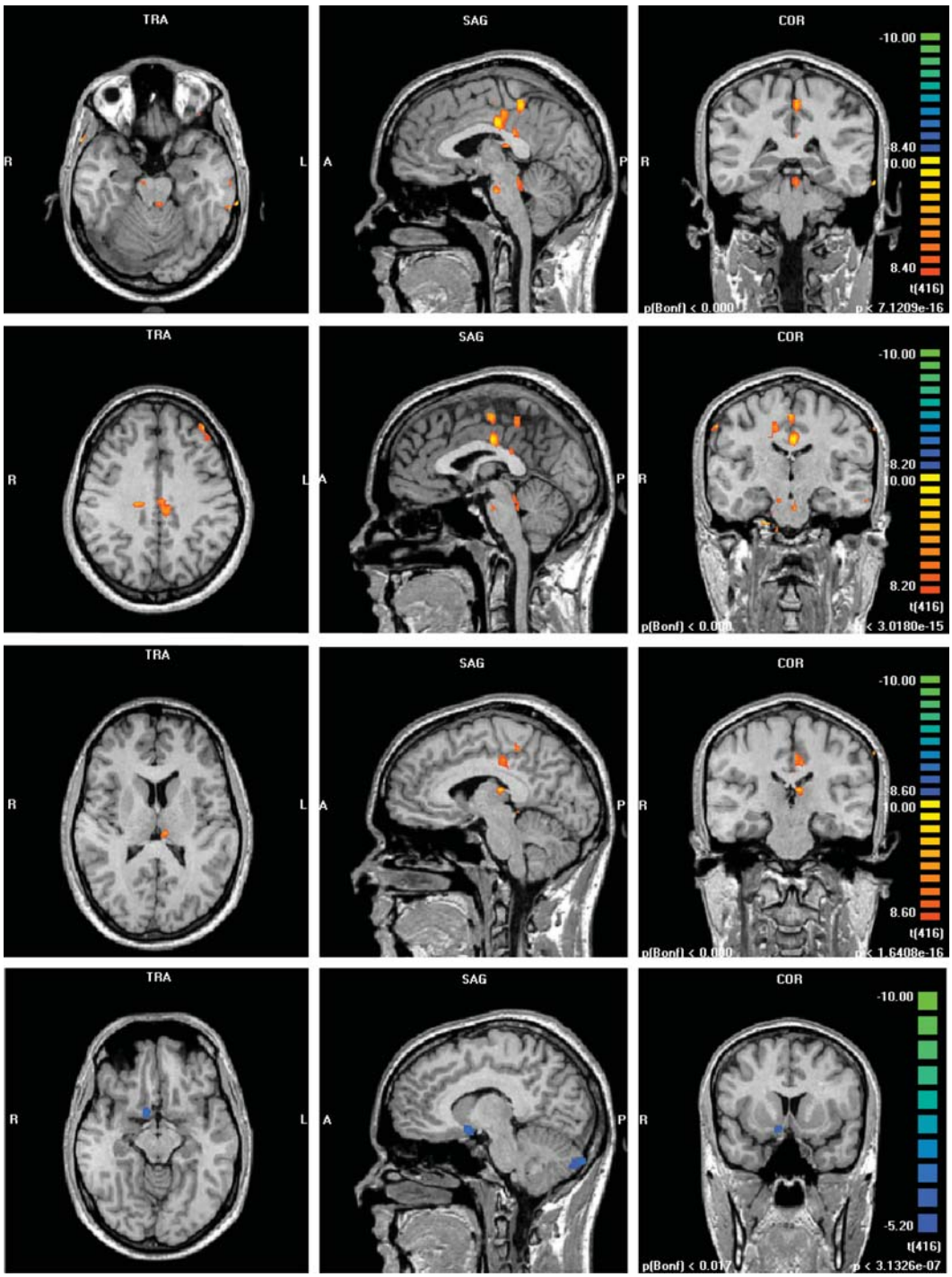

Figure 4 Functional MRI results of the GLM multistudy: merged display pattern of activations and deactivations.
Activations are displayed in red to yellow; deactivations are displayed in blue to green. The left row shows axial slices, the middle row different sagittal slices and the right row coronal slices with activated areas in the GLM multistudy. The fourth horizontal row shows deactivations found in the GLM multistudy in axial, coronal and sagittal views.

left posterior cingulated gyrus and the left insula. Deactivations were found in the right nucleus accumbens and the right cerebellar hemisphere.

Discussion

The mode of action of VNS is still poorly understood. BOLD fMRI has been studied previously in patients with either treatment-resistant depression or epilepsia using the NCP® [2, 16, 17, 21, 23, 24, 31]. We have reviewed recent fMRI/VNS studies (Table 2) and the field is very inconsistent due to missing standardisations in the methods and diverse experimental designs. Nevertheless, there is some agreement across the studies regarding neuroanatomical structures involved in processing VNS signal.

Recently, Kraus et al. [13] reported BOLD fMRI deactivations of limbic and temporal brain structures using non-invasive tVNS techniques in healthy subjects. The

Table 1 Descriptions of evaluated regions of the brain.

Characterisation of tVNS-induced cerebral activations and deactivations		
Brain region	Event	Side
Brainstem (including locus coeruleus)	↑	Left
Thalamus	↑	Left ≫ right
Hypothalamus		
Putamen		
Nucleus caudatus		
Orbitofrontal cortex		
Prefrontal cortex	↑	Left
Precentral gyrus		
Postcentral gyrus	↑	Bilateral
Anterior cingulate gyrus		
Posterior cingulate gyrus	↑	Left
Insula	↑	Left
Amygdala		
Hippocampus		
Parahippocampus		
Nucleus accumbens	↓	Left
Parietal lobe		
Occipital lobe		
Cerebellar hemisphere	↓	Left

A positive correlation in the GLM multistudy is displayed in the column "Event" with a ↑ and a negative BOLD contrast with a ↓. The column "Side" indicates the ipsi (=left), contralaterally (=right) or bilaterally activated cortical area. Activation in the left thalamus was significantly higher than the right thalamus.

authors proposed that tVNSs applied to the inner side of the tragus travel along the ramus auricularis nervi vagi towards the brainstem where the signals are processed. However, Kraus et al. [13] could not show brainstem activations that are considered as mandatory for further subcortical and cortical activities. Choosing more specific parameter settings and impulses suitable for parasympathetic nerve system activation (see above), our study showed a more robust activation in the left locus coeruleus (LC), a brainstem nucleus that has recently been related to clinical depression [1]. The LC is the major location of norepinephrine in the brain and some antidepressants are believed to act in this area as norepinephrine reuptake inhibitors. Furthermore, the LC has been studied in relation to VNS. In an experimental animal study, Groves et al. [9] demonstrated direct neuronal responses from the LC following acute challenge of VNS and outlined a pre-eminent impact of the LC for VNS. Krahl et al. [15] showed that lesioning the LC in rats minimises VNS-induced seizure suppression. The projections of the LC are far, e.g., within the brainstem, to the cerebellum, the thalamus and the hypothalamus, the amygdala and the cortex. The LC receives a constant and excitatory input from the prefrontal cortex, an area responsible for executive functions, such as determining good and bad or social control. Interestingly, our study has shown a significant activation of the prefrontal cortex during acute tVNS. Morphometric [26] and functional imaging data [5] have outlined that functional anatomical abnormalities can be related to the onset of depressive disorders. Depressed patients suffer from decreases in cortical thickness, neuronal sizes and area volume.

The thalamus is believed to relay information selectively to various parts of the cortex. It also plays an important role in regulating states of sleep and is involved in consciousness. Using tVNS, we produced a bilateral (left ≫ right) activation of this area.

We identified the left posterior cingulated gyrus, a part of the limbic system, as another activated brain area. Functional imaging studies consistently found that emotional stimuli activate the posterior cingulate cortex [18]. This region may mediate interactions of emotional and memory-related processes.

Moreover, we observed significant deactivations in the right nucleus accumbens (NAc). This nucleus is thought to play an important role by acting as a "motivation relay" between the limbic system and systems involved in motor controls. Furthermore, it seems to be deeply involved in reward, pleasure and addiction generation processes. The NAc has recently been studied in relation to therapy-refractory depression. Schlaepfer et al. [30] used deep brain stimulation to stimulate this nucleus along with ventral striatum areas. Their findings, in three patients with refractory depression, suggest that stimulating the NAc might be a new promising approach for treating refractory depressive conditions.

Conclusion

Many studies have revealed that VNS clearly has effects on the brain. As there is some agreement with reviewed fMRI studies on VNS and our results concerning neuroanatomical structures involved in processing VNS signals, our device and parameter settings are feasible and suitable for future scientific tVNS procedures. Our device and the parameters can activate cortical as well as subcortical brain areas including the brainstem.

Current state-of-the-art technologies for therapy and diagnosis involve clinical infrastructure (e.g., for imaging) or require surgical interventions. Due to the consistency of our results with traditional VNS, we suggest that this non-invasive tVNS of the ramus auricularis nervi vagi at the left tragus would open new promising applications to

Table 2 Summary of the current literature of fMRI studies of vagus nerve stimulation (VNS).

VNS-induced cerebral activity alterations in various fMRI studies							
	Bohning et al. (2001) [2]	Lomarev et al. (2002) [17]	Sucholeiki et al. (2002) [31]	Narayanan et al. (2002) [24]	Liu et al. (2003) [16]	Mu et al. (2004) [21]	Nahas et al. (2007) [23]
Condition	Treatment-resistant depression	Treatment-resistant depression	Intractable partial seizure	Medically refractory epilepsy	Complex partial seizure	Treatment-resistant depression	Treatment-resistant depression
Struture							
Brainstem						↓lf	
Thalamus				↑bl			
Hypothalamus	↑lf	↑lf					
Cerebellar hemisphere							↓bl
Frontal lobe			↑bl				
Prefrontal cortex						↑bl	
Superior frontal gyrus							
Cingulate gyrus					↓bl	↓	↓rg
Orbitofrontal gyrus	↑bl	↑bl				↑bl	
Postcentral gyrus			↑rg	↑lf			↑lf
Entorhinal gyrus							
Temporal lobe	↑lf	↑lf		↑rg	↑lf	↑rg	↑bl
Insula				↑bl	↑	↑lf	
Amygdala	↑lf	↑lf			↑		
Hippocampus					↑		
Parahippocampal gyrus							
Nucleus accumbens							
Parietal lobe	↑bl	↑bl					
Occipital lobe	↑bl	↑bl		↑lf			
Putamen						↑lf	
Remarks							Active VNS group

Depending on the stimulation and scanning protocols, different brain areas are found to be involved. There is some agreement across the studies regarding neuroanatomical structures involved in processing VNS signal. Abbrevations: bl, bilateral; lf, left; rg, right.

diagnose and treat neuropsychiatric conditions, such as treatment-resistant depressions or epilepsy.

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