The Neuropsychology of transcutaneous Vagus Nerve Stimulation

Dissertation

zur Erlangung des akademischen Grades

doctor rerum naturalium (Dr. rer. nat.)

genehmigt durch die Fakultät für Naturwissenschaften der

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Eingereicht am 24. Mai 2019

Verteidigt am 22. Oktober 2019

Zusammenfassung

In dieser Dissertation werden neuropsychologische Effekte der transkutanen Vagusnervstimulation sowie ihr therapeutisches Potential im klinisch-neuropsychologischen Bereich untersucht. Die ersten beiden Studien zielen auf GABAerge Neuromodulation durch tVNS ab. Diese wird als einer der neuronalen Wirkmechanismen der tVNS diskutiert, doch die Evidenzlage ist noch ungenügend. Es wird eine Modulation der automatischen Motorinhibition durch tVNS gezeigt, welche eng mit der GABA-Konzentration im motorischen Kortex assoziiert ist. Andererseits findet sich kein Effekt von tVNS auf visuelle bistabile Perzeption, die eng mit der GABA-Konzentration im visuellen Kortex assoziiert ist. Da die Ergebnisse der beiden Studien kein konsistentes Bild ergeben, ist in dieser Arbeit ein zusätzlicher Studienentwurf enthalten, in dem per MEG gemessene Hirnoszillationen als Indikator für GABAerge Neuromodulation durch tVNS genutzt werden sollen. In der dritten Studie kommt eine Aufgabe zum Einsatz, in der Antwortkonflikte erzeugt werden. Es wird gezeigt, dass tVNS die globale und konfliktspezifische Performanz verbessern sowie frontomediale Theta-Aktivität, einen elektrophysiologischen Index für Konfliktkontrolle, verstärken kann, was das klinisch-neuropsychologische Potenzial der tVNS verdeutlicht. In der vierten Studie werden tonische Pupillengröße und ereigniskorrelierte Pupillenantworten als Indikator für noradrenerge Neuromodulation durch tVNS und als potentieller Biomarker für tVNS-Responsivität untersucht. Es zeigt sich kein systematischer Einfluss der tVNS auf die Pupillenmaße. In einem zusätzlichen Bericht wird exploriert, ob Vagus-sensorisch evozierte Potenziale (VSEP) helfen können, Responder und Nichtresponder zu identifizieren. Im Gesamtfazit hat diese Arbeit das klinische Potenzial der tVNS bestätigt. Die Wirkmechanismen und Determinanten für erfolgreiche Anwendung der tVNS sind jedoch noch ungenügend verstanden.

Abstract

This thesis focuses on neuropsychological effects of transcutaneous vagus nerve stimulation (tVNS) and its potential as a prospective treatment for neuropsychological deficits. The first two studies target behavioral and electrophysiological indices that might support a GABAergic neuromodulation by tVNS. GABAergic neuromodulation is one of the assumed mechanisms of action of tVNS, but the evidence in its favor is sparse. It is shown that tVNS modulates automatic motor inhibition, a process associated to GABA in the motor cortex, but not visual bistable perception, which is associated to GABA in the visual cortex. Moreover, the former effect is restricted to the contralateral brain hemisphere relative to the stimulation, suggesting a possible lateralization of the effect. Since these findings are not fully consistent with respect to GABAergic neuromodulation, a study proposal is enclosed in which GABAergic neuromodulation will be further scrutinized using MEG. The third study investigates effects of tVNS on executive control of action. It is shown that tVNS enhances global and conflict-specific behavioral performance in a cued go-nogo-change task as well as conflict-related electrophysiological frontal midline theta responses, rendering it a promising candidate treatment for executive control deficits. The fourth study probes the potential of pupillometry as a non-invasive marker for tVNS responsiveness. No systematic modulation of pupil size readouts by tVNS is found. In an additional short report, it is explored whether vagus-sensory evoked potentials (VSEP) can help to separate responder and non-responder groups of individuals. It is concluded that tVNS is a promising clinical method, and its further investigation for neuropsychological applications is promising. On the other hand, its mechanisms of action are still poorly understood and warrant further investigation.

Abkürzungsverzeichnis

ACC: anterior cingulate cortex
ACh: acetylcholine
ADHD: attention deficit hyperactivity disorder
Ag/AgCl: silver/silverchloride
AIC: Akaike information criterion
ANOVA: analysis of variance
BDNF : brain-derived neurotrophic factor
BF: Bayes factor
BIC: Bayes information criterion
BNC: Bayonet Neill–Concelman (cable connector)
cm: centimeter
CSF: cerebro-spinal fluid
CV: coefficient of variation
DNV: dorsal nucleus of the vagus
ECG: electrocardiography
EEG: electroencephalography
EMG: electromyography
ER: error rate
ERPD: event-related pupil dilation
FDA: U. S. Food and Drug Administration
fMRI: functional magnetic resonance imaging
GABA: gamma-aminobutyric acid
HDI: highest density interval
HPA: hypothalamic-pituitary-adrenal axis
Hz: Hertz
IIR: infinite impulse response
IOR: inhibition of return
ISI: inter-stimulus interval
ITI: inter-trial interval
iVNS: invasive vagus nerve stimulation
kHz: Kilohertz
kΩ: Kiloohm
LC: locus coeruleus
LRP: lateralized readiness potential
M: mean
mA: milliampere
MCI: mild cognitive impairment
MCMC: Markov chain Monte Carlo
MEG: Magnetoencephalography
MRI: magnetic resonance imaging

ms: millisecond
µs: microsecond
N1: first negative potential deflection
N2: second negative potential deflection
NA: nucleus ambiguus
NCE: negative compatibility effect
NE: norepinephrine
NTS: nucleus tractus solitarii
P1: first positive potential deflection
P2: second positive potential deflection
PD: percept duration
PI: principal investigator
PPI: prepulse inhibition
RPD: readiness potential difference waves
RT: reaction time
s: second
sAA: salivary alpha-amylase
SD: standard deviation
SEM: standard error of the mean
SFM: structure from motion
SICI: short-interval intracortical inhibition
SMA: supplementary motor area
SOA: stimulus onset asynchrony
SpV N: Spinal trigeminal nucleus
TMS: transcranial magnetic stimulation
TNS: trigeminal nerve stimulation
tVNS: transcutaneous vagus nerve stimulation
USA: United States of America
VNS: vagus nerve stimulation
VSEP: vagus-sensory evoked potential

Abbildungsverzeichnis

Inhalt

1 General Introduction

1.1 Prolegomena

This thesis is concerned with the *neuropsychology of transcutaneous vagus nerve stimulation* (tVNS). I want to clarify upfront that I am by no means claiming to describe or define this field comprehensively – this would be a presumptuous claim about a single person's work of a few years. However, in this thesis I try to cover different aspects of the field and to give recommendations for its further development. The studies described herein answer some questions and raise many new ones, thus I hope that they will spark further research and turn out to be fruitful contributions to the emerging neuropsychology of tVNS – hence the title.

1.2 About tVNS

TVNS is electrical stimulation of the vagus nerve (10th cranial nerve) through the skin (transcutaneously). It is a rather new method (Ventureyra, 2000, is commonly considered the first describing publication), that was developed to pose an alternative to direct or invasive vagus nerve stimulation (iVNS) for the treatment of drug-refractory epilepsy, depression and other disorders (Bauer et al., 2016; Hein et al., 2013; Lehtimäki et al., 2013). In contrast to iVNS, where a stimulation electrode is surgically wrapped around the vagus nerve in the neck, tVNS can be applied to different locations of the outer ear innervated by the auricular branch of the vagus nerve, mostly the cymba conchae or the tragus through electrodes attached to the skin (Kraus et al., 2013; Peuker & Filler, 2002; Yakunina, Kim, & Nam, 2017). It is also possible to apply tVNS externally to the neck (cervical tVNS, Brock et al., 2017; Simon & Blake, 2017), but the focus in this thesis will be on auricular tVNS. In the following, the abbreviation 'tVNS' will denote auricular transcutaneous vagus nerve stimulation unless further specified, 'iVNS' will denote invasive / surgical vagus nerve stimulation, and 'VNS' will denote the general concept of vagus nerve stimulation.

1.3 Historical sketch

The historical roots of VNS reach back more than one-hundred years. In the 18th and 19th century, it was believed that epilepsy was caused by excessive blood flow to the brain ('venous hyperaemia'), and epileptic patients were treated by manual compression of the carotid arteries in the neck to suppress blood flow. In the late 19th century, American neurologist James L. Corning developed his 'carotid fork' (Figure 1.), a device to facilitate carotid compression, which was later augmented by stimulation electrodes. Corning intended to stimulate cervical branches of the vagus nerve, which runs next to the carotid artery in the neck, in order to decrease heart rate and, subsequently, blood flow to the brain. Even though Corning reported good treatment successes, the method was not widely accepted at the time due to safety concerns and lack of reproducibility of therapeutic success (Lanska, 2002).

VNS fell into oblivion and was only further investigated decades later, when animal studies demonstrated that iVNS could synchronize or desynchronize brain oscillations in cats and dogs (Yuan & Silberstein, 2016). This motivated further research in humans. The first controlled clinical trials of iVNS as an antiepileptic treatment (Penry & Dean, 1990; Uthman et al., 1993) reported, on average, substantial reductions in seizure frequency, even though relevant proportions of patients did not respond, i.e., did not show improvements. Following a number of further clinical trials, iVNS, applied to the left cervical vagus nerve, was approved by the US Food and Drug Administration (FDA) for management of pharmacoresistant epilepsy in 1997 (Morris et al., 2013). During epilepsy studies, it had been noticed that patients' mood was enhanced following iVNS treatment (Harden et al., 2000), which led to studies in patients with depression and, in 2005, to FDA approval of iVNS for treatment of pharmacoresistant depression (Cristancho, Cristancho, Baltuch, Thase, & O'Reardon, 2011). More recently, iVNS has been under investigation as a candidate treatment for conditions that are not neurological in the narrower sense, such as heart failure (De Ferrari et al., 2010), arthritis, inflammatory bowel disease (Levine, Koopman, Faltys, Zitnik, & Tak, 2014), and chronic pain (Lange et al., 2011).

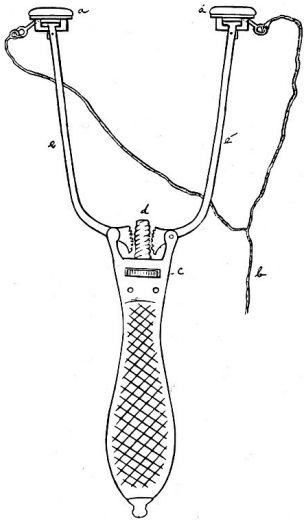

Figure 1. James L. Corning's carotid fork with stimulation electrodes. Reprinted from Lanska (2002).

In the meantime, non-invasive cervical and auricular tVNS approaches had been developed. Cervical tVNS is conceptually similar to Corning's initial approach of transcutaneuosly stimulating the vagus nerve in the neck, next to the carotid artery. It is mostly under investigation for headache management today (Goadsby, Grosberg, Mauskop, Cady, & Simmons, 2014; Nesbitt, Marin, Tompkins, Ruttledge, & Goadsby, 2015). The currently most widespread commercially available cervical tVNS device (gammaCore®, Desitin GmbH[1], Figure 2.) is hand-held and delivers sinusoidal alternating current with a broadband amplitude-modulated frequency spectrum (Nesbitt et al., 2015). Auricular tVNS is currently more established in research and clinical practice (my observation). It is under investigation for a wide range of clinical applications and has been receiving attention as a neuromodulation method to influence cognition and behavior in healthy individuals as well (described in more detail below). The most widespread commercially available auricular tVNS device (NEMOS®, tVNS technologies[2], Figure 2.) delivers current in rhythmic square pulses (Yuan & Silberstein, 2016).

[1] www.desitin.de

[2] www.t-vns.de

Figure 2. Commercial devices for administration of auricular (NEMOS®) and cervical (gammaCore®) tVNS. The small ear picture indicates the vagally innervated area of the auricle: Cymba conchae (Cy), cavum conchae (Ca), and tragus (T). Reprinted from Yuan & Silberstein (2016).

In the USA, the gammaCore® device has received FDA approval for headache management in 2017[3]. The NEMOS® device has received European clearance (CE certification, which indicates legal conformity and safety, but not necessarily clinical efficacy) as a treatment for epilepsy and depression in 2010 and for chronic pain in 2012 (Yuan & Silberstein, 2016).

[3] FDA submission no. DEN150048

1.4 Clinical and preclinical tVNS studies

Epilepsy

There have been several studies of tVNS in patients with pharmacoresistant epilepsy. An early pilot study (Stefan et al., 2012) found that seizure frequency was reduced in five out of seven patients after 9 months of tVNS therapy, and that tVNS was well tolerated. Similarly, another pilot study (He, Jing, Wang, et al., 2013) found seizure frequency reductions in 9 out of 14 children with epilepsy during a 6-month tVNS trial. A more recent, placebo-controlled clinical trial in 27 epilepsy patients (Bauer et al., 2016) corroborated that tVNS decreased seizure frequency after 20 weeks of daily treatment. However, only about half of the patients were classified as responders (here defined as seizure frequency reduction > 25%). A larger-scale clinical trial of tVNS in epilepsy is pending, and the evidence for the effectiveness of tVNS in epilepsy is insufficient as of now (Boon, De Cock, Mertens, & Trinka, 2018).

Depression

A placebo-controlled pilot study in patients with depression (Hein et al., 2013) found that two weeks of tVNS decreased depression severity as measured by standard inventories. This finding has been replicated later in a larger patient sample (Rong et al., 2016). However, this study identified only about one third of the patients enrolled as responders (here defined as reduction in the Hamilton-Depression Rating Scale score >50% after four weeks of treatment). Neuroimaging studies in depression patients found that tVNS altered functional brain connectivity in the default mode network (Fang et al., 2016; Liu et al., 2016) and led to insula activations that were correlated to the clinical effectiveness of tVNS treatment (Fang et al., 2017).

Tinnitus

Tinnitus is a third clinical field in which several tVNS studies exist. A pilot study (Lehtimäki et al., 2013) found that 10 days of tVNS, combined with sound therapy, ameliorated patient-reported tinnitus severity and attenuated auditory event-related fields in the patients' MEG signal. Similarly, another pilot study found clinically meaningful amelioration of patient-reported tinnitus severity in four out of 10 patients after 20 days of combined tVNS and sound therapy (De Ridder, Vanneste, Engineer, & Kilgard, 2014). This has been replicated in a larger sample (30 patients), 15 of whom

were classified as responders (here defined as patient-reported symptom relief) to combined tVNS and sound therapy (Shim et al., 2015). A further pilot study administering tVNS (without sound therapy) for six months could not replicate any clinically meaningful effect (Kreuzer et al., 2014).

Other diseases

A pilot study of tVNS in schizophrenia found no effect on symptom severity (Hasan et al., 2015). Moreover, it has been suggested to study tVNS as a potential treatment for attention deficit hyperactivity disorder (ADHD, Beste et al., 2016), autism spectrum disorders (Jin & Kong, 2017), Alzheimer's dementia (Jacobs, Riphagen, Razat, Wiese, & Sack, 2015), and post-operative cognitive dysfunction (Xiong et al., 2009). The idea that tVNS might be a promising treatment in Alzheimer's dementia has received support through recent evidence that tVNS can recover impaired microglia function in a mouse model of Alzheimer's dementia (Huffman et al., 2019; Kaczmarczyk, Tejera, Simon, & Heneka, 2018), and there is an ongoing clinical trial of tVNS as a treatment for mild cognitive impairment (MCI)[4]. For ADHD, trigeminal nerve stimulation (TNS) has been suggested as an alternative or complementary treatment to tVNS, and a recent study found promising clinical improvements (McGough et al., 2019).

All of the above studies assumed that tVNS effects are primarily mediated by central neuromodulation, i.e., effects on neurotransmission and neuroplasticity in the brain. As discussed in more detail below, it is assumed that tVNS has also peripheral-autonomic effects, and a number of studies have focused on the clinical potential of these effects.

A study in patients suffering from chronic pelvic pain (Napadow et al., 2012) found that tVNS ameliorated patient-reported pain intensity and anxiety. Antinociceptive effects of tVNS have been replicated in some studies but not in others, and remained overall rather inconsistent between studies and individuals (De Icco et al., 2018; Janner, Klausenitz, Gürtler, Hahnenkamp, & Usichenko, 2018; Laqua, Leutzow, Wendt, & Usichenko, 2014; Usichenko, Laqua, Leutzow, & Lotze, 2017).

Some studies investigated cardiac consequences of peripheral-autonomic modulation through tVNS. It has been found to reduce sympathetic nerve activity, indexed through heart rate variability and resting musle activity (Clancy et al., 2014; Murray, Clancy,

[4] ClinicalTrials.gov Identifier: NCT03359902

Deuchars, & Deuchars, 2016; Ylikoski et al., 2017) and to have beneficial effects on atrial fibrillation (Stavrakis et al., 2015; Yu et al., 2013). Cardiac effects of tVNS depend on stimulation parameters, such as pulse width and stimulation frequency (Badran, Mithoefer, et al., 2018). These findings suggest that tVNS has clinical potential for cardiological conditions, but again, one of the key challenges is low consistency of effects between individuals (Murray, Atkinson, Mahadi, Deuchars, & Deuchars, 2016).

Taken together, these studies indicate that tVNS bears clinical potential for a wide range of conditions. One of the key challenges for its further development appears to be the lack of interindividual consistence in treatment success between individuals. Those differences are currently not well understood, and they may depend on anatomy, current physiological state, and stimulation parameters.

1.5 Mechanisms of action of VNS

Anatomy

The vagus nerve is the 10th cranial nerve. Vagus is latin for wandering or excursive, and in fact the vagus nerve has a wide distribution in the body, both anatomically and functionally (Figure 3.). It carries afferent and efferent fibres. One of its central functions is parasympathetic innervation of the heart, lungs, and gastrointestinal organs. Further vagus efferents innervate the voluntary muscles of the larynx and pharynx. Afferent vagus nerve fibres carry visceral information from the lungs, heart, gastrointestinal tract, taste information, and sensory information from the concha of the outer ear, through the auricular branch of the vagus nerve. The cervical vagus nerve runs parallel to the carotid artery in the neck, before entering the skull through the jugular foramen. In the brainstem, vagus nerve fibres terminate in four brain projection areas: Nucleus ambiguus (NA), dorsal nucleus of the vagus (DNV), Nucleus tractus solitarii (NTS), and spinal trigeminal nucleus (SpV N). NA and DNV give rise to parasympathetic and efferent fibres of the vagus nerve, whereas NTS and SpV N receive afferent information (Berthoud & Neuhuber, 2000; Clancy, Deuchars, & Deuchars, 2013; Rutecki, 1990).

Herein lies the most prominent anatomical difference between iVNS and auricular tVNS: iVNS in the neck (and potentially also cervical tVNS) can, in principle, reach all types of vagus nerve fibres, and subsequently all vagus brain projection areas, since all fibres pass through the neck, whereas auricular tVNS is restricted to the (afferent) auricular branch of the vagus nerve and, subsequently, the afferent brain projection areas, NTS and SpV N (Clancy et al., 2013). For cervical VNS, the recruitment of different types of vagus nerve fibres likely depends on stimulation parameters, especially stimulation frequency and current intensity (Rutecki, 1990). Whether and how stimulation of certain vagus nerve fibre types has specific consequences for therapeutic effects is not fully understood, but evidence from rodent studies suggests that optimal anti-inflammatory effects of iVNS can be achieved by activating efferent fibres while inhibiting afferent fibres of the vagus nerve (Borovikova et al., 2000; Patel, Saxena, Bellamkonda, & Butera, 2017).

Physiology

The assumed physiological mechanisms of VNS are mostly discussed in terms of modulations of central neurotransmission and neural plasticity, particularly in the locus coeruleus-norepinephrine (LC-NE) system. There is a second line of research, focusing on – presumably acetylcholine-mediated – peripheral-autonomic effects. However, both lines of research are widely independent, and, to the best of my knowledge, no human and very few rodent studies have considered central-neuromodulatory and peripheral-autonomic VNS effects and their interaction simultaneously. Moreover, direct neurophysiological studies (e.g., invasive recordings or neurochemical analyses) in humans and rodents have been carried out almost exclusively for iVNS but not for tVNS, therefore models of physiological mechanisms of tVNS have to be inferred from iVNS studies, and direct physiological evidence for physiological effects of tVNS is sparse. It will be reviewed separately in the next section.

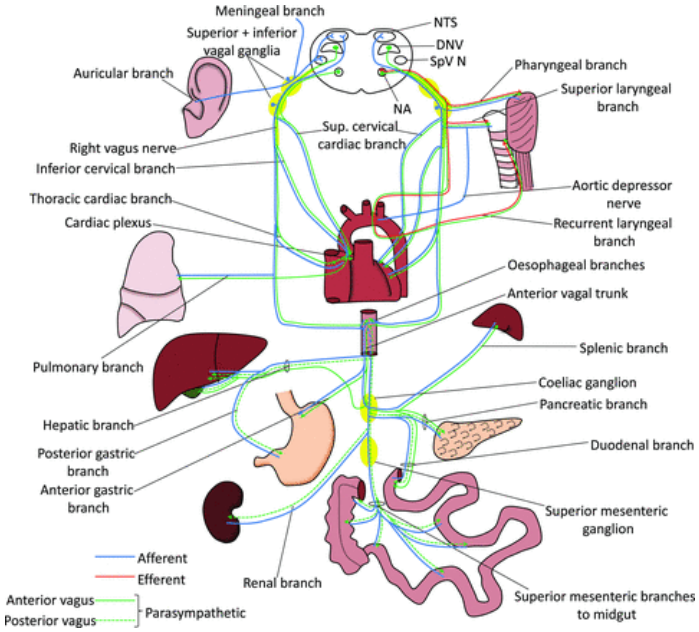

Figure 3. Overview of functional vagus nerve anatomy. On top, the four brainstem projection areas of the vagus nerve are shown. NTS: nucleus tractus solitarii; DNV: Dorsal nucleus of the vagus; SpV N: Spinal trigeminal nucleus; NA: Nucleus ambiguus. Reprinted from Clancy, Deuchars, & Deuchars (2013).

It has been found that iVNS entails concentration shifts for several neurotransmitters, most prominently norepinephrine (NE). NE is an anatomically plausible mediator for VNS effects, since the locus coeruleus (LC), the main source of NE in the human and rodent brain, is a downstream projection area of the NTS, which is one of the brain projection areas of afferent vagus nerve fibres (Aston-Jones & Cohen, 2005b; Berthoud & Neuhuber, 2000). Two studies using electrophysiological single-cell recordings in rodents found that iVNS increased firing of NEergic, and, subsequently, serotonergic neurons, and decreased firing of dopaminergic neurons in the ventral tegmental area (Manta, Dong, Debonnel, & Blier, 2009; Manta, El Mansari, Debonnel, & Blier, 2013). Invasive electrophysiological recordings in rodents have corroborated increased firing of NEergic neurons in the LC and hippocampus and serotonergic neurons in the dorsal raphe nuclei following iVNS (Dorr & Debonnel, 2006; Groves, Bowman, & Brown, 2005; Hulsey et al., 2017; Raedt et al., 2011). Similarly, increased levels of NE and brain-derived neurotrophic factor (BDNF) as measured by microdialysis in the rodent medial prefrontal cortex and subsequent neurochemical analysis have been shown following iVNS (Follesa et al., 2007). Lesioning the LC in rodents abolished antidepressant-like effects of iVNS as measured through the forced-swim test (Grimonprez, Raedt, Portelli, et al., 2015). Despite this abundant evidence for an

NEergic pathway of iVNS in rodents, findings in human patients have been less consistent. Neurochemical analyses of cerebrospinal fluid (CSF) of patients receiving iVNS found no evidence for increased NE metabolites (Ben-Menachem et al., 1995; Carpenter et al., 2004), and effects of iVNS on non-invasive indices of NEergic activity (P300 component of the event-related potential and pupil size) have been inconsistent (De Taeye et al., 2014; Jodoin, Lespérance, Nguyen, Fournier-Gosselin, & Richer, 2015; Schevernels et al., 2016).

As a second mechanism of action, interactions of iVNS with gamma-aminobutyric acid (GABA) transmission have been reported by some studies. In the cerebro-spinal fluid (CSF) of human patients receiving iVNS, increased levels of GABA have been found (Ben-Menachem et al., 1995). By means of metabolic neuroimaging, it has been shown that long-term iVNS increased GABA receptor density in frontal and frontotemporal areas in epileptic patients, and that the receptor density increase was highly correlated to seizure reduction (Marrosu et al., 2003), and that long-term iVNS increased resting EEG power in the gamma (20-50 Hz) band, which is, in turn, related to local GABA concentration (Marrosu et al., 2005). Along these lines, it has been found that pharmacological increases in GABA transmission in the rodent NTS reduced susceptibility to seizures (Walker, Easton, & Gale, 1999). Another rodent study found a protective effect of iVNS on GABAergic neurons following traumatic brain injury (Neese et al., 2007).

Finally, central and peripheral acetylcholine (ACh, which was historically referred to as ‘Vagusstoff’/’vagus substance’) transmission is likely also involved in VNS mechanisms of action, even though the evidence supporting this involvement is rather sparse and indirect. It has been shown in rodents that electrophysiological effects of iVNS on cortical synchrony and excitability were disrupted after infusion of the muscarinic (i.e., one of the receptor types of ACh) antagonist scopolamine, so the study authors concluded that these VNS effects were mediated by muscarinic receptor activation (Nichols et al., 2011). Further rodent studies found effects of iVNS on peripheral inflammatory responses (Borovikova et al., 2000) and on central neural plasticity in the auditory cortex (Engineer, Møller, & Kilgard, 2013; Engineer et al., 2011; Morrison et al., 2019), which the authors attributed to cholinergic neuromodulation induced by iVNS, respectively. Moreover, effects of iVNS on

myocardial function (Lewis et al., 2001), heart rate (Buschman et al., 2006), and heart rate variability (Hirfanoglu et al., 2018) have been found, providing evidence for peripheral-autonomic effects of iVNS. It is assumed that these peripheral effects of iVNS are mediated through vagal activation of the hypothalamic-pituitary-adrenal axis (HPA) as well as ACh release in the intestine and spleen (Bonaz, Sinniger, & Pellissier, 2016). In turn, peripheral-autonomic activation can have upstream effects in the brain, such as fostering neural plasticity in the hippocampus via the NTS and medial septum, as a recent rodent study has shown (Suarez et al., 2018).

In sum, there is solid evidence for an involvement of the LC-NE system in the mechanisms of action of iVNS, even though findings in rodents could not always be translated to humans as of now. There is accumulating evidence for GABAergic and parasympathetic-cholinergic pathways of iVNS, but the number of studies explicitly probing these pathways is still limited. Some studies have found effects of iVNS on serotonergic and dopaminergic transmission, but this evidence has remained rather anecdotal.

tVNS studies

The above findings on physiological mechanisms of VNS all originate from studies employing iVNS, which raises the question of how well they translate to tVNS. Several fMRI studies in healthy humans and tinnitus patients consistently found increased NTS and LC activations following tVNS (Dietrich et al., 2008; Frangos, Ellrich, & Komisaruk, 2015; Kraus et al., 2013; Yakunina et al., 2017; Yakunina, Kim, & Nam, 2018). Part of the studies reported activations in the spinal trigeminal nucleus, dorsal raphe nuclei, and insula (Dietrich et al., 2008; Kraus et al., 2007). For other brain areas, such as the thalamus and nucleus accumbens, increased as well as decreased activation has been reported following tVNS (Dietrich et al., 2008; Frangos et al., 2015; Kraus et al., 2013). It has been found that tVNS increased short-interval cortical inhibition (SICI) in the motor cortex, a transcranial magnetic stimulation (TMS) readout informative of GABA transmission, and two recent studies reported tVNS-induced increases in salivary alpha-amylase (sAA), a marker of central NEergic transmission (Ventura-Bort et al., 2018; Warren et al., 2018).

As for cholinergic-parasympathetic effects, it has been shown that tVNS can decrease heart rate (Badran, Mithoefer, et al., 2018), and increase heart rate variability (Clancy et al., 2014; Sclocco et al., 2019; Tran et al., 2018). Both findings suggest a shift towards parasympathetic preponderance. Moreover, anti-neuroinflammatory effects of cervical tVNS have been shown in rodents (Kaczmarczyk et al., 2018). For cardiovascular effects of tVNS, a mediating role of the NTS has been discussed as well (Chen et al., 2015; Murray et al., 2016).

In sum, empirical evidence supports the assumption that the effects of tVNS on NEergic and GABAergic neuromodulation as well as its cholinergic-parasympathetic effects are comparable to the effects of iVNS.

The role of stimulation parameters

Prior to the administration of tVNS, a number of decisions must be made regarding the side (left ear, right ear) and location (tragus, cymba conchae, ear canal, etc.) of stimulation electrodes, stimulation frequency, pulse width, current intensity, on-off cycle, pulse shape and polarity (sinusoidal, sqare-shaped, etc.), and overall treatment duration. How these parameters affect neural and clinical effects of the stimulation is not well understood, which is partly due to the multidimensionality of the problem – if every one of these eight parameters had only two levels to vary between, the parameter space would comprise $2^8 = 256$ parameter combinations to be compared, and most of the above parameters are much more variable in reality. However, a number of studies have tackled the question, and their findings highlight some important aspects of parameter-effect relationships in tVNS and iVNS.

For clinical applications, both iVNS and tVNS are almost exclusively administered to the left vagus nerve or left ear, respectively, due to anticipated adverse cardiac effects, since the right vagus nerve sends efferent fibres to the heart (Nemeroff et al., 2006). These concerns are most likely unfounded, considering that several studies have applied right-sided or bilateral tVNS and iVNS in animals and humans without reporting any adverse cardiac effects (He et al., 2013; Krahl, Senanayake, & Handforth, 2003; Premchand et al., 2014; Wang, Yu, et al., 2015; Wang, Zhou, Sheng, Yu, & Jiang, 2015), and that (beneficial) cardiac effects have also been found for left-sided tVNS

(Chen et al., 2015; Clancy et al., 2014). Nonetheless, left-sided application of tVNS and iVNS is the current standard.

Within the auricle, tVNS can be administered to the vagally innervated area, i.e., the tragus, the cymba conchae, the cavum conchae, or the ear canal (Peuker & Filler, 2002). It has been reported that tVNS in the anterior vs. posterior wall of the ear canal produced different activations as measured by fMRI (Kraus et al., 2013), but this finding has not been replicated as of now. Another fMRI study that systematically compared brain activations following tVNS at different locations in the auricle found that cymba conchae, ear canal, and tragus stimulation produced activations of vagus projection areas, but the most consistent activations were achieved with cymba conchae stimulation (Yakunina et al., 2017).

The most systematic exploration of iVNS parameter space to date has been carried out in rodents (Hulsey et al., 2017). Using invasive electrophysiology, the authors showed monotonous positive relationships between iVNS current intensity (0 to 2.5 mA) and LC activation; stimulation frequency (7.5 to 120 Hz) and LC activation (the total number of driven spikes was comparable across frequencies, but at higher frequencies the firing rate was increased, i.e., the LC spiked faster); and pulse width (30 to 500 μs) and LC activation. In sum, the study found that the increase in LC spiking per iVNS stimulation pulse is, within the tested parameter range, approximately a linear function of total charge per pulse (pulse width × current). Findings in humans have been consistent with this, yet less systematic: Monotonous relationships between iVNS pulse width and fMRI-measured brain activation (Mu et al., 2004) and between iVNS frequency and brain activation (Lomarev et al., 2002) have been reported. However, increased LC spiking or increased activity in other vagus projection areas are probably not linearly related to clinical benefits, such that simple conclusions (such as ‘more charge, more clinical efficacy’) cannot be drawn from these studies. In fact, several studies assessing iVNS effects on neural plasticity in rodents rather found an inverted U-shaped effect, in that moderate iVNS intensities (e.g., 0.5 mA) induced the highest level of neural plasticity, whereas both higher and lower intensities had a weaker or no effect (Borland et al., 2016; Morrison et al., 2019; Zuo, Smith, & Jensen, 2007).

Some animal studies suggest that iVNS does not always have an activating effect on the vagus nerve, but that a nerve block can be achieved as well through certain current waveforms (Vuckovic, Tosato, & Struijk, 2008) or extremely high stimulation frequencies in the kilohertz range (Patel et al., 2017).

Overall treatment duration is highly variable between studies: tVNS studies in healthy individuals typically only apply a single session of stimulation, with a duration of ~15 minutes to 1h (e.g., Sellaro, de Gelder, Finisguerra, & Colzato, 2018), whereas patients enrolled in iVNS studies have typically received several months of iVNS at experiment time. Anecdotal reports suggest that the clinical efficacy of iVNS unfolds over a period of approximately 18 months (De Taeye et al., 2014), but a systematic investigation is pending.

In sum, the relationship between iVNS parameter space and LC activation in rodents is relatively well understood. It is unclear, however, how well these findings translate to humans, to tVNS, and to clinical efficacy. Most tVNS studies use commercially available stimulation devices, which typically have as standard parameters a frequency of 25 Hz, 200 µs pulsewidth, 30s on / 30s off cycle, and variable current intensities up to 3 mA (e.g., Sellaro et al., 2018).

1.6 Cognitive-behavioral studies of tVNS

The assumed neuromodulatory effects of tVNS have attracted the interest of cognitive neuroscientists, and a number of studies in healthy individuals have probed effects of tVNS on cognitive-behavioral measures. Studies can be roughly grouped into those that investigated effects on executive control in the wider sense, effects on social-emotional functions, and effects on memory. The first group of studies chiefly discuss tVNS effects on executive control as a consequence of NEergic and GABAergic neuromodulation. Part of the studies in the second group invoke the polyvagal theory (Porges, 2001), which states that social interaction and emotion recognition is controlled by autonomic regulation through the vagus nerve. The third group of studies discuss NE-mediated plasticity as one of the mechanisms mediating effects of tVNS on memory. In sum, the studies reviewed below have shown effects of tVNS in a variety of cognitive-behavioral domains in healthy individuals, but some of them faced similar

problems as the clinical studies reviewed above, i.e., lack of reproducibility and consistency of effects.

Studies on executive control

It has been found that tVNS enhanced behavioral performance (reaction times) in an action cascading task, in which participants reacted to the position of a visual stimulus on the screen, but were required to switch between different position-response mappings in a subset of trials, prompted through auditory signals (Steenbergen et al., 2015).

A study employing a serial reaction time task with alternating predictable and unpredictable response sequences found no global effect of tVNS on behavioral performance, but a decrease in a particular trial sequence effect: performance impairments when a stimulus was identical to the stimulus two positions before, a concept analog to inhibition of return (IOR), which typically occurs in spatial attention paradigms (Klein, 2000). This sequential IOR was decreased under tVNS, but only during predictable response sequences (Jongkees, Immink, Finisguerra, & Colzato, 2018).

Another study involving a choice reaction time task and an Eriksen flanker task found that tVNS enhanced post-error slowing in both tasks, an established behavioral index of performance monitoring and error processing (Sellaro, van Leusden, et al., 2015).

In a study on inhibitory control, tVNS was found to decrease false alarm rates in a go/nogo task with the additional requirement that stimuli had to be mentally rotated. The same study did not find effects of tVNS on performance in a backwards inhibition paradigm, in which cues prompted switching between three task sets, and trial sequence effects were studied (Beste et al., 2016).

In a variant of the Simon task, it has been shown that the sequential modulation of the Simon effect, i.e., modulation of the effect of response conflict on reaction time through conflict in the previous trial, was increased under tVNS, along with increased amplitudes in the N2-P3 event-related potential complex (R. Fischer, Ventura-Bort, Hamm, & Weymar, 2018).

Studies on social-emotional functions

A study involving a Cyberball task, i.e., a virtual ball-throwing game against purported other players (who were really computer-controlled confederates), found no enhancement of prosocial behavior through tVNS (Sellaro, Steenbergen, Verkuil, van Ijzendoorn, & Colzato, 2015).

However, it has been found that tVNS enhanced emotion recognition in a 'reading the mind in the eyes' task, i.e., rating emotional states from photos of human eyes regions (Colzato, Sellaro, & Beste, 2017). Using a similar task, this emotion recognition enhancement through tVNS has been replicated for face but not body images (Sellaro et al., 2018).

One subscale of subject-reported flow experience as measured by the Flow Short-scale, absorption, was found to be decreased under tVNS, whereas the other subscale, fluency, was not modulated (Colzato, Wolters, & Peifer, 2018).

Finally, one study reported that tVNS enhanced divergent thinking, a cognitive process subserving creativity (Colzato, Ritter, & Steenbergen, 2018).

Studies on memory and fear

In a sample of elderly healthy individuals, tVNS enhanced recall in an associative face-name memory task (Jacobs et al., 2015).

Another study demonstrated that tVNS accelerated extinction of conditioned fear (Burger et al., 2016). However, these effects did not generalize to psychophysiological indices of fear extinction, such as eyeblink startle reflex and skin conductance response in a later study (Burger et al., 2017), and three further studies could not consistently replicate effects of tVNS on fear extinction (Burger et al., 2019; Burger et al., 2018; Genheimer, Andreatta, Asan, & Pauli, 2017).

1.7 Recent trends in VNS research

Physiological effects of VNS can depend on the precise timing of the stimulation relative to physiological states. Early studies in cats and dogs found that effects of iVNS on heart rate depended on the phase of the cardiac cycle at the time of stimulation pulses

(Jalife & Moe, 1979; Levy, Lano, & Zieske, 1972). Some recent studies have focused on the dependence of tVNS effects on the respiratory cycle. It is a long-standing theory that LC activity and excitability are sensitive to the respiratory cycle (Melnychuk et al., 2018), and indeed it has been found that fMRI-measured brain activations in vagus projection areas as well as modulations of heart rate variability were stronger when tVNS was administered during exhalation compared to sham and inhalation, possibly reflecting an interaction between respiration-locked LC activations and the electrical stimulation (Garcia et al., 2017; Napadow et al., 2012; Sclocco et al., 2019).

A second emerging line of research is closed-loop administration of VNS. So far, it has been demonstrated that heart rate control can be achieved using closed-loop iVNS systems, where heart rate is continuously monitored using electrocardiography (ECG) and iVNS is activated when the ECG data fulfills certain criteria, e.g., when heart rate exceeds a threshold (Romero-Ugalde et al., 2018; Tosato, Yoshida, Toft, Nekrasas, & Struijk, 2006; Zhou et al., 2010). To my knowledge, there are no further reports of closed-loop VNS applications so far. However, closed-loop cortical stimulation systems for epilepsy have been tested successfully (Fountas et al., 2005), and closed-loop iVNS and tVNS systems for clinical applications may be developed in the not too distant future.

The third recent trend in VNS research is the search for biomarkers predictive of stimulation responsiveness. As pointed out above, two key challenges for further development and investigation of VNS are the high proportion of non-responders in clinical and preclinical studies, and the multidimensionality of the parameter space. The ideal biomarker will be easy to measure, reliably separate responders from non-responders, and be sensitive to stimulation parameters in responders, such that stimulation protocols can be individually optimized. Furthermore, it should covary with clinical efficacy of the stimulation. Several candidate biomarkers have been tested, including ECG, EEG, fMRI, pupillometry, and CSF readouts (Clancy et al., 2014; De Taeye et al., 2014; Fang et al., 2017; Raedt et al., 2011; Schevernels et al., 2016). The biomarker problem will be further detailed in chapters 5 and 6.

1.8 Outline

This thesis incorporates four main studies and three additional short reports. Study 1 investigates behavioral and electrophysiological effects of tVNS on automatic motor inhibition using a subliminal response priming task. Study 2 asks for modulations of visual bistable perception dynamics through tVNS. The overall aim of studies 1 and 2 is to test for neuropsychological consequences of GABAergic neuromodulation, which is assumed to be one of the mechanisms of action of tVNS. Few studies have explicitly investigated this GABAergic modulation so far. I pick up previous research showing that both experimental paradigms – subliminal response priming and visual bistable perception dynamics – are tightly coupled to GABA levels in the supplementary motor area (SMA) and the visual cortex, respectively, therefore a modulation of the behavioral and electrophysiological readouts can be expected, given a consistent GABAergic neuromodulation through tVNS. Study 3 employs a cued go-nogo-change task and scrutinizes behavioral and electrophysiological effects of tVNS, especially frontal midline theta activity during response conflicts. This study does not focus on any particular neurotransmitter system that might be affected by tVNS, but rather has a functional focus and asks whether tVNS might be a candidate treatment for clinical deficits in executive control and conflict monitoring. Study 4 uses tonic and event-related pupil size measurement to capture the temporal evolution and interindividual consistency of modulations of the LC-NE system through tVNS. The potential of pupil size measurements as a biomarker for tVNS responsiveness is discussed. Three additional short reports investigate effects of tVNS on prepulse inhibition (PPI) of the acoustic startle reflex as well as the brain oscillations measured by magnetoencephalography (MEG) and vagus-sensory evoked potentials (VSEP) as candidate predictive biomarkers for tVNS responsiveness.

2 Study 1: GABA-associated effects of tVNS in the motor system

A modified version of this chapter, excluding the section ‘Earlobe-sham stimulation as a potential confounding factor’ of the discussion, has been published as:

Keute, M., Ruhnau, P., Heinze, H. J., & Zaehle, T. (2018). Behavioral and electrophysiological evidence for GABAergic modulation through transcutaneous vagus nerve stimulation. *Clinical neurophysiology*, *129* (9), 1789-1795.

A modified version of the section ‘Earlobe-sham stimulation as a potential confounding factor’ of the discussion has been published separately as:

Keute, M., Ruhnau, P., & Zaehle, T. (2018). Reply to" Reconsidering Sham in Transcutaneous Vagus Nerve Stimulation studies". *Clinical neurophysiology*, *129* (11), 2503.

2.1 Abstract

Transcutaneous vagus nerve stimulation (tVNS) has been hypothesized to modulate gamma-aminobutyric (GABA) transmission in the human brain. GABA in the motor cortex is highly correlated to measures of automatic motor inhibition that can be obtained in simple response priming paradigms. To test the effects of tVNS on GABA transmission, we measured tVNS-induced alterations in behavioral and electrophysiology during automatic motor inhibition. Participants were 16 young, healthy adults (8 female). We combined a subliminal response priming paradigm with tVNS and EEG measurement. In this paradigm, automatic motor inhibition leads to a reversal of the priming effect, a phenomenon referred to as the negative compatibility effect (NCE). We computed the NCE separated by response hands, hypothesizing a modulation of the left-hand NCE. Using EEG, we measured readiness potentials, an established electrophysiological index of cortical motor preparation. As hypothesized, for the ipsilateral hand/contralateral hemisphere, compared to sham stimulation, tVNS increased the NCE and modulated the electrophysiological readiness potentials. Our results indicate that tVNS is selectively affecting the GABAergic system in the motor system contralateral to the stimulated ear as reflected in a behavioral and electrophysiological modulation. This is the first combined behavioral and electrophysiological evidence for direct GABAergic neuromodulation through tVNS.

2.2 Introduction

Previous research has shown that tVNS can impact a variety of cognitive and behavioral functions, such as response inhibition, action cascading, and memory (Beste et al., 2016; Jacobs et al., 2015; Steenbergen et al., 2015), such that it bears potential beyond its already established clinical applications in epilepsy, depression, and pain: since it is a treatment with low cost, low effort and low risk, it can be considered as a therapeutic option for conditions far less pervasive than drug-refractory epilepsy, and prospectively, it might be used as a treatment for neuropsychological deficits or even to optimize neuropsychological functioning in healthy individuals.

The effects of both methods of vagus nerve stimulation are assumed to be mediated by concentration shifts of the neurotransmitters norepinephrine (NE), gamma-aminobutyric acid (GABA), and acetylcholine (ACh) in the central nervous system (Van Leusden, Sellaro, & Colzato, 2015). Previous studies suggested that these neurotransmitter modulations concomitantly account for cognitive-behavioral effects of VNS without further delineating the effects of individual transmitters. However, dedicated studies of neuromodulatory mechanisms of action have been carried out almost exclusively for iVNS. Therefore, direct evidence for effects of tVNS on neurotransmission is sparse. Neuroimaging studies have shown that brain activations elicited by tVNS are similar at large to those elicited by iVNS (Assenza et al., 2017; Dietrich et al., 2008; Frangos et al., 2015; Liu, Mosier, Kalnin, & Marks, 2003; Mu et al., 2004), such that common neuromodulatory mechanisms of invasive and transcutaneous VNS can be assumed.

Central VNS effects are mainly mediated by the nucleus tractus solitarii (NTS), which is a key brain projection area of the vagus nerve. The NTS, in turn, projects to a number of brainstem and forebrain areas, including the locus coeruleus (LC), which is the main source of norepinephrine (NE) in the brain and presumably mediates NEergic effects of VNS. In support of this, short-term effects of iVNS on cortical and hippocampal NE concentration have been found (Ben-Menachem et al., 1995; Follesa et al., 2007; Raedt et al., 2011; Roosevelt, Smith, Clough, Jensen, & Browning, 2006). Furthermore, other transmitter systems show responsiveness to VNS, for instance, neurobiological studies involving both rodents and humans with epilepsy receiving iVNS showed long-term modulations of the dopamine and serotonin system (Carpenter et al., 2004; Hammond et al., 1992; Manta et al., 2013).

Effects of VNS on GABA transmission have been less frequently investigated than noradrenergic effects. In rats, no immediate hippocampal GABA modulation was observed after repeated short trains of iVNS (Raedt et al., 2011). After long-term iVNS, however, cortical $GABA_A$ receptor density as well as GABA levels in cerebrospinal fluid in human epilepsy patients were increased, and additionally, the receptor density increase was highly correlated with seizure reduction (Hammond et al., 1992; Marrosu et al., 2003).

There is, to our knowledge, only one previous study explicitly investigating a GABAergic pathway of tVNS. Using transcranial magnetic stimulation (TMS), Capone

et al. (2015) demonstrated reduced cortical excitability in healthy participants after one hour of tVNS as measured through increased short-interval intracortical inhibition (SICI). SICI is a TMS protocol informative of $GABA_A$ activity within the motor cortex. The SICI increase indicates increased GABA concentration (Stagg et al., 2011). Furthermore, the reported effect was restricted to the motor cortex contralateral to the stimulated ear (i. e., to the right motor cortex following stimulation of the left ear). Another study found evidence for a hemisphere-specific mechanism of action of VNS, in that an increase in gamma band power was found in the EEG of iVNS-treated epilepsy patients that was stronger on the right side of the scalp, i. e., contralateral to the stimulation (Marrosu et al., 2003). In the present study, we aimed to assess whether the effect of tVNS on cortical excitability suggesting an effect of tVNS on GABA activity in the motor cortex (Capone et al., 2015) would translate into a GABA-associated behavioral effect. We used a subliminal motor priming paradigm, in which the direction of arrows appearing on the screen has to be indicated by the subject, with the target arrows being preceded by a subliminal masked arrow serving as a prime. When the prime-target stimulus-onset asynchrony (SOA) is chosen within a time window of ca. 100 – 200 ms, a negative compatibility effect (NCE) occurs, i. e., faster reactions in incompatibly than in compatibly primed trials. Electrophysiologically, the NCE is reflected in characteristic patterns of lateralized readiness potentials (LRP) (Eimer & Schlaghecken, 1998). The LRP is a difference waveform composed of multiple components from EEG sensors capturing activity from the motor cortex. It is indicative of cortical motor tendency at a given point in time, i. e., whether a movement of the left or right hand is prepared in the motor cortex (Eimer, 1998). The LRP is triphasic in compatible trials and biphasic in incompatible trials, each phase corresponding to either motor preparation or motor inhibition for one response and the opposite for the other response. The second phase of the compatible-trial LRP is referred to as the reversal phase and considered the electrophysiological substrate of automatic inhibition of the initially primed response, which causes the NCE (Seiss, Klippel, Hope, Boy, & Sumner, 2014).

The inhibitory process causing the NCE has been shown to be mediated by the supplementary motor area (SMA) (Sumner et al., 2007) and to be robustly negatively correlated to GABA concentration in the SMA as measured by magnetic resonance spectroscopy (Boy, Evans, et al., 2010). If tVNS modulates GABA transmission in the

contralateral motor cortex (Capone et al., 2015), we expect automatic motor inhibition to be modulated in the right motor cortex following left-ear tVNS, resulting in modulated automatic inhibition of left-hand responses. Therefore, our analyses focus on the comparison between compatibly primed (i. e., inhibited) and incompatibly primed (i.e., facilitated) left-hand responses. In the following, we refer to the intraindividual reaction time (RT) difference between compatibly primed and incompatibly primed left-hand trials as the left-hand NCE.

We hypothesized that tVNS, administered to the left ear, will increase GABA transmission of the right motor cortex that, in turn, will result in a modulated left-hand NCE and altered LRP components for trials with a left-hand response.

2.3 Methods

Subjects and general procedure

Participants were 16 healthy adults (8 female). Age varied from 20 to 28 years (M 25.1 ± 2.4). All participants were free from any current or past neurological or psychiatric disorder, were right-handed and had normal or corrected-to-normal vision (all by self-report). Before the experimental sessions, written informed consent was obtained from all subjects. The experiment was carried out in accordance with the declaration of Helsinki, and approved by the local ethics committee. Each participant underwent two experimental sessions at least 48 hours apart, one involving tVNS, and one involving sham stimulation. For each subject, both sessions were scheduled at the same daytime. Subjects received money (8 € / hr) or course credit as a reimbursement for participation. The order of sham stimulation and tVNS was randomized across all participants.

Electrical stimulation

TVNS was administered to the cymba conchae of the left ear, sham stimulation to the left earlobe (cf. Figure 4.). Due to cardiac safety concerns, administration of tVNS is, by convention, limited to the left ear (Chen et al., 2015). The cymba conchae has recently been demonstrated to be the optimal location for tVNS (Yakunina et al., 2017). Two conventional neurostimulation electrodes were used (Ambu Neuroline[5]) that were cut manually to a size of 4×4 mm. The two electrodes were placed 1 cm apart (center-to-

[5] www.ambu.com

center) with the anode being more rostral, and fixated using Genuine Grass EC2 adhesive electrode cream (Natus Neurology[6]). Stimulation current was delivered as monophasic square pulses at a pulse width of 200 µs using a medical stimulation device (Digitimer DS7[7]) triggered via a BNC cable by custom code written in Matlab (MathWorks[8]).

Pulse frequency was set to 25 Hz in trains of 30s, each followed by 30s without stimulation. Stimulation intensity was set to 8 mA, if tolerable for the subject, and else individually adjusted below pain threshold. Mean stimulation intensity delivered was 5.9 ± 1.6 mA for tVNS and 7.5 ± 0.8 mA for sham stimulation. These stimulation settings are in the range of standard parameters used in clinical trials and therapy (Dietrich et al., 2008). In each experimental session, electrical stimulation began 25 minutes prior to the task and ended at task onset.

6 www.natus.com

7 www.digitimer.com

8 ww.mathworks.com

Experimental task and analysis

The experimental scenario was created using Presentation software (Neurobehavioral Systems[9]) and presented on a 24 inch LCD screen with a vertical refresh rate of 60 Hz. Participants were seated at a distance of 70 cm to the screen. Each trial consisted of a prime double arrow, presented for one frame (16 ms), a single blank frame, followed by a random pattern mask (7 frames), two blank frames and the target (7 frames) (cf. Figure 4.). Target stimuli were presented with a vertical shift of 1.5° visual angle, randomly either above or below fixation, to avoid confounding effects of retinal afterimages. After each target stimulus, participants indicated the direction of the target arrow by pressing the left control or right enter key on a standard PC keyboard (lower left and lower right key). If no response occurred within 1.5 s, the trial was counted as missed. After a wrong or missed response, a red 'x' was presented as feedback. Participants were instructed that they would see double arrows on the screen each preceded by a tangle of lines and that they were to indicate the direction of each double arrow with a left or right button press, respectively, as fast and as accurately as possible. Participants were not informed about the occurrence of prime stimuli. The task consisted of three blocks, each with 96 trials and a short (subject-terminated) break between blocks. Trials with wrong or missed responses were repeated at the end of each block to make sure that 288 (3×96) correct trials per subject would be available for analysis. The response priming task took about 15 minutes. After the task in the second session, participants were debriefed about the primes. For data analysis, we focused on left-hand responses and analyzed the trial types and computed subject-wise median RT for the two trial types (left / compatible; left / incompatible). Subsequently, the negative compatibility effect on reaction times (RT-NCE) for each subject was computed as the subject-wise difference between the median RT in compatible and incompatible trials and compared between sham and tVNS using a two-way repeated measures ANOVA (RT as dependent variable, stimulation (sham vs. tVNS) and compatibility as within-subject independent variables).

[9] www.neurobs.com

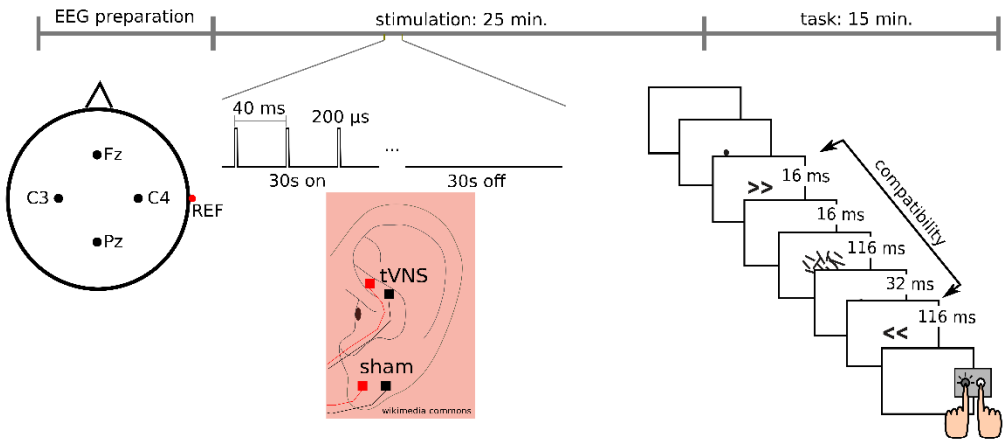

Figure 4. Experimental design. After EEG preparation, electrical stimulation (tVNS or sham) was administered for 25 minutes in trains of 30 s ON (pulse width 200 µs, pulse-onset interval 40 ms/frequency 25 Hz) and 30 s OFF. After stimulation offset, the experimental task began. Ear image reprinted from Wikimedia Commons.

EEG acquisition and analysis

Electroencephalogram (EEG) was recorded from four scalp electrodes (Fz, C3, C4, Pz), referenced to the right earlobe, at a sampling rate of 1 kHz using a BrainAmp amplifier (BrainProducts, DE). EEG data were analyzed using FieldTrip (Oostenveld, Fries, Maris, & Schoffelen, 2011). The raw data were bandpass filtered (0.3 to 45 Hz Butterworth filter) and segmented into single trials, time-locked to the prime (time window: -200 to 600 ms relative to the prime). EEG data from one subject could not be analyzed due to technical recording problems.

To attain an index of cortical motor preparation in the NCE task, usually LRPs are computed (Eimer, 1998). LRPs are obtained by extracting single trials as epochs out of the continuous EEG signal from electrodes in the vicinity of the motor cortex (here: C3 and C4), subtracting one electrode from the other separately for each response hand, then subtracting these difference waveforms for left- and right-hand responses from each other (Eimer & Schlaghecken, 1998). In order to assess cortical response preparation for left-hand trials, we only performed the first step, i. e., computed subject-wise C4-C3 difference waves for each left-target trial, time-locked to prime onset, separately by prime-target compatibility and target direction. In the following, we will refer to these C4-C3 difference waves as readiness potential difference waves (RPD). RPDs for each trial type were subjected to baseline correction (baseline 100 to 0 ms before the prime). Data from a 100ms time window around the local extrema of the

RPDs were averaged over time and compared between sham and tVNS using dependent-sample t-tests.

Furthermore, we determined the latencies of the reversal phase from RPDs in compatible trials, using a jack-knife approach (Miller, Ulrich, & Schwarz, 2009). We computed as many grand average waveforms as there were participants in the sample, leaving out one participant at a time. From the 15 resulting grand average waveforms, we determined the latency of the local maximum within a time window from 250 to 450 ms after prime presentation, in which we expected the reversal phase of the RPD. Plausibility of the determined latencies was assessed by visual inspection. Latencies were compared between sham and tVNS using dependent-sample t-tests. Before testing for significance, t-values were divided by (n-1) in order to correct for the artificial variance reduction introduced by the jack-knife method (Kiesel, Miller, Jolicœur, & Brisson, 2008).

2.4 Results

Behavioral Results

Figure 5.A shows the mean ± standard error of individual NCE magnitudes, which was increased by 5.1 ms after tVNS (t_{15} = 2.36, p = .032), as hypothesized.

In a repeated measures ANOVA of RT, we found a main effect of compatibility ($F_{1,15}$ = 18.39, p < .001) with higher RTs in compatible trials (434 vs. 411 ms), i. e., a consistent NCE was present across stimulations. Stimulation had no main effect on RT ($F_{1,15}$ = 0.19, p = .664). There was a significant compatibility×stimulation interaction ($F_{1,15}$ = 5.57, p = .032), i. e., the NCE was modified by the stimulation.

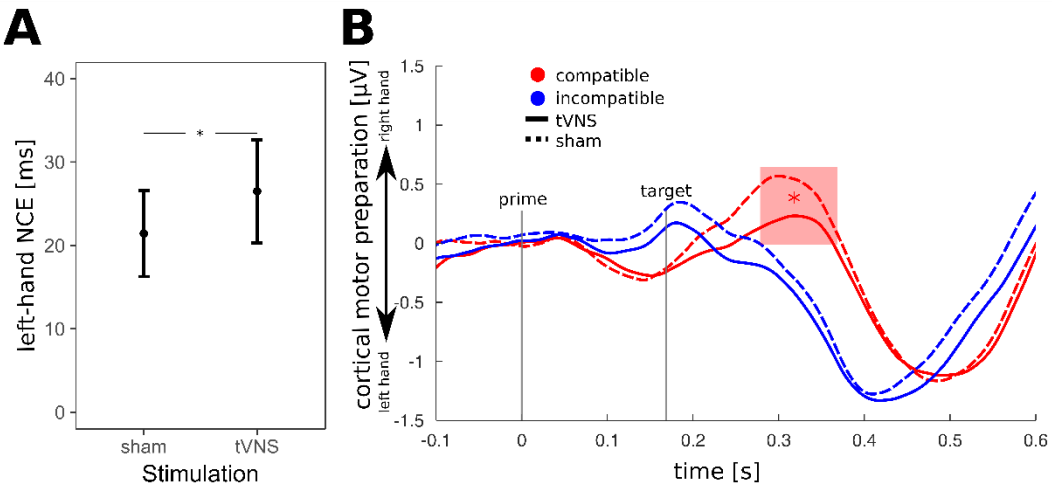

Figure 5. A: NCE on RT. Mean ± standard error of individual differences between RT in compatible and incompatible trials. A positive value indicates longer RTs in compatible trials. B: Prime-locked readiness potential difference waves (RPDs) from left-hand trials. The red window indicates a significant difference between sham and tVNS for the compatible-trial RPD (p = .049).

EEG Results

Analysis of the EEG data (Figure 5.B) revealed a selective, tVNS-induced modulation of the RPDs in compatible left-hand response trials. For compatible trials, we found a significant difference between the sham and tVNS condition for a time window from 280 to 380 ms after prime presentation, i. e., during the reversal phase (t_{14} = -2.15, p = .049, red window in Figure 5.). No such difference was found for incompatible trials (130 to 230 ms after the prime, t_{14} = -1.34, p = .203). Furthermore, the latency of the reversal phase in compatible trials was significantly longer after tVNS (sham: 300 ms, tVNS: 319 ms, t_{14} = 3.19, p = .004, t-value corrected by division through (n-1)).

2.5 Discussion

Summary of results

The present study investigated behavioral and electrophysiological consequences of assumed GABAergic neuromodulation through tVNS in the contralateral motor cortex / ipsilateral hand relative to the stimulation. This was motivated by previous findings of hemisphere-specific tVNS effects on brain oscillations (Marrosu et al., 2003) and cortical excitability (Capone et al., 2015). Specifically, the latter finding suggests a GABA modulation in the motor cortex contralateral to the tVNS stimulation side. In our

study, we assessed behavioral effects by means of a subliminal priming task whose outcomes are known to be highly correlated to GABA concentration in the dorsal medial frontal cortex (supplementary motor area) (Boy, Evans, et al., 2010). In the analysis, we focused on trials with left-hand responses because we expected a GABA-mediated effect on automatic motor inhibition of initially primed left-hand responses, taking place in the motor cortex of the right hemisphere. As hypothesized, we demonstrated direct GABAergic effects of tVNS – it modulated the NCE of the ipsilateral hand relative to the stimulated ear accompanied by modulations of the readiness potential difference waves arising from the contralateral hemisphere. To our knowledge, the present study is the first to demonstrate effects on electrophysiology in healthy humans after a single session of tVNS.

Connections to existing literature

Given that tVNS is assumed to cause an increased GABA concentration in the human central nervous system, we would have expected the NCE to be reduced, since the GABA-NCE correlation shown by Boy, Evans et al. (2010) is negative. However, we found the ipsilateral-hand NCE to be increased after tVNS, which might point to a relationship between tVNS and GABA transmission that is not strictly linear as previously assumed. Nonlinear, especially U-shaped, relationships between VNS parameters or neurotransmitter concentrations on one side and behavioral or physiological effects on the other side have been found repeatedly (Aston-Jones & Cohen, 2005b; Clark, Krahl, Smith, & Jensen, 1995; Clark, Naritoku, Smith, Browning, & Jensen, 1999; Yerkes & Dodson, 1908). Therefore, similar effects of tVNS are possible with behavioral improvements at certain optimal stimulation intensities and impairments when deviating from these optimal settings. Furthermore, it is possible that GABA activity is not globally increased by tVNS, but rather differentially modified in different brain regions, as has been found before (Greenhouse, Noah, Maddock, & Ivry, 2016). Finally, the role of GABA for the NCE is paradoxical, in that more GABA in the SMA leads to less inhibition, reflected in a decreased NCE (Boy, Evans, et al., 2010). Our results indicate more inhibition (increased NCE) following tVNS. Increased inhibition in the primary motor cortex following tVNS, associated with higher GABA level in the primary motor cortex, has been shown before (Capone et al., 2015), and our findings appear to be in line with this. It is conceivable that the reported effects in our

study are also mediated through the primary motor cortex rather than the SMA, which might account for the apparent incompatibility between our findings and previous literature (Boy, Evans, et al., 2010).

Inspection of the EEG results yields further interesting insights. LRPs, which are computed as the difference between left-hand and right-hand RPDs, typically have a triphasic (compatible priming) or biphasic (incompatible priming) shape in the NCE task. The first phase (priming phase) represents initial activation of the primed response, and the second phase (reversal phase) represents inhibition of the initially primed response and activation of the prime-opposite response. Since the prime-opposite response is the target response in incompatible trials, inhibition of the initially primed response facilitates the correct response in these trials (Eimer & Schlaghecken, 2003). This bi- and triphasic shape can be seen also in the left-hand RPDs in Figure 5. B. The reversal phase amplitude in compatible left-hand trials was significantly decreased, alongside an increased latency of the reversal phase, i. e., the phase that represents an inhibition of the left-hand response after its initial, prime-induced activation (Seiss et al., 2014). This finding is difficult to interpret, because a decreased reversal phase amplitude appears to contradict the increased behavioral NCE. The tVNS-induced decrease of the reversal phase amplitude, together with its longer latency, indicates that automatic motor inhibition reaches its maximum later in response to tVNS and might thus cause a longer-lasting inhibition of the initially primed response, leading to an increased NCE (Schlaghecken, Rowley, Sembi, Simmons, & Whitcomb, 2007). This interpretation is speculative, however, since the mechanism mediating between readiness potentials and the behavioral NCE is not known in detail.

Limitations

We ran exploratory post-hoc analyses of the right-hand NCE as well. It remains an open question whether the effect that we report actually represents a lateralization of GABAergic effects of tVNS, in the sense that tVNS has no effect on GABA transmission in the ipsilateral hemisphere / contralateral hand. Even though we did not find any tVNS effects in right-hand trials in exploratory post-hoc analyses, neither on the NCE (t_{14} = 0.55, p = .590), nor on the amplitude (t_{14} = 0.79, p = .789) or latency ($t_{14,\ corrected}$ = 1.07, p = .152) of the RPD reversal phase, we do not have sufficient evidence for the absence of a GABAergic effect of tVNS in the ipsilateral hemisphere /

contralateral hand, and our study lacks statistical power to subject a possible lateralization to a rigorous statistical analysis. This is partly due to the fact that we only used trials with compatible or incompatible prime-target combinations. Using neutral priming and prime-only trials, we might have obtained more detailed insights into the effects of tVNS on automatic motor inhibition in the ipsilateral and contralateral hemisphere, since the processing of the prime-target combinations used in our experimental design engages the motor and perceptual at both local and global levels and does not allow for a detailed separation of processes in one or the other hemisphere (Schlaghecken, Bowman, & Eimer, 2006). Considering the intraindividual right-hand NCE differences between the sham and tVNS session, we found that they have a significantly greater interindividual variability than the respective differences of the left-hand NCE ($F_{15,15}$ = 7.69, p < .001), with substantial positive and negative right-hand NCE differences in part of the subjects, whereas apart from experimental manipulation, the magnitude of the NCE was found to be an intraindividually stable trait (Boy, Evans, et al., 2010). A replication of our findings in a larger sample, with the above modifications to the experimental paradigm, would be desirable.

Another constraint is that tVNS is exclusively administered to the left ear, due to safety concerns. If a lateralization of tVNS effects can be corroborated by future research, it would be quite obvious that tVNS is not used to its full potential as long as it is important, since the concerns are most likely unfounded (Chen et al., 2015; De Couck et al., 2017). There is one recent study in which tVNS was administered to the right ear in humans (De Couck et al., 2017), without reporting any undesired cardiac effects. Future tVNS research should therefore consider to further investigate lateralized behavioral consequences of both left- and right-ear tVNS in the motor domain, and perhaps also on visual, auditory or higher cognitive processes.

Earlobe-sham stimulation as a potential confounding factor

The publication of this study entailed a comment (Rangon, 2018), in which the possibility was raised that the way sham stimulation was realized in our study and most other tVNS studies could confound results. Specifically, Rangon (2018) questioned the widespread use of earlobe stimulation as a sham condition in transcutaneous vagus nerve stimulation (tVNS) studies, arguing that the unexpected results in our study – an increased behavioral effect, even though we expected a decreased one – might be a

consequence of the earlobe-sham stimulation, which implies that the earlobe stimulation would have had a stronger effect than tVNS on GABA transmission and, subsequently, on our parameters of interest. As mentioned above, tVNS as a method is still under scrutiny. Not only have its mechanisms of action remained unclear, but also the effects and interactions of various stimulation parameters, including stimulated ear (left vs. right), stimulation site (cymba conchae vs. tragus), current intensity, frequency, and on-off cycle are largely unknown. Moreover, finding the appropriate comparison condition for tVNS-related data is not trivial, but essential to draw conclusions from the effects found. A sham (placebo) condition is necessary to exclude that stimulation effects are merely caused by sensation of the electrical current. Therefore, the ideal sham condition and the tVNS condition will be undistinguishable for participants, while the former will not have any systematic effect on the brain circuits innervated by the vagus nerve. For tVNS, there are four ways the sham condition has been realized so far: Mounting the stimulation device but delivering no current (e.g., Hein et al., 2013), delivering current at a lower frequency or intensity (e.g., Bauer et al., 2016), stimulating the scapha, i.e., the middle area of the outer ear margin (e.g., Fang et al., 2016), and stimulating the earlobe (e.g., Beste et al., 2016). In recent tVNS studies, the earlobe seems to be the most frequently used locus for sham stimulation (our observation). Both the earlobe and the scapha are free from vagal innervation and solely innervated by the greater auricular nerve (GAN) (Peuker & Filler, 2002). However, Rangon (2018) argues that, being a long-standing acupuncture point, the earlobe may not be as neutral in response to electrical stimulation as assumed in terms of central nervous and behavioral effects, rendering it unsuitable for sham stimulation. To support this, she refers to an fMRI study (Romoli et al., 2014), in which electrical stimulation of the upper scapha yielded a mere somatosensory activation, whereas stimulation in the notch between the antitragus and antihelix led to a more widespread activity increase. However, none of those stimulation sites were near the earlobe, and the activations following stimulation of the antitragus-antihelix notch might as well be due to its proximity to the vagally innervated area of the auricle (Peuker & Filler, 2002). Notwithstanding, some human fMRI studies have shown activations of the somatosensory, and partly insular, areas following earlobe stimulation, but there are no reports of activation in the assumed key target areas of tVNS - the nucleus of the solitary tract and the locus coeruleus (Badran, Dowdle, et al., 2018; Frangos et al., 2015; Yakunina et al., 2017). Furthermore, earlobe

stimulation did not modulate the EEG spectrum in humans (Heffernan, 1996). Finally, another study that applied several days of earlobe stimulation in rats found positive effects on cognition (Kuo, Lin, Tang, Cheng, & Hsieh, 2016). However, there was no placebo condition and the generalizability of a rodent to the human earlobe is questionable. In sum, the evidence reviewed does not support the hypothesis of a systematic behavioral effect of earlobe stimulation in humans, and even less an influence on GABA transmission. Therefore, we do not see sufficient reason to abolish it as a sham condition in tVNS studies in general. Furthermore, Rangon's (2018) suggestion to administer sham stimulation to the cymba conchae, but at a lower frequency (as in Bauer et al. 2016), entails two problems: Firstly, we do not know whether and how a low-frequency stimulation affects the vagus nerve compared to a higher-frequency stimulation. See for instance Hein et al. (2013) for a tVNS effect in depressed patients using 1.5 Hz as a stimulation frequency. Secondly, and more importantly, subjects can easily feel the frequency difference. These problems may not be relevant for a long-term clinical assessment of epileptic seizures as in Bauer et al. (2016), but in the investigation of cognitive and behavioral short-term effects of tVNS, they might introduce serious biases. Finally, there is another reason why stimulating the earlobe might not be the ideal sham condition – the subject's sensation of earlobe stimulation appears to be different from tVNS, as the higher tolerated currents for earlobe stimulation and anecdotal subject reports in our study indicate (Keute, Ruhnau, Heinze, & Zaehle, 2018). This creates a dilemma: Should we apply the same current intensity in tVNS and sham stimulation and tolerate different sensations or vice versa? This dilemma could be resolved by applying sham stimulation to the scapha instead of the earlobe. Even though we have not systematically compared both options, our experience from pilot trials is that the sensation difference between scapha stimulation and tVNS is smaller than between earlobe stimulation and tVNS, so we will consider it for future experiments. Another design option worth considering is to run experimental paradigms twice per session, once before and once during or after each tVNS and sham stimulation. This could help to see whether sham stimulation has in itself an influence on the parameters of interest. In sum, the evidence supporting objections against earlobe-sham stimulation appears rather inconsistent. Given the problems that each method of sham stimulation entails, we believe that earlobe, or potentially scapha stimulation, should be further applied unless more consistent evidence speaking against

these locations emerges. Experimental designs with repeated measures might help bypass some (but not all) of the problems discussed.

2.6 Conclusion

In this study, we show for the first time that tVNS has behavioral and electrophysiological consequences specifically associated to GABAergic neuromodulatory effects on the motor system contralateral to the stimulation.

3 Study 2: GABA-associated effects of tVNS in the visual system

A modified version of this chapter has been published as:

Keute, M., M., Boehrer, L., Ruhnau, P., Heinze, H. J., & Zaehle, T. (2019). Transcutaneous vagus nerve stimulation (tVNS) and the dynamics of visual bistable perception. *Frontiers in Neuroscience*, *doi: 10.3389/fnins.2019.00227*.

3.1 Abstract

Transcutaneous vagus nerve stimulation (tVNS) is widely used for clinical applications, but its mechanism of action is poorly understood. One candidate pathway that might mediate the effects of tVNS is an increase in GABAergic neurotransmission. In this study, we investigated the effect of tVNS on visual bistable perception, which is highly coupled to GABA. Participants were 34 healthy young subjects. We used a static (Necker cube) and a dynamic (structure from motion) bistable perception task. Each subject underwent tVNS as well as sham (placebo) stimulation for ~45 min. We analyzed effects of tVNS on percept durations by means of Bayesian multilevel regression. We found no evidence for a modulation of bistable perception dynamics through tVNS in either task, but the analyses do not ultimately confirm the null hypothesis either. We discuss different possible implications of our finding and propose that GABAergic effects of tVNS should be further investigated using more direct measures of GABA concentration, and, more generally, that a better understanding of the mechanisms of action of vagus nerve stimulation is needed. Finally, we discuss limitations of our study design, data analysis, and conclusions.

3.2 Introduction

In the previous chapter, I described effects of tVNS on automatic motor inhibition (Keute, Ruhnau, Heinze, et al., 2018), a process tightly coupled to GABA concentration in the motor cortex (Boy, Evans, et al., 2010). Effects of tVNS on other processes associated to GABA have been found, such as cortical excitability (Capone et al., 2015a), action cascading (Steenbergen et al., 2015), response inhibition (Beste et al., 2016), and divergent thinking (Colzato, Ritter, et al., 2018). To further corroborate the engagement of a GABAergic pathway through tVNS, in this study we examined effects of tVNS on the dynamics of visual bistable perception, which is highly correlated to GABA concentration in the visual cortex (Van Loon et al., 2013).

Bistable perception means switching between multiple perceptual interpretations of an unchanging sensory (e. g., visual) input (Blake & Logothetis, 2002). Ambiguous figures are a well-known example of visual stimuli resulting in bistable perception, but there are

dynamic, binocular and auditory examples of bistable perception as well (Pressnitzer & Hupé, 2006). Individuals differ with respect to bistable perception dynamics, and several covariates for interindividual variation have been identified, such as structural characteristics of the parietal cortex (Kanai, Bahrami, & Rees, 2010) and genetic contributions (Miller et al., 2010; Shannon, Patrick, Jiang, Bernat, & He, 2011). The inhibition account of bistable perception states that it arises from reciprocal inhibition of different stimulus-selective neural populations in the visual cortex (Blake & Logothetis, 2002; Wang, Arteaga, & He, 2013). Alternative accounts have been proposed that emphasize interactions between perceptual and cognitive processes rather than low-level perceptual inhibition (Sterzer, Kleinschmidt, & Rees, 2009). In favor of the inhibition account, however, it has been found that GABA concentration in the visual cortex as measured by magnetic resonance spectroscopy is positively correlated with perceptual stability, i.e., the average timespan during which perceptual interpretation remains constant, across several visual bistable perception tasks. Furthermore, pharmacological increase of $GABA_A$ activity through administration of lorazepam increased perceptual stability (Van Loon et al., 2013). Motivated by these findings, van Leusden et al. (2015) proposed to study effects of tVNS on bistable perception in order to further establish the link between tVNS and GABA-associated behavioral and perceptual effects.

Besides this GABA-dependence of perceptual stability in visual bistable perception, other neurotransmitter systems have been found to be involved. Percept duration is positively correlated to pupil diameter at the time of perception switch (Einhäuser, Stout, Koch, & Carter, 2008), which is a reliable marker of NE activity (Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010). Moreover, an influence of the dopamine (Schmack et al., 2013) and serotonin (Kondo et al., 2011) systems has been discussed.

In the present study, we investigated tVNS effects on static as well as dynamic visual bistable perception. Given that tVNS is assumed to increase GABAergic transmission, we expected bistable perception to be stabilized, i.e., a prolonging of perception epochs between two switches.

3.3 Methods

Participants and procedure

Thirty-four healthy volunteers (20 female) participated in the study. They were between 18 and 33 years of age (mean: 23.1 ± 3.0). All participants were right-handed, free from current or past neurological or psychiatric diseases, were under no medication (except for oral contraceptives) and had normal or corrected-to-normal vision. Written informed consent was obtained from all participants prior to the experiment. They received money (€8/hr) or course credit as a reimbursement for participation. The study was carried out in accordance with the declaration of Helsinki and approved by the ethics committee of the medical faculty at the University of Magdeburg.

Each participant underwent two experimental sessions, one involving active tVNS at the cymba conchae of the left ear and one involving sham (placebo) stimulation at the left ear lobe (cf. Figure 6.). The order of tVNS and sham stimulation was randomized across participants. Both sessions were scheduled at the same daytime and at least 48 hours apart at constant light conditions.

Each experimental session consisted of two experimental tasks, each run once before (pre) and once during stimulation (online). The order in which the two tasks were presented was randomized across sessions, but held constant within one session (i. e., between the pre and online run).

All stimuli were presented on a 24 inch screen at a vertical refresh rate of 60 Hz. Participants were seated at a distance of 70 cm to the screen. Responses were given by pressing the left and right control button on a PC keyboard. All experimental tasks were coded and run in Matlab 2015[10] using Psychtoolbox 3[11].

In the static bistable perception task, a Necker cube (Kornmeier & Bach, 2005) was presented on the screen for 300 s. The cube consisted of black lines presented on a white background and subtended a visual angle of 7.0°. In this task, two spatial orientations of the cube can be perceived, in which either of the two central vertices can appear to be in front, i.e., closer to the observer. Participants were instructed to initially

[10] www.mathworks.com

[11] www.psychtoolbox.org

indicate whether they perceived the left or the right vertex to be closer by pressing the corresponding key and to indicate every switch in perceptual interpretation by pressing the key corresponding to the perception after the switch.

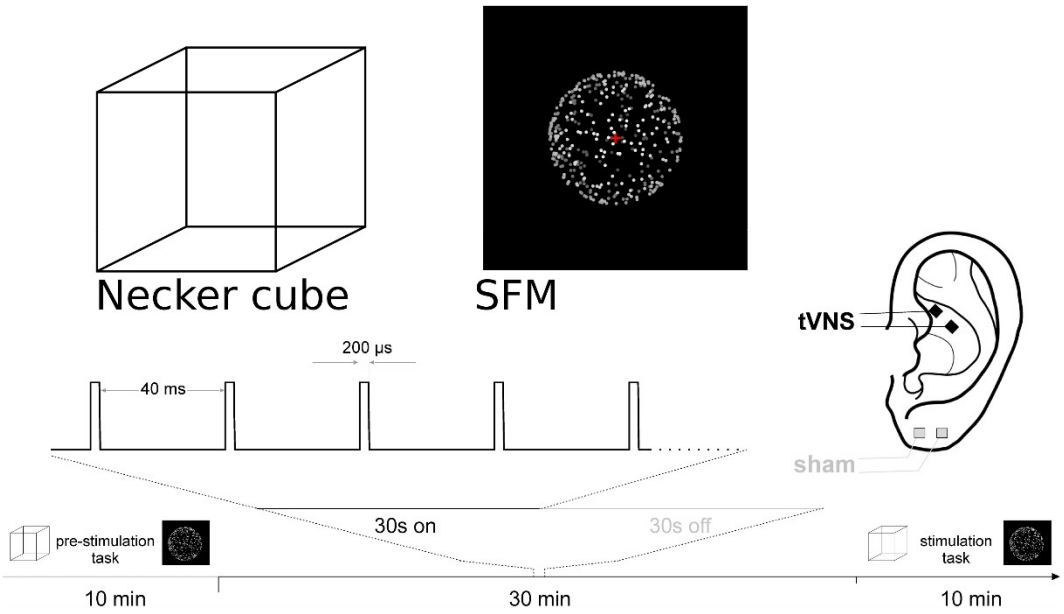

Figure 6. Experimental tasks and experimental design.

For the dynamic bistable perception task, referred to as structure from motion (SFM), a circular cloud of left- and right-moving dots was presented on the screen with a central fixation cross. These moving dots are perceived as an either left- or right-rotating sphere with the bistable perceptive interpretation being the direction of rotation (left vs. right) (Van Loon et al., 2013). Again, participants were asked to indicate the perceived direction of rotation initially and after every perceptual switch by pressing the associated key. The two dot clouds moved at an angular velocity of 23°/s around the vertical axis. The individual dot size was 6.6 arcmin in width and height. All dots were equal in luminance (white) on a grey background. The dot clouds covered a circular area with a diameter of 15.6° visual angle. After an initial presentation of a fixation cross, the task was presented for 300 s.

After the first run of the two tasks, electrical stimulation started and was administered for 30 min prior to the second run of the tasks to give stimulation effects time to unfold. Stimulation continued throughout the online run of the tasks (Figure 6.).

For stimulation, medical Ag/AgCl electrodes (Ambu Neuroline[12]), cut to a size of 4×4 mm and mounted on a piece of silicone at a center-to-center distance of 1 cm were used. Electrical conductance between the electrode and the skin was established using a small amount of Genuine Grass adhesive electrode cream (Natus Neurology[13]). For tVNS, the electrodes were placed in the cymba conchae of the left ear, for sham stimulation at the left earlobe. Across conditions and participants, the anode was placed more rostral. Stimulation pulses were generated by a medical stimulation device (Digitimer DS7[14]) at a current intensity of 3 mA and a pulse width of 200µs, triggered by an Arduino Uno circuit board[15] programmed to a stimulation cycle of 30s stimulation at 25 Hz, followed by a 30s break.

Data analysis

We analyzed percept durations (PD), which were computed as the time difference between two reported switches. When the same key was pressed multiple subsequent times, only the first press was counted, such that all PD values describe the time span between two changes in perception. The time before the first and after the last keypress was excluded from the analysis. Furthermore, PDs shorter than 200 ms were considered lapses and excluded from further analysis. We excluded subjects if they had carried out 2 keypresses or less, i.e., no percept switches, in at least one of the four runs of a task. Furthermore, we excluded subjects if the time between their first and last keypress was shorter than 150 s, i.e., if less than half of the runtime was available for analysis, in at least one of the four runs of a task. We computed mean PDs for each subject in each run. Data from 29 subjects for the Necker cube task and from 25 subjects for the SFM task entered the final analysis.

We analyzed mean PDs by means of Bayesian multilevel regression using the *brms* library in R[16] and Stan[17] (Bürkner, 2017). We constructed a linear model of PD with time (pre- vs. post-stimulation), stimulation (sham vs. tVNS), and time×stimulation interaction as fixed effects. As random effects, we specified subject-wise random

12 www.ambu.com

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15 www.arduino.cc

16 www.r-project.org

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intercepts to account for repeated measures. We used weakly regularizing Gaussian priors ($\mu = 0$, $\sigma = 15$) for the model coefficients of all three fixed effects (McElreath, 2016). Posterior distributions of the parameters have been obtained by Markov chain Monte Carlo (MCMC) sampling in Stan (Gelman, Lee, & Guo, 2015) with 5000 iterations per chain, the first 2000 iterations being discarded as 'warm-up' iterations, and four independent sampling chains. Since our effect of interest was the time×stimulation interaction, we compared the model with interaction to a model without it using Bayes factors. Moreover, we report the posterior distribution of the interaction model coefficient as estimated in the 12000 iterations of the MCMC procedure, alongside the 95% highest density interval (HDI), i.e., the 2.5% and 97.5% percentiles of the posterior effect size distribution.

3.4 Results

Necker cube

Mean overall PD for the static bistable perception task (Necker cube) was 9.0 s. The Bayesian sampling procedure estimated a mean time×stimulation interaction of *3.0 s*. The 95% HDI was $-2.7 < b < 8.7$ (Figure 7. A-C). Bayes factor model comparison favored the model without interaction over the model with interaction (BF: 2.9).

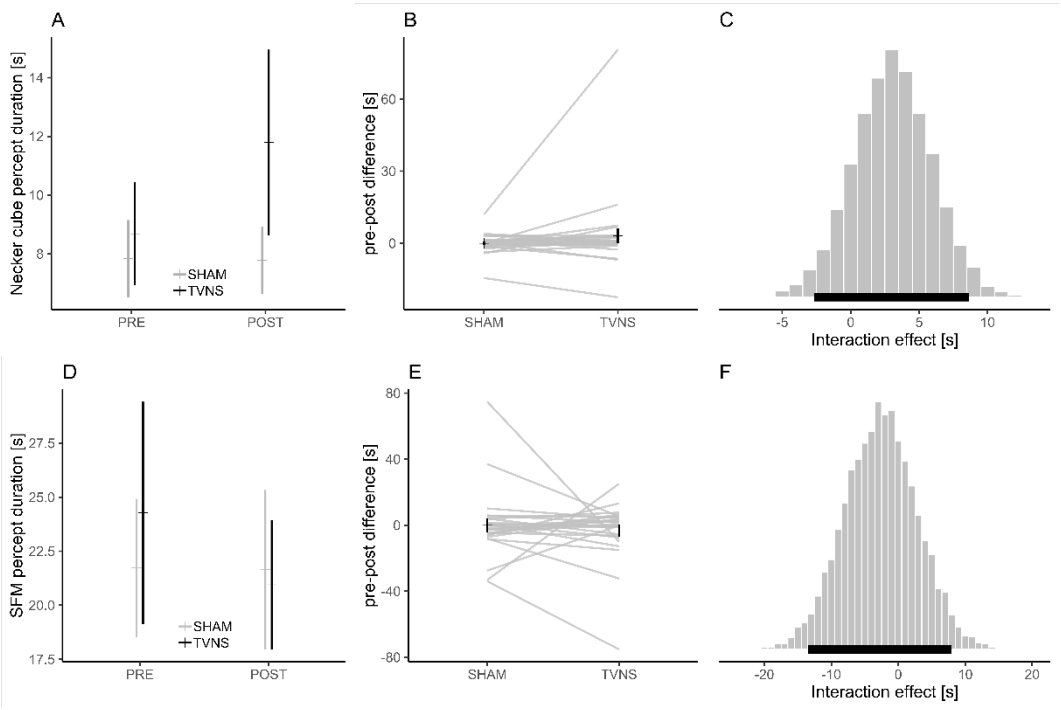

Figure 7. A: Mean ± SEM of percept durations in the Necker cube task; B: Mean ± SEM of pre-online change in percept durations in the Necker cube task, gray lines: individual subjects; C: Posterior distribution of time × stimulation interaction in the Necker cube from the Bayesian multilevel model, black bar: 95% highest density interval of interaction effect; D-F: Equivalents for the SFM task.

Structure from motion

Mean overall PD for the dynamic bistable perception task (SFM) was 22.1 s. The Bayesian sampling procedure estimated a time×stimulation interaction of *-2.6 s*. The 95% HDI was -13.4<*b*<7.9 (Figure 7. D-F). Bayes factor model comparison favored the model without interaction over the model with interaction (BF: 2.5).

Correlations between tasks

Collapsed over all task runs, PDs were moderately correlated (ρ = .42, p < .001) between both tasks. Spearman's ρ is reported because individual percept durations in both tasks differed significantly from the normal distribution (both p < .005 in Lilliefors-Kolmogorov-Smirnov tests).

3.5 Discussion

In this study, we asked whether tVNS affects the dynamics of visual bistable perception. As suggested previously, an increase of GABAergic activity through tVNS should

result in slower dynamics of visual bistable perception (Van Leusden et al., 2015). We analyzed percept durations using Bayesian multilevel regression analyses. Bayesian analyses, other than most inferential statistical methods, allow in principle to accept the null hypothesis, based on the quantiles of the posterior distributions or Bayes factors (Kruschke, 2013). In our analyses, the 95% HDI of coefficient distributions in both analyses included zero, but both intervals were rather wide. Moreover, Bayes factors favored a model without interaction effect for both experimental tasks, but the magnitude of both Bayes factors was rather low (< 3). In sum, we find evidence for a null effect of tVNS on the dynamics of visual bistable perception, which is, however, not fully conclusive. On the other hand, no tendency toward a non-zero effect size is apparent from either experimental task, so we tentatively accept the null hypothesis. The moderately high correlation between the two tasks indicates that they capture similar processes underlying bistable perception (Carter & Pettigrew, 2003).

Even though our results are not fully conclusive, they allow for several interpretations. First, tVNS might have a different effect on GABA transmission in different parts of the brain. Even though there is no a priori reason to assume that GABAergic effects of tVNS are different between the motor and visual cortex, the results from our recent study (Keute, Ruhnau, Heinze, et al., 2018), alongside another study investigating the effects of tVNS on cortical excitability (Capone et al., 2015a) indicate that effects of tVNS on GABA transmission might have a more complex spatial distribution in the brain than just a whole-brain increase, but a systematic investigation of this is pending. Therefore, we cannot rule out that tVNS affects GABA transmission in the motor but not in the visual cortex. We suggest that the spatial distribution of GABAergic effects of tVNS should be investigated using more direct physiological measures such as, e.g., magnetic resonance spectroscopy. Moreover, it seems to be an oversimplification of the mechanism of action of tVNS if hypotheses about its behavioral or physiological effects are derived simply based on increases of NE, ACh, and GABA. Further central and peripheral candidate pathways of both tVNS and iVNS have been found, including serotonergic (Dorr & Debonnel, 2006; Grimonprez, Raedt, Baeken, Boon, & Vonck, 2015), plasticity-promoting (Biggio et al., 2009; Borland et al., 2016), anti-inflammatory (Kaczmarczyk et al., 2018; Ottani et al., 2009), and peripheral autonomic (Clancy et al., 2014) mechanisms. An integrative model of these mechanisms and their interaction is pending.

Second, despite the robust correlation (Van Loon et al., 2013), GABA in the visual cortex is not the only neurotransmitter system with an influence on visual bistable perception. Other neurotransmitters, such as dopamine (Schmack et al., 2013) and norepinephrine (Einhäuser et al., 2008; Hupé, Lamirel, & Lorenceau, 2009) are potential mediators of visual bistable perception dynamics. Norepinephrine is considered an important target neurotransmitter of tVNS (Badran, Dowdle, et al., 2018). Even though a tVNS-induced increase in norepinephrine transmission should have a stabilizing effect on bistable perception (Einhäuser et al., 2008), i.e., should have the same direction as a tVNS induced increase in GABA transmission, interactions between neurotransmitter systems may be more complex. Moreover, bistable perception dynamics underlie numerous inter- and intraindividual variations, such as gender, personality traits, practice (Scocchia, Valsecchi, & Triesch, 2014), genetic differences (Miller et al., 2010; Schmack et al., 2013; Shannon et al., 2011), or clinical conditions (Vierck et al., 2013).

Third, there are limitations to our experimental design. Several participants had to be excluded based on the criteria described above, which might indicate that the parameters of our experimental paradigm have not been optimally tuned. Longer stimulus presentations and improved control of visual attention, e.g., by using a chin-rest, might improve the overall data quality. However, given our data, there is no apparent reason to assume that this would have led to the discovery of a tVNS effect.

In sum, we do not find any positive evidence for a tVNS effect on visual bistable perception, but our data remain inconclusive inasmuch as they do not ultimately confirm the null hypothesis either. We did not find evidence for a simple link between tVNS, GABA transmission and stabilized bistable perception.

4 Study 3: Effects of tVNS on executive control of action

A modified version of this chapter has been published as:

Keute, M., Barth, D., Liebrand, M., Heinze, H. J., Kraemer, U. & Zaehle, T. (in press) Effects of transcutaneous vagus nerve stimulation (tVNS) on conflict-related behavioral performance and frontal midline theta activity. *Journal of Cognitive Enhancement.*

4.1 Abstract

Several previous studies have highlighted the potential of transcutaneous vagus nerve stimulation (tVNS) to enhance executive control of action. In the present study, we tested for effects of tVNS on behavioral performance and frontal midline theta activity during response conflicts. Frontal midline theta reflects transient activation of the posterior mid-frontal cortex in situations requiring increased executive control of action. It is an established marker for response conflict monitoring.

We carried out a combined behavioral and electroencephalography (EEG) within-subjects experimental study employing a cued go-nogo-change task. Twenty-two healthy young adults participated.

We found that tVNS enhanced global behavioral accuracy, i.e., decreased the proportion of erroneous and missed responses, compared to sham (placebo) stimulation, and reduced conflict costs on behavioral performance in go/change response conflicts. Furthermore, in trials eliciting go/stop conflicts, frontal midline theta was enhanced under tVNS. During go/change conflicts, a stronger subsequent drop in frontal theta power was observed under tVNS.

These findings corroborate the potential of tVNS to enhance executive control of action. For the first time, we show an effect of tVNS on frontal midline theta activity, which suggests that tVNS interacts with the neural mechanisms underlying conflict monitoring.

We conclude that tVNS is a promising method to enhance executive control and recommend the further investigation of tVNS as a candidate treatment of clinically relevant executive control deficits.

4.2 Introduction

It is a crucial human ability to adopt and maintain goals as well as behavioral routines and strategies to achieve these goals. Many routines, such as car-driving, can be carried out in a highly automatized way, i.e., without need to allocate a high amount of cognitive resources to them. Notwithstanding, humans are able to constantly monitor the environment and flexibly adapt to changes, which is described by the closely related concepts of executive and cognitive control of action (we will use executive control / EC in the following) (Logan, 1985; Posner, Snyder, & Solso, 2004). A central aspect of EC is conflict monitoring, i.e., the ability to detect environmental requirements to override a prepotent behavioral response, and to implement an adapted one (Botvinick, Braver, Barch, Carter, & Cohen, 2001). In the electroencephalogram (EEG), frontal midline theta activity (FMΘ), i.e., a transient spectral power increase in the theta band (~4-8 Hz) over fronto-central electrodes, is an established marker for conflict monitoring and for other types of mental effort such as working memory load, anxiety, and error monitoring (Cavanagh & Shackman, 2015; Klimesch, 1999; Nigbur, Ivanova, & Stürmer, 2011). The anterior cingulate cortex (ACC) has been suggested as a key brain region involved in the detection of response conflicts and initiation of control mechanisms, and as a generator of FMΘ (Asada, Fukuda, Tsunoda, Yamaguchi, & Tonoike, 1999; Botvinick, Cohen, & Carter, 2004; Gevins, 1997; Hajihosseini & Holroyd, 2013; Luu, Tucker, & Makeig, 2004), whilst other studies found sources of FMΘ in the adjacent mid-cingulate cortex or pre-supplementary motor area (Cavanagh & Frank, 2014).

EC deficits can be caused by multiple factors: non-pathological conditions such as exhaustion, lack of cardiovascular fitness, and normal aging (Baumeister, 2002; Manard, François, Phillips, Salmon, & Collette, 2017; Weinstein et al., 2012), neurodevelopmental or neurodegenerative disorders (Greene, Hodges, & Baddeley, 1995; Hornberger, Piguet, Kipps, & Hodges, 2008; McKinlay, Grace, Dalrymple-Alford, & Roger, 2010; Ozonoff & Jensen, 1999; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005) psychiatric conditions (Lesh et al., 2013), and many more. EC deficits have been linked to decreased FMΘ (Wang, Lo, et al., 2015). It has been shown repeatedly that pathological EC deficits are associated with lower patient-reported quality of life (QOL), and, conversely, amelioration of those deficits with higher QOL

(Brown & Landgraf, 2010; Cotrena, Branco, Shansis, & Fonseca, 2016; Ness et al., 2008; Sherman, Slick, & Eyrl, 2006).

In this study, we assessed the potential of transcutaneous vagus nerve stimulation (tVNS) as a prospective means to improve EC and ameliorate EC deficits. To this end, we investigate effects of tVNS on behavioral performance and FMΘ during response conflicts in a cued go-nogo-change task in healthy young adults.

Through electrical stimulation of the vagus nerve in the outer ear and downstream central nervous and peripheral effects, tVNS has shown effectiveness as an adjunct treatment for epilepsy (Bauer et al., 2016), depression (Hein et al., 2013), tinnitus (Lehtimäki et al., 2013), and chronic pain (Chakravarthy, Chaudhry, Williams, & Christo, 2015). Several recent studies found that tVNS can also modulate cognitive-behavioral functions that are related to EC: tVNS decreased false alarm rates in a response inhibition paradigm with high working memory load (Beste et al., 2016); it decreased reaction times in an action cascading paradigm (Steenbergen et al., 2015); it increased post-error slowing in an Eriksen Flanker task and an auditory choice reaction time task (Sellaro, van Leusden, et al., 2015), and it enhanced conflict-related behavioral adjustments in a Simon conflict task (R. Fischer et al., 2018). It also increased intracortical inhibition (Capone et al., 2015) as well as automatic motor inhibition (Keute, Ruhnau, Heinze, et al., 2018). Taken together, these findings indicate that tVNS has the potential to improve EC in healthy individuals and potentially ameliorate EC deficits, even though the evidence is not fully consistent so far.

In this study, we used a cued go-nogo-change task with five cue-target combinations, two of which are expected to elicit response conflicts. This task allows us to study behavioral and electrophysiological responses associated with go-/nogo-conflicts and go-/change-conflicts as well as sustained attention, reflected in overall behavioral performance. A recent study employing the same task (Liebrand, Kristek, Tzvi, & Krämer, 2018) showed that behavioral performance was reduced in trials with conflict-eliciting cue-target combinations. Moreover, the same study showed patterns of lateralized spectral power changes in the mu and beta bands (~9-25 Hz) reflecting response preparation. Conversely, in the present study we focus on the consequences of tVNS on behavioral performance and conflict-related FMΘ.

4.3 Methods

Subjects and general procedure

We carried out a placebo-controlled, single-blind, within-subject experimental study. Twenty-two healthy young adults (16 females) participated. Mean age was 23.8 years (range 21 to 28). Written informed consent was obtained from all participants. The study was carried out in accordance with the Declaration of Helsinki and has been approved by the ethics committee of the medical faculty at the Otto von Guericke University Magdeburg. Inclusion criteria were: right-handedness; no history of neurological or psychiatric disease or brain injury; no pregnancy; no medical or recreational drug use (except for oral contraceptives); no alcohol consumption at the day of the experiment and the day before; no pacemakers or other metal implants (all by self-report). All participants had normal or corrected-to-normal vision. They were reimbursed with money (8€/h) or course credit. Each participant was tested on two days, one involving tVNS, the other involving sham stimulation, in pseudo-randomized order. The two experimental sessions for each participant were scheduled at least 48h apart to enable full wash-out of any stimulation effects and at approximately the same daytime to avoid any circadian confounding effects. Total time per session (incl. EEG and tVNS/ sham preparation, pre-task electrical stimulation, and experimental task) was ~3h. All participants had no prior experience with tVNS.

Transcutaneous Vagus Nerve Stimulation (tVNS)

TVNS was administered to the cymba conchae of the left ear. For electrical stimulation, we used conventional neurostimulation electrodes (Ambu Neuroline[18]), cut to a size of 4x4 mm. We assembled those electrodes on a small piece of ear silicone 1 cm apart (center-to-center) with the anode rostral of the cathode and fixated them to the ear using Genuine Grass EC2 adhesive electrode cream (Natus Neurology[19]). For sham stimulation, we chose the left earlobe instead of the cymba conchae, which is free of vagal innervation (Bermejo et al., 2017; Peuker & Filler, 2002) and does not engage any of the target brain areas of tVNS (Hein et al., 2013; Keute, Ruhnau, & Zaehle, 2018). Default stimulaton intensity was 3 mA. In six tVNS sessions and one sham session, subjects reported that stimulation at 3 mA was painful, so we reduced intensity stepwise

[18] www.ambu.com

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by max. 0.9 mA until it was tolerable. Current was delivered in square pulses at a pulse width of 200 µs, a frequency of 25 Hz, and a 30s/30s on-off cycle by a medical stimulation device (Digitimer DS7[20]) triggered by custom code running on an Arduino Uno circuit board[21]. Each participant received stimulation 30 min. prior to the task, and stimulation continued throughout the task (~46 min).

Experimental paradigm

Participants performed a cued go-nogo-change-task (Liebrand et al., 2018), comprising 960 trials. They were instructed to respond to colored triangle-shaped target stimuli as fast and as accurate as possible (see Figure 8.A).

Black targets required the standard 'go' response (pressing the right 'String'-key on a PC keyboard with the right index finger). Green targets required the 'go-change' response (pressing the left 'String'-key with the left index finger). Red targets required no behavioral response ('stop'). Each target stimulus was preceded by a colored square-shaped stimulus serving as a probabilistic cue. A black cue predicted a black target 100% of the time. A green cue predicted a black (75% of the time) or green (25% of the time) target, and a red cue predicted a black (75% of the time) or red (25% of the time) target. Thus, five types of cue-target combinations resulted: certain-go (black cue / black target), change (green / green), no-change (green / black), stop (red / red), and no-stop (red / black). Black, green and red cues appeared in one third of the trials each. Overall, the standard response was highly prepotent (it was required in about 83% of the trials), so that we expect any target requiring a non-standard response (i.e., green/change and red/stop targets) to elicit a response conflict between the required and the prepotent response.

Cue and target appeared for 100 ms each, with a fixed stimulus onset asynchrony (SOA) of 1.1 s. The interval between two consecutive trials (reponse offset to cue onset) was randomly jittered between 1.3 and 1.6 s. There was a 20 s break every 80 trials. Before the actual experiment, participants could practice the procedure in 12 trials with feedback. After the training, no feedback was provided anymore. Participants were instructed to keep their gaze on a central horizontal fixation line above which the stimuli were presented throughout the experiment. The task instruction was to react as fast and

[20] www.digitimer.com

[21] www.arduino.cc

accurately as possible, and not to give any response before appearance of the target. The experimental task was built and run using Presentation 21 (Neurobehavioral Systems[22]).

EEG recording and data processing

EEG was recorded from an EEG cap with 64 pre-mounted Ag/AgCl electrodes (EasyCap[23]) according to the international 10/20 system. FCz served as online reference and AFz as ground electrode. Electrooculogram (EOG) was recorded bipolarly from the outer canthus of each eye for horizontal eye movements (hEOG) and from above and below the right eye for vertical eye movements and eyeblinks (vEOG). Electrode impedances were kept below 5 kΩ. EEG was recorded using a BrainAmp amplifier (Brain Products, DE) at a sampling rate of 500 Hz and with an online lowpass filter (250 Hz).

Offline data analysis was carried out using FieldTrip (Oostenveld et al., 2011). Data were bandpass filtered (0.1 to 40 Hz). Severely corrupted channels were identified by visual inspection and removed from the data. Upon visual inspection and rejection of segments containing gross artifacts, we performed an independent component analysis using the FastICA algorithm (Hyvärinen & Oja, 2000), visually identified and removed components containing eye, muscle, and stimulation artifacts. Data were visually inspected again, and segments with remaining gross artifacts were rejected. Previously removed channels were reconstructed using spline interpolation and data were re-referenced to the average of all channels. EEG preprocessing and artifact correction were carried out blind to the stimulation (sham / tVNS).

Data were cut into single trials. Time-frequency analysis was performed using Fast Fourier Transformation (FFT) over Hann-tapered time windows of 500 ms length, at frequencies ranging from 2 to 36 Hz in steps of 2 Hz. Time windows moved along the epochs from -0.5 to 2.5 s relative to the cue stimuli in steps of 100 ms. Resulting spectral power values were log-transformed ($10*\log_{10}$), so that differences between values have decibel (dB) units. We used the pre-cue epoch (-750 to -250 ms) as baseline. The EEG and tVNS setup and the stimulation paradigm are illustrated in Fig. 1A.

[22] www.neurobs.com

[23] www.easycap.de

Statistical Analyses

We analyzed behavioral data within a linear mixed-effects regression (LMER) framework using trial-wise data. This approach has several advantages compared to analyzing subject-wise average values: It takes into account all available information, e.g., intraindividual trial-to-trial variability, or varying numbers of trials across subjects, and it provides flexibility for testing effects within and between experimental sessions (Baayen, Davidson, & Bates, 2008; Frömer, Maier, & Abdel Rahman, 2018; Jaeger, 2008). We tested for main effects of *stimulation* (sham vs. tVNS) and *condition* (certain-go vs. no-change vs. no-stop vs. go-change vs. stop) and for the interaction between stimulation and condition. To account for learning and exhaustion, we also included *session* (first vs. second, this was orthogonal to stimulation due to pseudo-randomization of sham and tVNS) and *block* (1-80, each block containing 12 trials) as fixed effects. To test for statistical significance of the fixed effects, we compared a baseline model containing all main effects of interest to reduced models where one main effect at a time was dropped. Interaction effects were tested by comparing the baseline model to an extended model containing the interaction effect. Model comparisons were carried out using likelihood-ratio tests (LRT). To account for repeated measures, we specified random intercepts per subject and nested random slopes between sham and tVNS sessions. This random effects structure was selected because it significantly improved the model fit compared to models with random intercepts only, following recommendations in the literature (Barr, Levy, Scheepers, & Tily, 2013). We used R and the *lme4* library (Bates, Mächler, Bolker, & Walker, 2015) to construct and test models.

We constructed an LMER model of reaction times (including only go-trials with correct responses) and a logistic generalized LMER model of response 'correctness'. A response was considered correct if no omission or commission error occurred. For the accuracy models, we excluded three subjects who performed at ceiling (i. e., overall accuracy > 99 % both in the sham and tVNS session). We report effect sizes as estimated by the models, and the test statistic (log-likelihood ratio / χ^2) and p-value as obtained in the likelihood-ratio test. Effect sizes are in ms for RT, and in log-odds ratio (logOR) for accuracy.

Statistical analysis of EEG data was carried out on trial-wise data using LMER models, analog to the analysis of RT. For FMΘ analyses, we averaged over a frontocentral electrode cluster (Fz, F1, F2, FCz, FC1, FC2, Cz, C1, C2), over theta frequencies (4-8 Hz), and over a time window from 200 to 600 ms post-target, based on visual inspection of the data and previous literature (Cavanagh & Frank, 2014).

We carried out additional beta-band band analyses over the cue-target interval, using the same pre-cue baseline, frequencies from 15 to 25 Hz, and an electrode cluster covering the sensorimotor cortex (FC1, FC3, FC5, C1, C3, C5, CP1, CP3, CP5, and the respective right-hemisphere homologues), following the beta-band analyses reported by Liebrand et al. (2018). For beta-band analyses, we calculated session-wise mean values across trials and performed statistical analyses over these mean values. Beta-band analyses were only carried out in order to replicate previous findings from the same task as a sanity check.

4.4 Results

Behavioral Performance

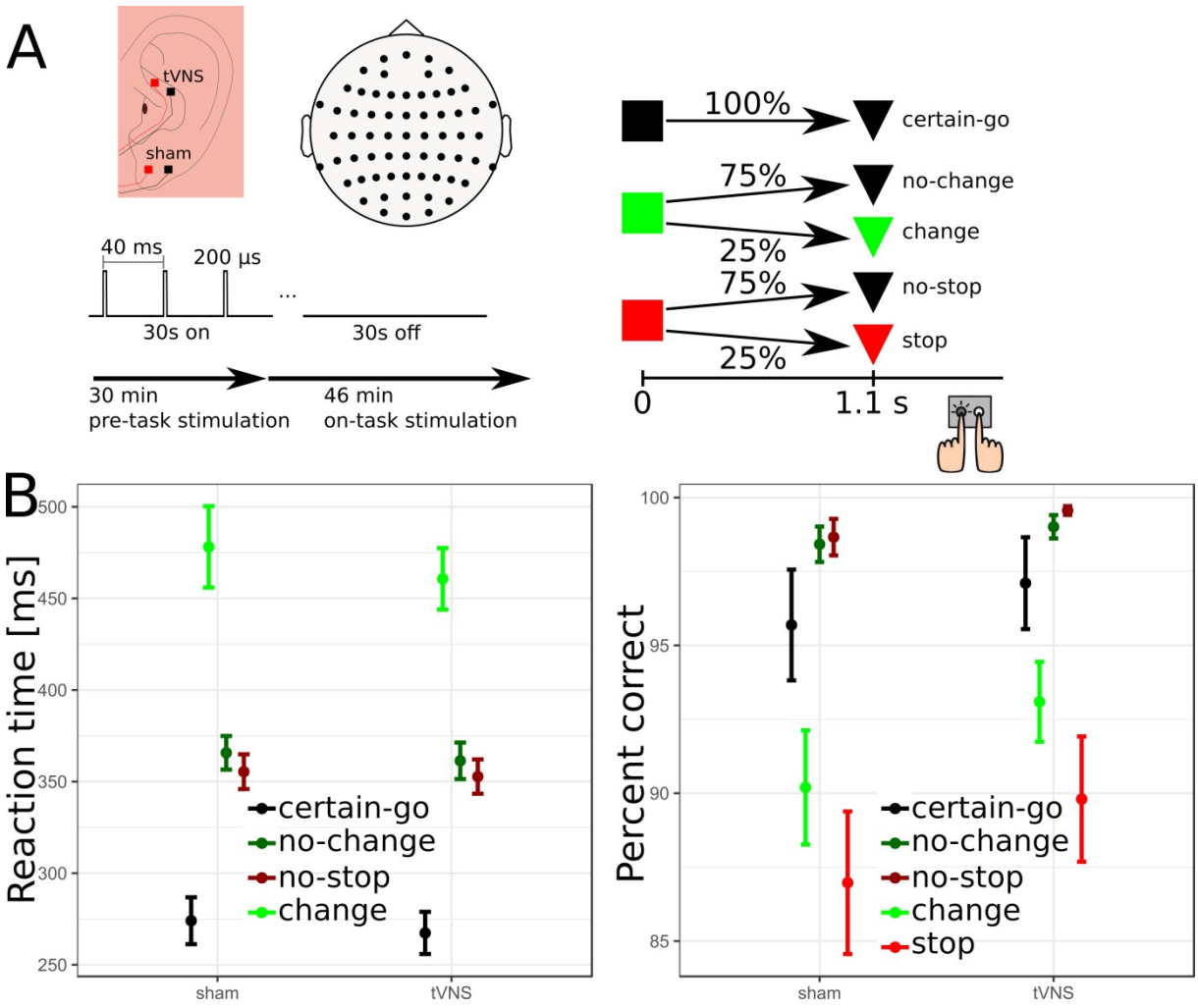

Figure 8. A: Left: Stimulation paradigm and EEG layout. Ear image reprinted from Wikimedia Commons. Right: Illustration of the experimental task. Subjects responded to triangle-shaped stimuli (black – right index finger response, green – left index finger response, red – no response). Target color was probabilistically predicted by square-shaped cue stimuli, presented 1.1 s before target onset. Five cue-target combinations were possible. B: Left: Mean ± standard error of subject-wise mean values for RT (correct responses only), Right: Mean ± standard error of subject-wise accuracy. Note that (parametric) standard errors for accuracies only give a rough estimation of the reliability of mean point estimation. For statistical analyses, we used logistic regression models of trial-wise response correctness, which are more appropriate to the non-normal (binomial) distribution of accuracy values. Three subjects that performed at ceiling (overall accuracy > 99% in both sessions) were excluded from accuracy visualization and analysis.

In the LMER analysis of RT (Fig. 1B, left panel), we found significant effects of *block* (speeding of 3.8 ms per block, $\chi^2 = 492.7$, $p < .001$), *session* (first session 20 ms slower, $\chi^2 = 7.2$, $p = .007$), and *condition* (no-change 91.5 ms, no-stop 82 ms, go-change 195.7 ms slower than certain-go, $\chi^2 > 1000$, $p < .001$). Across conditions, responses were, numerically, 5.7 ms faster in tVNS sessions, but the effect was not statistically significant ($\chi^2 = 0.7$, $p = .387$), and there was no tVNS×block interaction ($\chi^2 = 0.9$, $p = .323$). However, tVNS did interact with condition ($\chi^2 = 10.6$, $p = .014$). When we refitted the model to RTs in change and no-change trials only, this interaction was even clearer ($\chi^2 = 7.1$, $p = .008$), and it was driven by reduced change costs on RT, i.e., the RT difference between the change and no-change condition was 10.2 ms lower under tVNS compared to sham stimulation.

In the logistic LMER analysis of accuracy (Fig. 1B, right panel), we found significant effects of *block* (logOR -0.11 per block, $\chi^2 = 103.5$, $p < .001$), *session* (logOR for first session -0.37, $\chi^2 = 6.1$, $p = .013$), and *condition* (logOR for no-change: 1.1, no-stop: 1.5, go-change: -1.0, - stop: 1.4, all compared to certain-go, $\chi^2 = 259.3$, $p < .001$). There was no tVNS×block interaction ($\chi^2 = 1.2$, $p = .271$). Note that higher accuracies in the no-change and no-stop conditions compared to certain-go were due to a higher rate of omission errors in certain-go (in this condition, the required response was fully predictable, so we assume that participants' attention tended to fade during the cue-target interval in these trials). When only commission errors and correct responses were compared, accuracies in these conditions were lower than in certain-go.

Overall accuracy was higher in tVNS sessions (logOR 0.38, $\chi^2 = 5.2$, $p = .023$). There was a trend towards a tVNS×condition interaction, but it missed statistical significance ($\chi^2 = 9.4$, $p = .051$). When we refitted the model for correct responses and commission errors only (excluding missed responses), and separately for no-/change and no-/stop trials, we found a marginally significant tVNS×condition interaction for change and no-change trials ($\chi^2 = 3.7$, $p = .055$), i.e., the accuracy change costs tended to be lower under tVNS compared to sham stimulation (logOR -0.57). No such interaction was found for stop and non-stop trials ($\chi^2 = 0.3$, $p = .574$).

Cue-locked EEG results

In the cue-target interval, we found lateralizations of mu and beta power over the sensorimotor cortex, consistent with a previous study employing the same task (Liebrand et al., 2018): Beta power in the second half of the cue-target interval (0.5 to 1.1 s, compared to pre-cue baseline) differed between cues at right (i.e., ipsilateral to the standard response) sensorimotor electrodes (green < red < black, $F_{1,21}$ = 6.3, p = .02), but not at left (contralateral) sensorimotor electrodes ($F_{1,21}$ < 0.1, p = .925, Figure 9.).

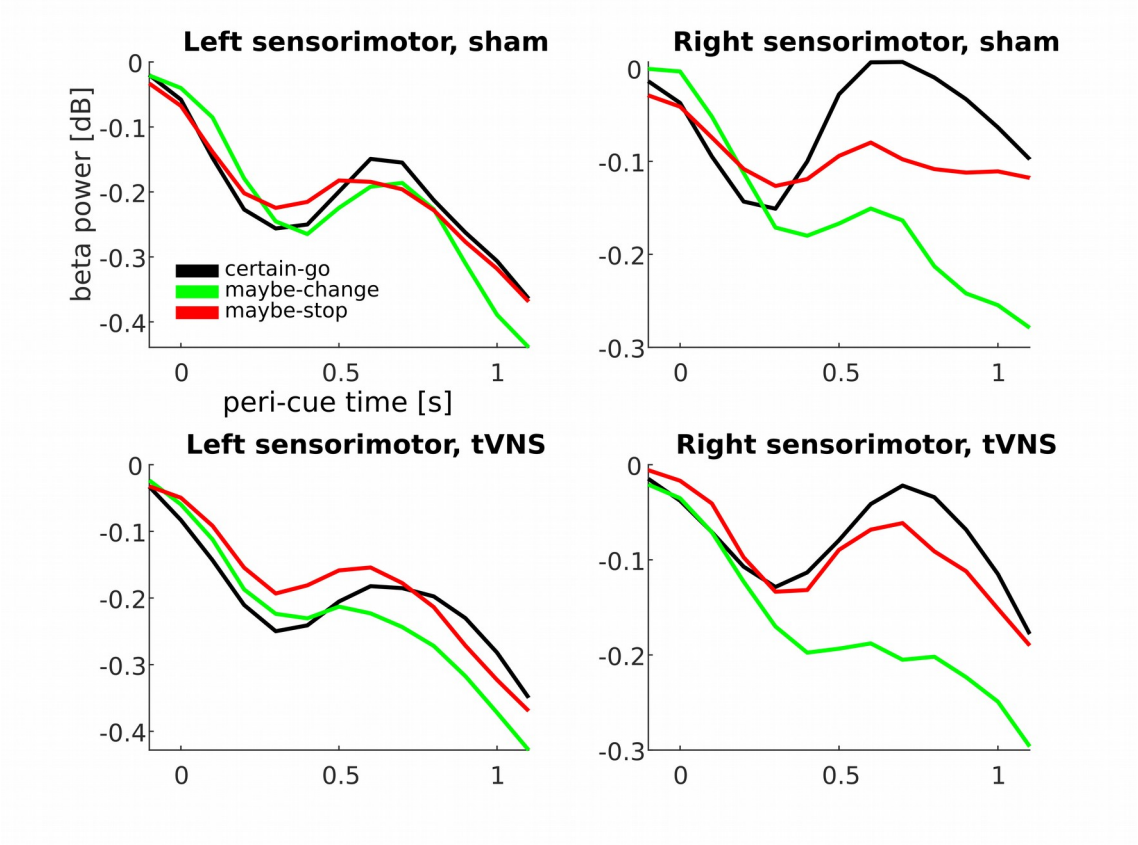

Figure 9. Beta power (15-25 Hz) in the cue-target interval over left sensorimotor (FC1, FC3, FC5, C1, C3, C5, CP1, CP3, CP5) and right sensorimotor (right-hemisphere homologues) electrodes. Note that FFT was carried out over 500 ms time windows, therefore smearing effects from target-locked beta activity can be present between 0.85 s and target onset (1.1 s).

This cue-locked power evolution reflects a lateralization of beta power, which is clearly apparent only in certaingo trials, as the inspection of t-value time courses reveals (Figure 10.). This is plausible given that lateralized beta power reflects response preparation (A. G. Fischer, Nigbur, Klein, Danielmeier, & Ullsperger, 2018), and that the certain-go condition is the only one that allows for an unambiguous response preparation. No systematic differences between sham and tVNS were found.

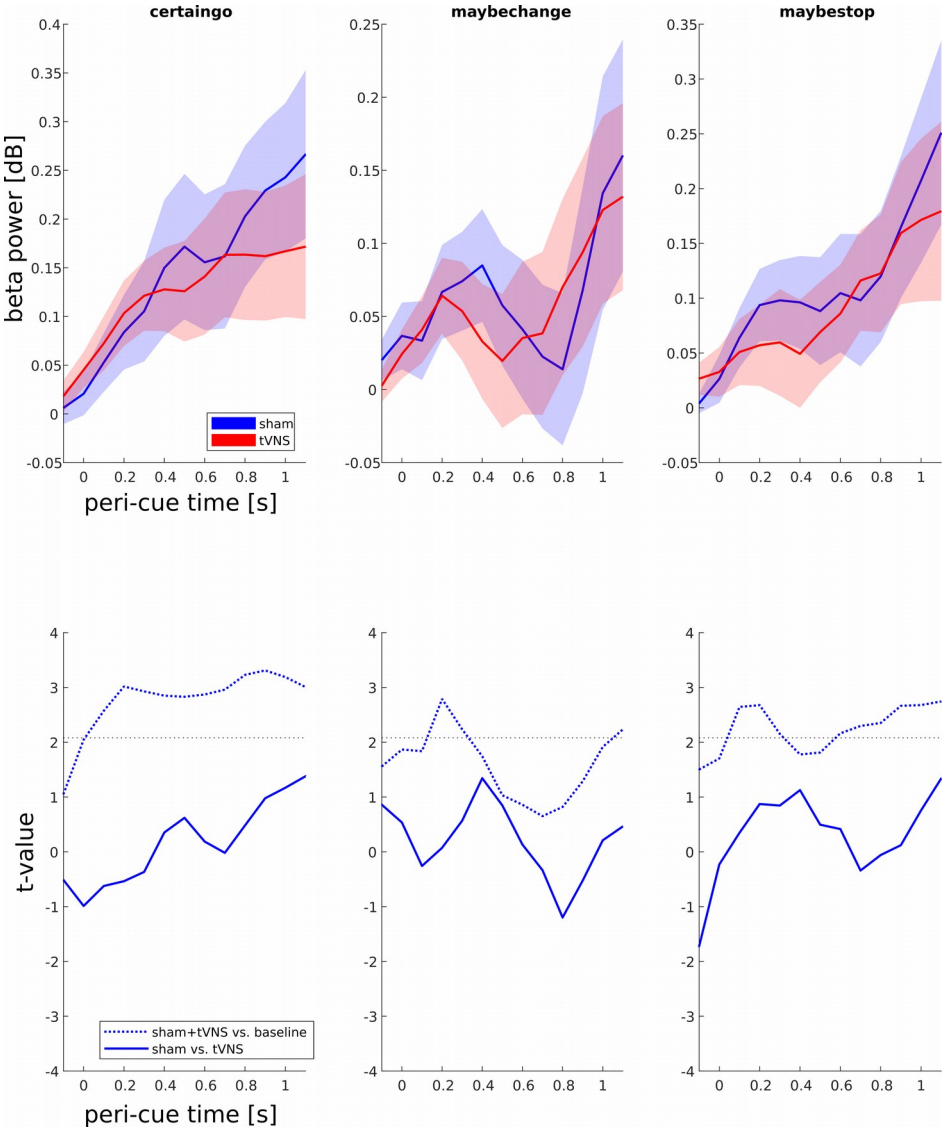

Figure 10. Beta power (15-25 Hz) lateralization in the cue-target interval, calculated as difference between right and left sensorimotor electrodes. Positive values indicate right>left. The lower row contains t-values comparing the subject-wise mean of tVNS and sham data to the pre-cue baseline (dashed lines) and sham to tVNS (solid lines).

Target-locked EEG results

Visual inspection of the target-locked time-frequency spectrum (Fig. 2A) revealed a transient increase in theta power (4-8 Hz) over fronto-central electrodes following stop and change stimuli over a time window of ~ 200 – 600 ms post-target, in line with previous findings (Cavanagh & Frank, 2014).

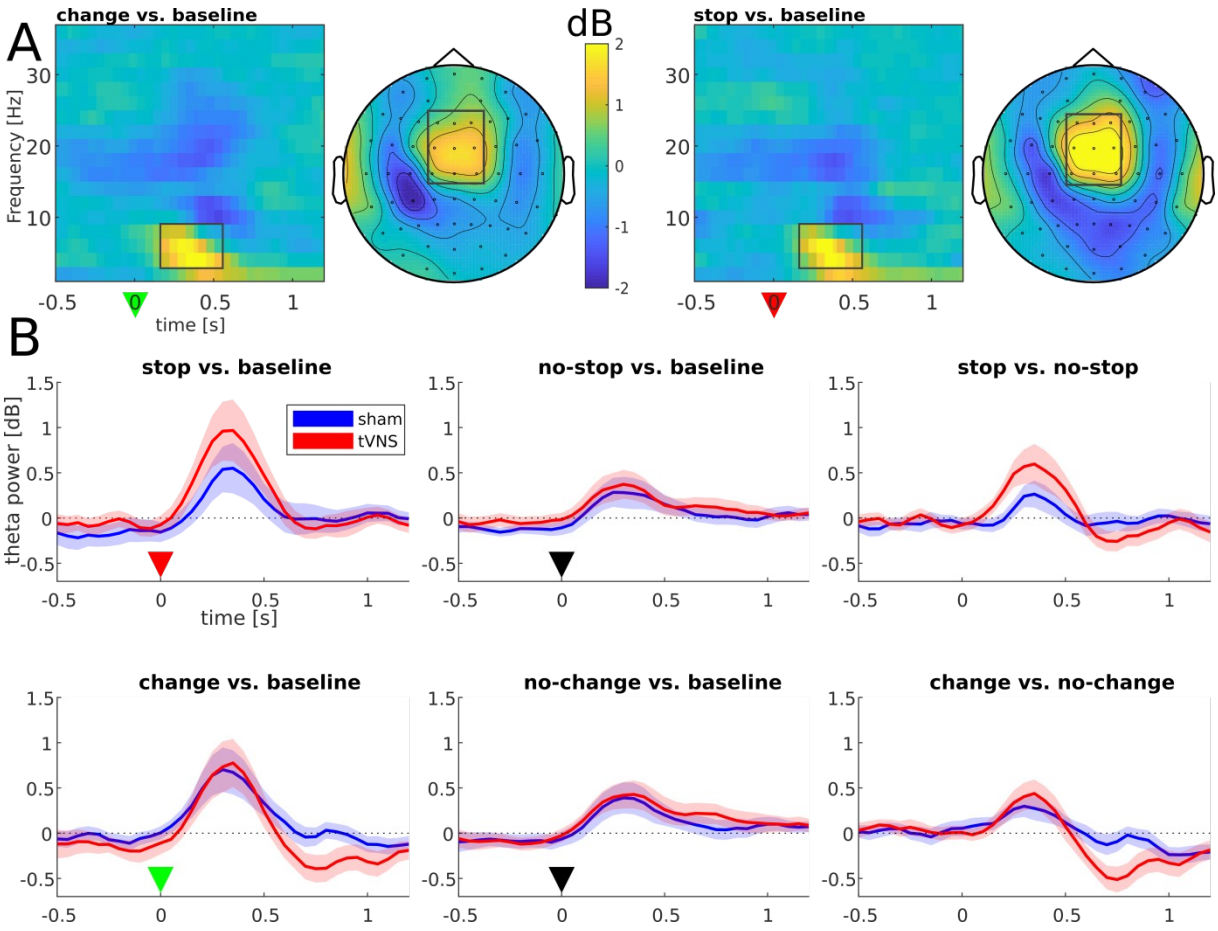

Figure 11. A: Baseline-corrected results of the time-frequency analysis, locked to change (left) and stop (right) target stimuli (Baseline: 750 – 250 ms pre-cue), averaged over sham and tVNS sessions. Panels show time-frequency data from 2 to 36 Hz, averaged over fronto-central electrodes (Fz, F1, F2, FCz, FC1, FC2, Cz, C1, C2), topographies show the theta band (4-8 Hz) between 200 and 600 ms after the target (FMΘ). B: Mean ± SEM of power change in the theta band at fronto-central electrodes.

Frequency- and channel-averaged fronto-central theta activity (Figure 11.B) showed a transient increase following target stimuli. Averaged over trials, over a time window from 200 to 600 ms post-target, and over sham and tVNS sessions, this increase was statistically significant for all targets (stop, no-stop, change, no-change, all $t_{21} > 2.0$, all $p < .029$), and it was greater for targets requiring non-standard responses (change, stop) than for those requiring standard responses (no-change, no-stop) (both $t_{21} > 1.95$, both $p < .032$), i.e., it was increased during response conflicts, in line with previous findings (Cavanagh & Frank, 2014).

Time-averaged FMΘ (200-600 ms post-target) in conflict (stop and change) trials was increased in tVNS sessions by 0.38 dB compared to sham sessions ($\chi^2 = 4.2$, $p = .040$). When stimulation effects were tested separately for stop and change trials, however, a

significant effect survived only in stop trials (χ^2 = 6.3, p = .012). In change trials, the same numerical trend was apparent, but it did not approach statistical significance (0.33 dB, χ^2 = 1.4, p = .228). However, following the FMΘ peak in change trials (600-1000 ms post-target), there was a stronger theta power decrease in tVNS compared to sham sessions (-0.24 dB, χ^2 = 3.9, p = .049), which was not apparent in stop trials (χ^2 = 0.1, p = .755). Statistical comparison of subject-wise mean values for the change vs. no-change and stop vs. no-stop contrasts (averaged over trials, right column of Fig. 2B) confirmed both effects, i.e., higher theta power during tVNS across the 200-600 ms post-target interval in stop trials (t_{21} = 2.97, p = .007) as well as lower theta power during tVNS across the 600-1000 ms post-target interval in change trials (t_{21} = -2.67, p = .014). In stop as well as change trials, FMΘ was higher in error compared to correct trials (change: 0.43 dB, χ^2 = 4.6, p = .033; stop: 0.4 dB, χ^2 = 4.1, p = .042), but this effect did not interact with stimulation (both χ^2 < 0.4, both p > .5).

4.5 Discussion

We studied effects of tVNS on behavioral performance and FMΘ during response conflicts. We found that, independently from stimulation, the overall pattern of RT, accuracy, and FMΘ across conditions was consistent with previous reports, in that response conflicts led to performance costs and elicited transient FMΘ responses (Cavanagh & Frank, 2014; Liebrand et al., 2018). Importantly, tVNS globally enhanced accuracy across conditions and reduced the performance costs of go/change response conflicts.

In the EEG data, we found a transient FMΘ response following target stimuli, which was increased in conflict (change, stop) compared to non-conflict (no-change, no-stop) trials. In stop trials, i.e., when any behavioral response had to be withheld, tVNS increased the FMΘ response. In change trials, the same numerical trend was apparent, but missed statistical approval. However, in change trials, tVNS led to a stronger drop of frontal theta power following the FMΘ peak.

During tVNS, behavioral accuracy was increased across conditions, suggesting that general adaptive control and sustained attention were enhanced. The effects on conflict-related FMΘ, especially the increased FMΘ activity in stop trials, additionally suggest a

more specific effect on the neural correlates of conflict monitoring and adaptation. Enhanced conflict adaptation during tVNS has been shown before (Beste et al., 2016; R. Fischer et al., 2018; Steenbergen et al., 2015). It appears somewhat paradoxical that we could show a behavioral enhancement of conflict adaptation for the change condition, manifested in reduced change costs on RT and accuracy, but not for the stop condition, whereas we have found increased FMΘ peaks under tVNS for the stop condition, but not for the sham condition. On the other hand, we found a stronger drop of frontal theta following the FMΘ peak in the change condition under tVNS, which was not apparent in the stop condition. The apparent dissociation between our behavioral and electrophysiological data complicates in-detail interpretation of our results. Nonetheless, we have shown that tVNS interacts with the neural mechanisms underlying conflict monitoring and adaptation, and it should be noted that behavioral indices of conflict cost are more difficult to obtain in stop conditions, since only accuracy but not RT data can be compared between conditions, unless the experimental paradigm allows, by design, for the calculation of stop-signal reaction times, which our paradigm does not. The stronger frontal theta drop in the change condition is difficult to interpret, and to the best of our knowledge, the specific functional role of this post-peak drop in FMΘ has not been investigated so far. Any in-depth interpretation is also limited by the fact that the effect of tVNS on the frontal theta drop was on the verge of statistical significance, which precludes too strong conclusions, so for now we will have to make do with the notion that tVNS appears to interact with the neural generators of FMΘ not only in the stop, but also in the change condition.

FMΘ has been characterized as the neural *lingua franca* of action monitoring processes (Cavanagh, Zambrano-Vazquez, & Allen, 2012), since it coincides with a broad range of situations requiring increased control, such as response conflict, error monitoring, memory load, and punishment (Cavanagh & Frank, 2014; Cavanagh & Shackman, 2015). It has been associated with behavioral readouts, such as the ability to override Pavlovian Learning Biases (Cavanagh, Eisenberg, Guitart-Masip, Huys, & Frank, 2013), behavioral adaptation to prediction errors in reinforcement learning (Cavanagh, Frank, Klein, & Allen, 2010), and performance cost of response conflicts (Cohen & Donner, 2013; Pinner & Cavanagh, 2017). Its neural generators have been consistently found in the anterior cingulate and mid-cingulate cortex and the adjacent pre-supplementary motor area, which further supports its role as a neurophysiological

correlate of action monitoring (Asada et al., 1999; Botvinick et al., 2004; Cavanagh & Frank, 2014; Gevins, 1997; Hajihosseini & Holroyd, 2013; Luu et al., 2004).

Studies in rodents have shown that (invasive) vagus nerve stimulation can increase hippocampal theta power, a neural correlate of memory encoding and retrieval processes (Broncel, Bocian, Kłos-Wojtczak, & Konopacki, 2017, 2018, 2019). Whether and how hippocampal theta is functionally related to FMΘ is controversial (Hsieh & Ranganath, 2014; Mitchell, McNaughton, Flanagan, & Kirk, 2008). However, the above rodent studies identified GABAergic and cholinergic neuromodulation as the mechanisms mediating the effect of vagus nerve stimulation on hippocampal theta (Broncel et al., 2018, 2019), both of which might also mediate the effect of tVNS on FMΘ (Hall, Barnes, Furlong, Seri, & Hillebrand, 2009; Onton, Delorme, & Makeig, 2005): Even though the mechanisms of action of tVNS and invasive vagus nerve stimulation are not fully understood, neuromodulatory effects in the norepinephrine, GABA, and acetylcholine neurotransmitter systems as well as peripheral-autonomic effects have been suggested repeatedly (Capone et al., 2015; Clancy et al., 2014; Hulsey et al., 2017; Keute, Ruhnau, Heinze, et al., 2018; Nichols et al., 2011; Raedt et al., 2011). All of these mechanisms have been shown to be involved in certain aspects of EC and adaptive behavior, e.g., GABA having a role for response inhibition (Quetscher et al., 2015), acetylcholine for behavioral flexibility (Picciotto, Higley, & Mineur, 2012), and norepinephrine for alertness, arousal, and adaptive behavior (Aston-Jones & Cohen, 2005b). Taken together, the assumed neural mechanisms of tVNS make effects on different aspects of EC plausible, and it appears questionable whether a single neural mechanism of tVNS can account for our findings of enhanced behavioral accuracy and increased conflict-related FMΘ during tVNS.

In sum, we have found that tVNS enhanced behavioral accuracy in a cued go-nogo-change task in young healthy adults. This study adds on previous findings that tVNS can enhance different aspects of EC, and extends them by demonstrating that tVNS can specifically interact with neural processes involved in conflict monitoring and adaptation as indexed by FMΘ.

We conclude that tVNS bears potential to enhance EC and conflict adaptation. We hope that future research will build on our and previous studies to investigate beneficial effects of tVNS on EC deficits in clinical populations.

5 Study 4: Pupillometry as a biomarker for tVNS responsiveness

A modified version of this chapter has been published as:

Keute, M., Demirezen, M., Graf, A., Mueller, N. G. & Zaehle, T. (2019). No modulation of pupil size and event-related pupil response by transcutaneous auricular vagus nerve stimulation (taVNS). *Scientific Reports*.

5.1 Abstract

Transcutaneous vagus nerve stimulation (tVNS) bears therapeutic potential for a wide range of medical conditions. However, previous studies have found substantial interindividual variability in responsiveness to tVNS, and no reliable predictive biomarker for stimulation success has been developed so far. In this study, we investigate pupil size and event-related pupil response as candidate biomarkers. Both measures have a direct physiological link to the activity of the locus coeruleus (LC), a brainstem structure and the main source of norepinephrine in the brain. LC activation is considered one of the key mechanisms of action of tVNS, therefore, we expected a clear increase of the pupillary measures under tVNS compared to sham (placebo) stimulation, such that it could serve as a prospective predictor for individual clinical and physiological tVNS effects in future studies.

We studied resting pupil size and pupillary responses to target stimuli in an auditory oddball task in 33 healthy young volunteers. We observed stronger pupil responses to target than to standard stimuli. However, and contrary to our hypothesis, neither pupil size nor the event-related pupil response nor behavioral performance were modulated by tVNS. We discuss potential explanations for this negative finding and its implications for future clinical investigation and development of tVNS.

5.2 Introduction

Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive electrical brain stimulation method that has been introduced as an alternative to direct or invasive vagus nerve stimulation (iVNS) (Ventureyra, 2000). TVNS can be administered externally to the neck (cervical tVNS) or the outer ear (auricular tVNS), which is partly innervated by the vagus nerve (Peuker & Filler, 2002). Both iVNS and tVNS can be employed as an adjunct therapy for pharmacoresistant epilepsy (Bauer et al., 2016; He, Jing, Zhu, et al., 2013; Stefan et al., 2012) and depression (Fang et al., 2016; Trevizol et al., 2015). TVNS has been attracting attention in recent years as a potential treatment for a variety of further conditions, including chronic headache (Barbanti et al., 2015; Magis, Gérard, & Schoenen, 2013), tinnitus (Lehtimäki et al., 2013), post-operative cognitive

dysfunction (Xiong et al., 2009), cerebral ischemia (Lu et al., 2017), and Alzheimer's disease (Kaczmarczyk et al., 2018). Moreover, several recent studies found effects of tVNS on cognitive and behavioral parameters, including response inhibition (Beste et al., 2016; Keute, Ruhnau, Heinze, et al., 2018), executive control of action (Steenbergen et al., 2015), and memory (Jacobs et al., 2015). These findings could pave the way for a prospective role of tVNS in neuropsychiatric and neuropsychological therapies.

As of now, the mechanisms of action of VNS are not fully understood, but accumulating evidence indicates a crucial role of the locus coeruleus – norepinephrine (LC-NE) system: Anatomically, the LC is a downstream projection area of the nucleus of the solitary tract, which is in turn one of the major brain projection areas of the vagus nerve (Fornai, Ruffoli, Giorgi, & Paparelli, 2011). A number of functional magnetic resonance imaging (fMRI) studies in humans consistently found LC activations following auricular tVNS (Assenza et al., 2017; Badran, Dowdle, et al., 2018; Frangos et al., 2015; Kraus et al., 2007; Yakunina et al., 2017). Moreover, increased levels of NE in the cerebrospinal fluid have been found in rodents after long-term iVNS (Follesa et al., 2007; Raedt et al., 2011; Roosevelt et al., 2006). Electrophysiological studies in rodents (Dorr & Debonnel, 2006; Groves et al., 2005; Hulsey et al., 2017) found immediate (i.e., beginning within a few milliseconds) LC spiking increases in response to iVNS, scaling with stimulation intensity, pulse width, and frequency.

The LC is the main source of NE in the brain. It has a central role in regulating arousal, attention and adaptive behavior (Berridge, 2008; Berridge & Waterhouse, 2003; Sara & Bouret, 2012). According to an influential model of LC-NE function (Aston-Jones & Cohen, 2005b), there are two functionally distinct modes of LC activity: Tonic activity, leading to a global increase in NE transmission, and phasic activity, leading to an upregulation of NE transmission in response to environmental requirements. Tonic LC activity has been linked to explorative, novelty-seeking, aroused and distractible behavior, whereas phasic LC activity promotes task-engagement and exploitative behavior (Aston-Jones & Cohen, 2005a; Usher, Cohen, Servan-Schreiber, Rajkowski, & Aston-Jones, 1999).

Next to invasive LC recordings, pupil size is considered the most reliable noninvasive marker of LC-NE activity, given constant luminance (Costa & Rudebeck, 2016; Joshi, Li, Kalwani, & Gold, 2016), with resting or tonic pupil size being indicative of tonic LC activity and pupillary responses to behaviorally relevant stimuli being indicative of phasic LC activity (Gilzenrat et al., 2010; Murphy, Robertson, Balsters, & O'connell, 2011). In this study, we combined tVNS with an auditory oddball paradigm whilst continuously measuring pupil size, asking for effects of tVNS on both tonic and phasic LC-NE activity as indexed through tonic pupil size and event-related pupil dilation (ERPD), respectively.

Our main interest in this study is to improve our mechanistic understanding of tVNS by establishing direct evidence for an effect of tVNS on LC-NE activity in humans. Furthermore, we are interested in pupil size as a candidate predictive biomarker for tVNS efficacy: Previous clinical studies of invasive and transcutaneous VNS in epilepsy and depression patients found that between one third and two thirds of patients did not respond to the stimulation, i.e., showed no amelioration of symptoms (Aaronson et al., 2013; Bauer et al., 2016; Carreno & Frazer, 2017; Fang et al., 2017). In order to exploit tVNS to its full potential, it will be necessary to predict individual treatment efficacy and to optimally adapt stimulation parameters. Tonic pupil size and/or ERPD might be used prospectively as an easy-to-use and inexpensive biomarker to identify responders to tVNS and to optimally tune stimulation parameters, given that a clear effect of tVNS on at least one pupillary parameter can be established.

Additionally, we will explore vagus-sensory evoked potentials (VSEP) as a further candidate biomarker for tVNS responsiveness and as a candidate explanatory variable for tVNS effects on pupil size measures (Fallgatter et al., 2003; Hagen et al., 2014). This additional analysis will be described in chapter 6.4.

5.3 Methods

Participants

Thirty-three healthy young adults (6 female) participated in the experiment. Age range was 21-30 years (M 24.4, SD 1.9). All had normal vision (visual defect of max. ± 1 diopter, no glasses or contact lenses could be used with the eyetracking hardware) and were free from any current or past neurological, psychiatric or ophthalmological condition and from any medical or recreational drug intake, except for oral contraceptives (all by self-report).

Procedure

We carried out a placebo-controlled, single-blind, randomized, within-subjects experimental study. Experimental sessions took place at the German Center for Neurodegenerative Diseases in Magdeburg. Each subject participated in two sessions, one involving sham (placebo) stimulation, and one involving real tVNS. For each subject, both sessions were scheduled in randomized order, at the same daytime and at least 48 hours apart, to enable full wash-out of any stimulation effects. As a reimbursement, subjects received course credit. The study was approved by the ethics committee of the medical faculty at the University of Magdeburg, and all experimental procedures were carried out in accordance with the Declaration of Helsinki.

Upon arrival, written informed consent was obtained from all participants. They were seated comfortably in a dimly lit room in an adjustable chair, with their head lying on a desk-mounted chinrest. Subjects were instructed to keep their gaze on a black fixation cross presented centrally against a grey background on a 24” screen at a distance of 70 cm throughout the experiment. Screen luminance had been adjusted in pilot sessions such that gaze could be kept on the screen comfortably over a longer time yet the fixation cross was clearly visible. Eye movements and pupil diameter were recorded continuously from the right eye at a sampling rate of 1000 Hz using a desk-based EyeLink 1000 eyetracker (SR Research[24]).

After an initial baseline measurement of pupil diameter (1 min), subjects performed an auditory oddball task (PRE-run, see below). After the first run, electrical stimulation (tVNS or sham stimulation, see below) started. During the first five minutes of

[24] www.sr-research.com

stimulation, subjects had no instruction other than keeping their gaze on the fixation cross (we refer to these first five minutes as ramp-up period). Subsequently, the second run (ON-run) of the oddball task was carried out. After this run, stimulation was turned off and the third run (POST-run) of the task began immediately. The experiment ended with another minute of resting pupil size recording. The experiment was controlled by custom Matlab (MathWorks[25]) code using Psychtoolbox 3[26] and the Eyelink add-in toolbox for eyetracker control.

Electrical stimulation

TVNS was administered to the cymba conchae of the left ear, sham stimulation to the left earlobe. Two conventional neurostimulation electrodes were used (Ambu Neuroline[27]) that were cut manually to a size of 4×4 mm. The two electrodes were mounted 1 cm apart (center-to-center) to a small piece of ear silicone, with the anode being more rostral, and fixated to the skin using Genuine Grass EC2 adhesive electrode cream (Natus Neurology[28]). Stimulation current was delivered as monophasic square pulses at a pulse width of 200 µs, pulse frequency of 25 Hz and current intensity of 3.0 mA using a medical stimulation device (Digitimer DS7[29]) triggered via a BNC cable by custom code running on an Arduino Uno circuit board[30]. Electrodes were mounted prior to the experiment, and stimulation parameters were tested. All subjects reported that stimulation with the above parameters was perceptible but not painful, both for sham stimulation and tVNS.

Auditory oddball task

In each run of the auditory oddball task, 300 auditory stimuli were presented through speakers, comprising 240 standard (500 Hz sinus tones of 60 ms duration) and 60 target (1000 Hz sinus tones of 60 ms duration) stimuli. Standard and target stimuli were presented in pseudo-randomized order, but two target stimuli were always separated by at least three standard stimuli. Inter-stimulus interval (ISI) was randomly jittered between 2.1 and 2.9 s. Subjects were instructed to press the space bar on a PC keyboard

25 www.mathworks.com

26 www.psychtoolbox.org

27 www.ambu.com

28 www.natus.com

29 www.digitimer.com

30 www.arduino.cc

with the right index finger after each target stimulus and to ignore the standard stimuli. Total duration of one run was ~13 minutes (with small differences because of the random ISI jitter). The oddball task was designed to resemble a previous task known to reliably elicit ERPD (Murphy et al., 2011).

Data analysis

Raw pupil sizes as measured by the eyetracker were linearly transformed from arbitrary units to millimeters (Hayes & Petrov, 2016). Eyeblinks and other artifacts were identified through a custom-made automatic Matlab procedure, verified by visual inspection and corrected by linear interpolation. On average, 7.7 (± 5.8) percent of the data were identified as artifacts and interpolated.

Data from the two baseline measurements (at the beginning and end of the experiment) were averaged over time (1 minute). Data from the ramp-up period (the first five minutes after stimulation onset, without task) were cut to five segments of 1 minute length, and each segment was averaged over time. Data from the three runs of the oddball task were cut to segments from -0.5 to 2.5 s relative to each stimulus. Target stimuli with missed responses were excluded from further analysis. The 0.5 s period preceding each stimulus served as trial-baseline. Event-related pupil dilation (ERPD) was computed as the mean percent change in pupil diameter over 1.5 s post-stimulus relative to the trial-baseline.

To capture the development of tonic pupil size over time-on-stimulation, we additionally computed the mean pupil size over the 2.5 s epochs following the standard stimuli, normalized as percent change to the pre-experiment baseline. Temporal variability of tonic pupil size was computed as coefficient of variation (CV) over the 2.5 s post-stimulus epochs in standard trials, averaged over trials. CV is the standard deviation over time, divided by the mean.

Analyses involving repeated measurements (i.e., pupil diameter or reaction times with multiple trials / measurements per subject) were analyzed using linear mixed-effects regression models. We specified random intercepts and random slopes between sham and tVNS per subject to account for repeated measurements. We used this random effects structure because we found that it fitted the data significantly better than random intercepts only, following recommendations in the literature(Barr et al., 2013). Fixed

effects were tested by comparing a full model (containing all fixed effects of interest) to reduced models using likelihood ratio tests, leaving out one fixed effect at a time(Bates et al., 2015). Models were fit using a maximum likelihood algorithm as implemented in Matlab. Next to the effect size as estimated by the model and the test statistic (likelihood ratio / χ^2). The test statistic in a likelihood ratio test is subjected to a χ^2 test because likelihood ratios asymptotically follow the χ^2 distribution under the null hypothesis (Wilks, 1938). Moreover, we report model comparisons based on Akaike's (AIC) and Bayes' information criterion (BIC), two indices of model fit based on model likelihood, penalized by the number of predictors in the model. The difference in AIC or BIC between two nested models (e.g., a model containing a certain fixed effect vs. a model without it) indicates the support for either model through the data (Burnham & Anderson, 2004). Note that, despite the name 'Bayes' in the BIC, these model comparisons do not perform Bayesian inference in the narrower sense, since they are based on penalized likelihood of the data (given the model), but do not incorporate prior and posterior probabilities of the models (given the data).

5.4 Results

Resting measurements

Mean overall pupil size during the baseline period was 2.9 mm (SD 1.3) and was not significantly different between sham and tVNS sessions (t_{32} = 0.92, p = .364, Figure 12.A). Pupil size decreased from the first (before stimulation onset) to the last (~13 min after stimulation offset) resting measurement by 11.9 percent (χ^2 = 25.2, p < .001), but was not significantly different between tVNS and sham (χ^2 = 1.3, p = .254), nor did the decrease over time interact with stimulation (χ^2 = 0.1, p = .738, Figure 12.A). Accordingly, model comparisons based on information criteria (positive values: supporting non-inclusion, negative values: supporting inclusion, for all reported information criteria in the following) favored the non-inclusion of stimulation main effect (ΔAIC = 0.7, ΔBIC = 3.6) and time-stimulation interaction (ΔAIC = 1.9, ΔBIC = 4.8) to the model.

During the rampup period (i.e., during the first five minutes on stimulation), normalized pupil size decreased by 3.7 percent points per minute (χ^2 = 45.6, p < .001, Figure 12.A),

but was neither different between sham and tVNS (χ^2 = 0.7, p = .397, Figure 12.A), nor did stimulation interact with time (χ^2 = 0, p = .892, Figure 12.A). Accordingly, model comparisons based on information criteria favored the non-inclusion of stimulation main effect (ΔAIC = 1.28, ΔBIC = 5.0) and time-stimulation interaction (ΔAIC = 2.0, ΔBIC = 5.8) to the model.

Considering the first seconds after stimulation onset, we found an initial increase in pupil size in response to both sham stimulation and tVNS (Figure 12.B, upper panel) compared to a 2 s baseline prior to stimulation onset. It can be seen that the increase lies above the (uncorrected) significance level both for tVNS and sham stimulation compared to baseline (pre-stimulation), but not for the sham vs. tVNS comparison (Figure 12.B, lower panel). This sensory-mediated increase in pupil size was negatively correlated with absolute pre-stimulation pupil size, in line with previous findings (Figure 12.B, right panels) (Murphy et al., 2011).

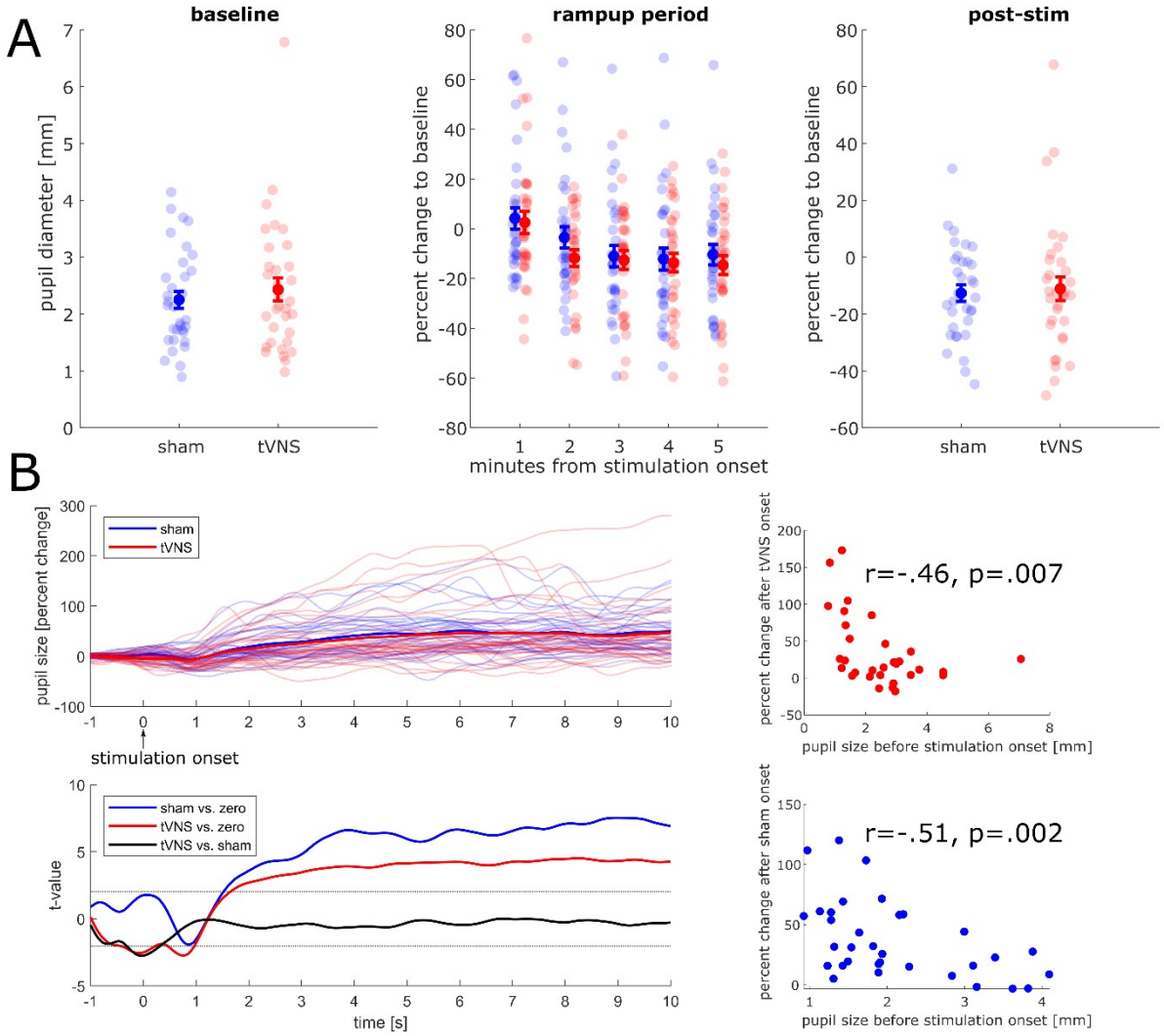

Figure 12. A: Pupil diameter during the baseline measurement (left), change to baseline during the first five minutes of stimulation, and in the post-task resting measurement (~13 min after stimulation offset). B: Left: Grand average pupillary response to stimulation onset and t-values. Dashed lines indicate t = ±2.04, i.e., the (uncorrected) two-tailed threshold for statistical significance at α = .05 and df = 32 (33 participants minus one). Right: relationship between mean pupil size in the 2s before stimulation onset and mean change in pupil size in the first 10s after stimulation onset. Negative correlations can be seen, consistent with previous studies.

Auditory oddball task

Figure 13.A shows the ERPD to target and standard stimuli in the pre-, on-, and post-run. The time- and trial-averaged ERPD was 5.4 percent points higher to target compared to standard stimuli (χ^2 = 353.5, p < .001) and decreased by 0.6 percent points per run between the pre-, online-, and post-run (χ^2 = 17.8, p < .001), i.e., there was a

stronger pupillary response to target stimuli compared to standard stimuli, and this response declined over time. Crucially, there was no significant difference between tVNS and sham ($\chi^2 = 0.5$, $p = .468$), and stimulation did not interact with run ($\chi^2 = 0$, $p = .939$) nor condition ($\chi^2 = 0.3$, $p = .861$), nor was there a three-way interaction between run, stimulation, and condition ($\chi^2 = 0.6$, $p = .756$). Additionally, model comparisons based on information criteria favored the non-inclusion of stimulation main effect (ΔAIC = 1.5, ΔBIC = 5.5), stimulation×run interaction (ΔAIC = 2.0, ΔBIC = 5.9), stimulation×condition interaction (ΔAIC = 2.0, ΔBIC = 5.9), and three-way interaction (ΔAIC = 3.4, ΔBIC = 11.4). Tonic pupil size during the three runs of the oddball task decreased by 0.1 mm per run ($\chi^2 = 4.2$, $p = .039$). There was no significant main effect of stimulation ($\chi^2 = 2.6$, $p = .108$) and, crucially, no run×stimulation interaction ($\chi^2 = 0$, $p = .977$). Model comparisons based on information criteria favored the non-inclusion of run×stimulation interaction (ΔAIC = 2, ΔBIC = 5.8) but were not conclusive on the non-inclusion of stimulation main effect (ΔAIC = -0.6, ΔBIC = 2.7).

Mean overall reaction time (RT) to target stimuli in the auditory oddball task was 0.395 s. RT did not significantly differ between sham and tVNS, nor between task runs, nor did task run interact with stimulation (all $\chi^2 < 4.7$, all $p > .095$). Omission errors to target stimuli were very infrequent (only one subject in one run had an error rate > 5 percent, Figure 13.B). No commission errors in response to standard stimuli occurred in any subject.

Mean overall tonic pupil size following standard stimuli (Figure 13.C) was 2.4 mm. It increased by 0.12 mm per run between the pre-, on-, and post-run ($\chi^2 = 29.5$, $p < .001$), but was not significantly different between sham and tVNS ($\chi^2 = 0.6$, $p = .448$), nor was there a run×stimulation interaction ($\chi^2 = 0$, $p = .938$). Model comparisons based on information criteria favored the non-inclusion of stimulation main effect (ΔAIC = 1.9, ΔBIC = 5.2) and run×stimulation interaction (ΔAIC = 1.4, ΔBIC = 4.7).

Mean overall temporal coefficient of variation in standard trials was 0.06, i.e., pupil size varied over time (standard deviation) by 6% relative to the mean pupil size. Temporal variability decreased over the three runs of the auditory oddball task by 0.002 per run ($\chi^2 = 15.6$, $p < .001$, Figure 13.D), but did not differ between sham and tVNS ($\chi^2 = 0.1$, $p = .773$), nor did run interact with stimulation ($\chi^2 = 0$, $p = .881$). Model comparisons

based on information criteria favored the non-inclusion of stimulation main effect (ΔAIC = 1.9, ΔBIC = 5.2) and run×stimulation interaction (ΔAIC = 2, ΔBIC = 5.2).

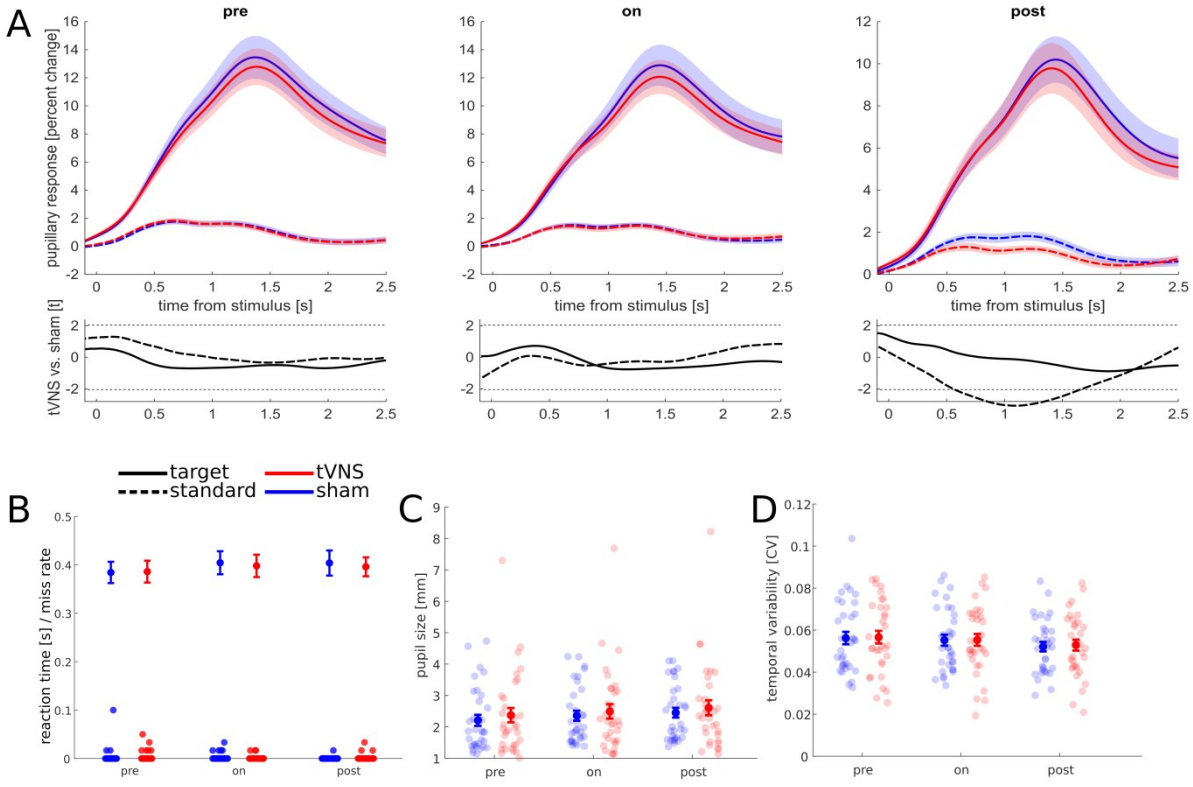

Figure 13. A: Upper panels: Pupillary response to standard and target stimuli in the auditory oddball task before (left), during (middle), and after stimulation (right). Lower panels: t-values comparing sham and tVNS. Dashed lines indicate t = ±2.04, i.e., the (uncorrected) two-tailed threshold for statistical significance at α = .05 and df = 32 (Thirty-three participants minus one). B: Mean ± standard error of RT to target stimuli (error bars) and omission error rate (dots). C: Tonic pupil size in the three task runs. D: Temporal variability of tonic pupil size in standard trials, expressed as coefficient of variation (see Methods).

Finally, we analyzed the evolution of tonic pupil size and ERPD over time-on-stimulation. To this end, we split standard and target trials from the on-run of the task in 20 blocks, respectively, and computed the mean tonic pupil size from standard trials (normalized to session baseline) and ERPD from target trials (normalized to pre-stimulus baseline). We found that tonic pupil size decreased by 0.1 percent points per block (χ^2 = 4.8, p = .029, Figure 14.A), without a main effect of stimulation (χ^2 = 0.7, p = .402) nor a block×stimulation interaction (χ^2 = 0.1, p = .790). Model comparisons

favored the non-inclusion of stimulation main effect (ΔAIC = 1.3, ΔBIC = 6.5) and block×stimulation interaction (ΔAIC = 2.0, ΔBIC = 6.9).

Similarly, we found that ERPD in target trials decreased by 0.05 percent points per block (χ^2 = 4.0, p = .047, Figure 14.B), without a main effect of stimulation (χ^2 = 0.2, p = .677) nor a block×stimulation interaction (χ^2 = 0.2, p = .672). Model comparisons favored the non-inclusion of stimulation main effect (ΔAIC = 1.8, ΔBIC = 7.0) and block×stimulation interaction (ΔAIC = 1.8, ΔBIC = 7.0).

Even though our main focus in this study were overall group-level effects, we carried out additional analyses to capture possible interindividual differences in pupillary stimulation responsiveness. These analyses are summarized in Figure 15.: We computed intra-session differences between the pre- and on-run of the auditory oddball task for tonic pupil size (pupil size over the 240 standard trials) and ERPD (change-to-baseline over the 60 target trials). It can be seen that for ERPD, the intra-session difference exceeded the threshold for uncorrected statistical significance only in a few sessions. For the intra-session change in tonic pupil size, there was considerably greater interindividual variability both in sham and tVNS sessions. However, intra-session changes in tonic pupil size were positively correlated between sham and tVNS sessions (r = .544, p = .001, Figure 15.B), whereas a clear dissociation could have supported an LC-NE mediated effect (it might have allowed to identify a responder subset of participants, albeit this would still have been rather weak and anecdotal evidence). Conversely, the relatively high correlation suggests that interindividual differences are driven by dispositional factors, such as pupillary responsiveness to somatosensory stimulation or general temporal variability in pupil size rather than by tVNS-induced LC-NE activation. For ERPD, this dissociation can be found (r = .270, p = .128, Figure 15.C), but it cannot be interpreted as evidence for an LC-NE-mediated effect, since the overall differences within both tVNS and sham sessions are small and mostly miss statistical significance in within-session comparisons.

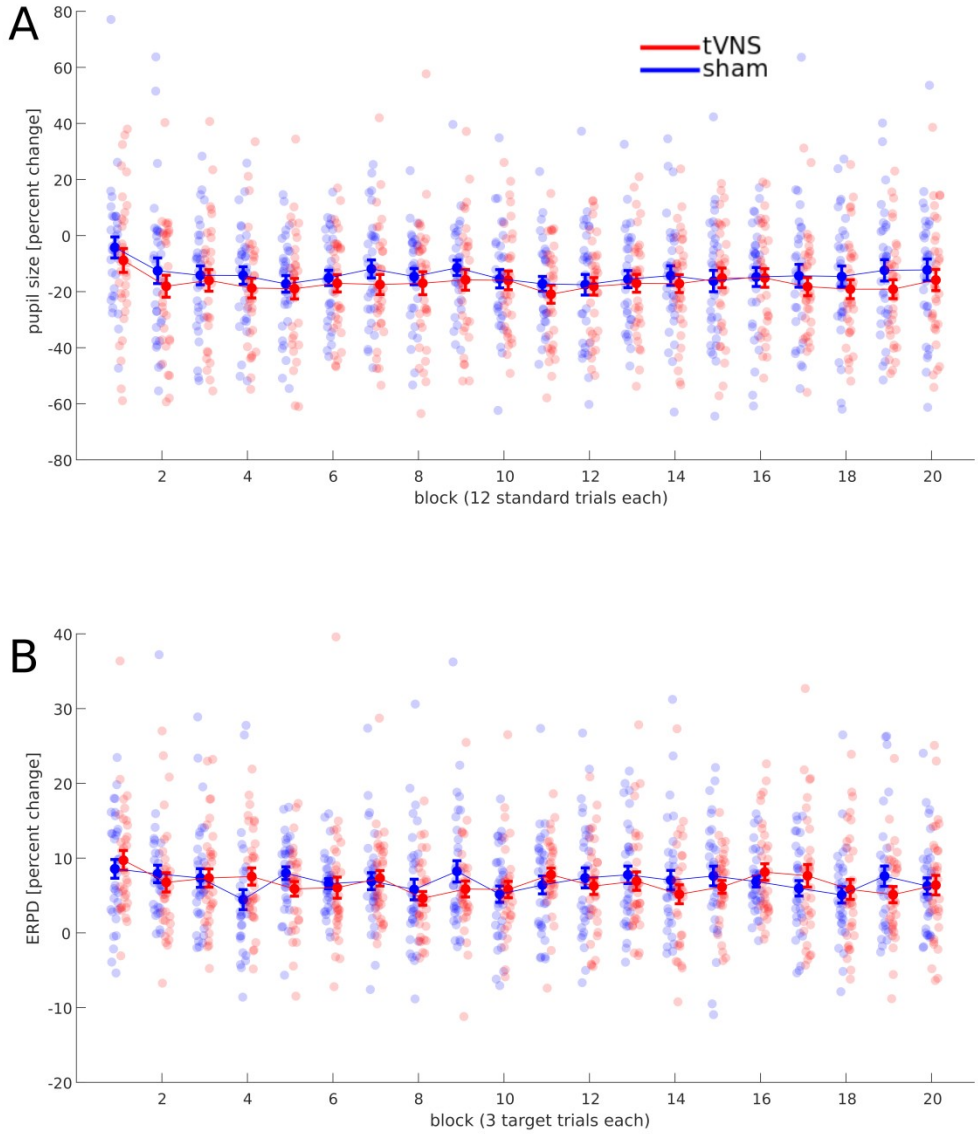

Figure 14. A: Evolution of tonic pupil size over time-on-task during the on-stimulation run of the auditory oddball task, relative to session baseline. B: Time-averaged pupillary responses to target stimuli over time-on-task, relative to pre-stimulus baseline.

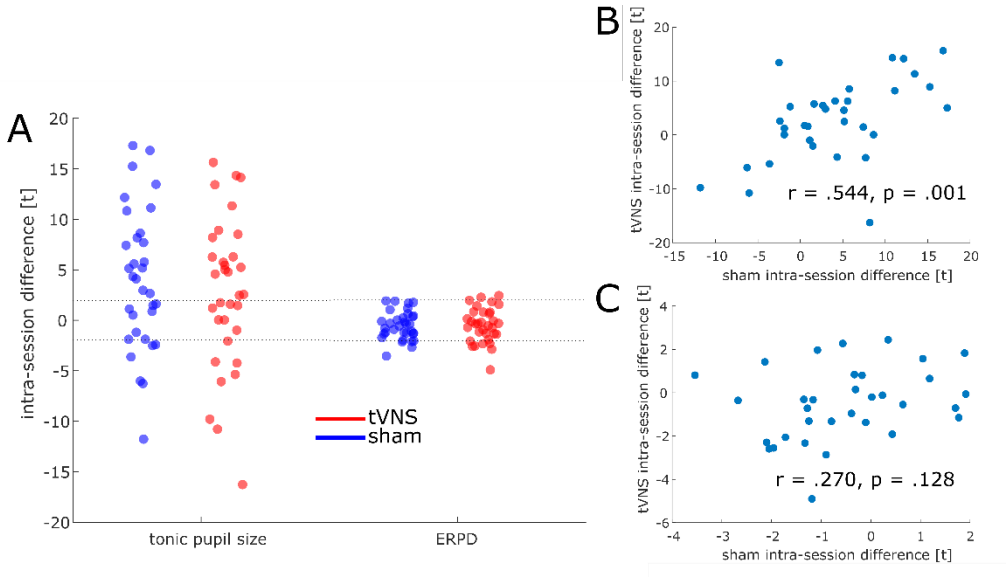

Figure 15. A: Within-session differences between the pre- and on-run of the oddball task for tonic pupil size and ERPD. The dashed lines mark the (uncorrected) two-tailed thresholds for statistical significance at α = .05 and df = 239 (tonic pupil size from 240 trials min) and df = 59 (ERPD from 60 trials), corresponding to the number of standard and target trials minus one. B: Relationship between intra-session difference in tonic pupil size in sham and tVNS sessions. C: Relationship between intra-session difference in ERPD in sham and tVNS sessions.

5.5 Discussion

We studied the effect of tVNS on tonic pupil size and ERPD. Given that previous studies consistently found LC activation following VNS (Frangos et al., 2015; Hulsey et al., 2017), we had a clear hypothesis that tVNS would increase pupil size, and that this modulation could be used prospectively as a qualitative (separating responders from non-responders) and quantitative (scaling with stimulation parameters in responders) predictive biomarker for tVNS responsiveness. However, our data do not support this hypothesis, in that we could not establish any systematic effect of tVNS on neither tonic pupil size nor ERPD.

To the best of our knowledge, there have been four previous studies combining VNS and pupillometry: One study in rodents (Bianca & Komisaruk, 2007) found that resting pupil size of both eyes was increased after unilateral iVNS. Likewise, a human study (Jodoin et al., 2015) found that resting pupil size but not pupillary light reflex was increased under iVNS. This finding could not be replicated by another human study (Schevernels et al., 2016), that found no effect of iVNS on resting pupil size, but a

(statistically non-significant) trend towards increased ERPD. The only published study of tVNS and pupil size to date (Warren et al., 2018) found no effect on resting pupil size in humans (discussed in more detail below). Our study replicates the findings from this study for tonic pupil size in a larger sample, and extends them by also taking into account ERPD.

Our hypothesis was built on previous findings that pupil size is a reliable marker of LC-NE activity (Gilzenrat et al., 2010; Murphy et al., 2011), but contrary to our hypothesis, we found no systematic effects of tVNS. The question of the 'missing link' arises – does it lie in the relationship between tVNS and LC-NE activation, or in the relationship between LC-NE activation and pupil size?

A possible explanation for our negative result could be that there is no relationship between tVNS and LC-NE activity whatsoever, but there is substantial evidence speaking against it: Several studies have found acute and sustained effects of iVNS on LC activity and NE concentration (Follesa et al., 2007; Hulsey et al., 2017; Landau et al., 2015; Raedt et al., 2011; Roosevelt et al., 2006). The number of vagus nerve fibres recruited by auricular tVNS is smaller than for iVNS, because the auricle is innervated only by afferent vagus nerve fibres (Burger & Verkuil, 2018; Peuker & Filler, 2002), yet a number of fMRI studies found LC activations following tVNS (Badran, Dowdle, et al., 2018; Frangos et al., 2015; Yakunina et al., 2017). Our data are in line with a similar, recent study (Warren et al., 2018), that did not find effects of tVNS on tonic pupil size nor on the P300 component of the event-related potential in a combined visual and auditory oddball task, which is considered a peripheral marker of LC-NE activity (Murphy et al., 2011). However, the same study found that tVNS increased salivary alpha-amylase (sAA), a peripheral marker of central NE level (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996). SAA increase after tVNS had been reported before (Ventura-Bort et al., 2018). In sum, there is solid evidence for a modulation of LC-NE activity through tVNS.

The relationship between LC-NE activity and pupil size, on the other hand, is also well established. Given constant luminance, pupil size, temporal variability of pupil size and ERPD are influenced by a variety of cognitive processes, including attention (Binda, Pereverzeva, & Murray, 2014; Gabay, Pertzov, & Henik, 2011; Wierda, van Rijn,

Taatgen, & Martens, 2012), mental effort (Alnæs et al., 2014; Granholm, Asarnow, Sarkin, & Dykes, 1996; Kahneman & Beatty, 1966), emotional arousal (Bradley, Miccoli, Escrig, & Lang, 2008), and behavioral relevance of stimuli (de Gee, Knapen, & Donner, 2014; Einhäuser et al., 2008). The mediation of these pupil-behavior relationships through the LC-NE system has been corroborated through electrophysiological LC recordings in monkeys (Costa & Rudebeck, 2016; Joshi et al., 2016) and rodents (Reimer et al., 2016) as well as pharmacological, behavioral and neuroimaging studies in humans (Alnæs et al., 2014; Gilzenrat et al., 2010; Hong, Walz, & Sajda, 2014; Keute, Ruhnau, Heinze, et al., 2018; Murphy, O'connell, O'sullivan, Robertson, & Balsters, 2014; Murphy et al., 2011; Phillips, Szabadi, & Bradshaw, 2000).

One possible explanation for our negative result is that pupil size is not exclusively dependent on the LC-NE system, but also on cholinergic transmission (Reimer et al., 2016). Given that VNS interacts with both the sympathetic / NEergic and parasympathetic / cholinergic central and peripheral nervous system (Bonaz, Picq, Sinniger, Mayol, & Clarençon, 2013; Borovikova et al., 2000; Kolman, Verrier, & Lown, 1975), it is possible that interactions between noradrenergic and cholinergic modulation mask the LC-NE-mediated effect of tVNS on pupil size. However, the interaction between NEergic and cholinergic effects of VNS is not well understood, and most studies so far have focused on either one, but not both, so we can only speculate about this.

Alternatively, it is also conceivable that we did not find the expected effects because we did not administer proper stimulation of the auricular branch of the vagus nerve. This seems unlikely, however, given that we have demonstrated behavioral and electrophysiological effects of tVNS previously using the same apparatus and similar parameters (Keute, Ruhnau, Heinze, et al., 2018), that electrical stimulation of the cymba conchae is a well-established tVNS method (Yakunina et al., 2017), and that our results are consistent with a similar, recent study (Warren et al., 2018). Moreover, the fact that stimulation onset (tVNS and sham alike) elicited a transient somatosensory pupillary response (Oka et al., 2007; Figure 12.B) and that all subjects reported that they felt the stimulation (tVNS and sham alike) renders it implausible that fundamental

technical flaws account for the negative result. In sum, the missing link between tVNS and pupil size / ERPD remains elusive and warrants further investigation.

Anyway, the absence of an effect of tVNS on both tonic pupil size and ERPD is a significant setback for the further development of targeted, individualized tVNS administration. However, there are other candidate biomarkers of tVNS efficacy, e.g., spectral power in the M/EEG (Hyvärinen et al., 2015; Lewine, Paulson, Bangera, & Simon, 2018), vagus-sensory evoked potentials (Hagen et al., 2014, cf. chapter 6.4), cardiac parameters such as heart-rate variability (Clancy et al., 2014), and fMRI readouts (Fang et al., 2017). As sophisticated, novel tVNS paradigms emerge, such as closed-loop (Romero-Ugalde et al., 2018), respiratory-gated (Sclocco et al., 2019), or parameter-optimized (Badran, Mithoefer, et al., 2018) tVNS, the search for such biomarkers will gain relevance, and we think that it should be a focus of future tVNS research.

6 Further studies

A modified version of section 6.3 has been pre-registered as:

Keute, M., Ruhnau, P., Heinze, H. J., & Zaehle, T. (pre-registered, 2019). Effects of transcutaneous vagus nerve stimulation (tVNS) on beta and gamma brain oscillations. *Cortex*. https://osf.io/q65pn

6.1 Overview

This chapter contains three short reports. The first one describes results from a pilot study probing the effects of tVNS on prepulse inhibition of the acoustic startle response. The second report contains a research proposal that aims to further clarify the occurrence, regional specificity, and possible lateralization of GABAergic neuromodulation through tVNS using brain oscillations as measured by MEG. The third report describes vagus-sensory evoked potentials (VSEP) that were measured from a subset of the participants in study 4. Relationships between VSEP components and tVNS effect on pupil size measures are explored.

6.2 tVNS and prepulse inhibition of startle

Introduction

Prepulse inhibition (PPI) is attenuation of behavioral responses to startle-eliciting stimuli through weak sensory stimuli (prepulses) immediately (i.e., by <500 ms) preceding the startle (Fendt, Li, & Yeomans, 2001). PPI represents a sensorimotor gating mechanism, by which irrelevant stimuli are kept out of awareness. When sensorimotor gating is activated through the prepulse, subsequent startle responses become attenuated as well (Braff, Geyer, & Swerdlow, 2001). In humans, the eye blink reflex is the standard way to quantify the startle response and PPI, since it is a reliable component of the startle response and easily measurable by electromyography (EMG) (Blumenthal, Elden, & Flaten, 2004).

Deficient PPI has been found in patients with schizophrenia, obsessive-compulsive disorder, Tourette's syndrome, and other neuropsychiatric disorders (Braff et al., 2001). Many studies have investigated neural correlates of sensorimotor gating and PPI. It has been found that pharmacological manipulations in norepinephrine, dopamine, serotonin, GABA, and glutamate/NMDA transmission can disrupt or enhance PPI (Fendt et al., 2001; Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001; Kumari, Soni, & Sharma, 2002; Phillips, Langley, Bradshaw, & Szabadi, 2000; Yamashita et al., 2006). Specifically, it has been shown that PPI depends on interactions between GABAergic

circuitry in the basal ganglia and mesolimbic dopaminergic transmission (Swerdlow, Braff, & Geyer, 1990).

As detailed in chapter 2, we were interested in probing GABA-associated neuropsychological readouts (Capone et al., 2015; Keute, Ruhnau, Heinze, et al., 2018). Furthermore, effects of VNS on serotonergic (Manta et al., 2009) and dopaminergic (Manta et al., 2013) transmission have been shown. Given these three transmitter systems through which tVNS might interact with the neural bases of sensorimotor gating and PPI, we explored effects of tVNS on the magnitude of PPI.

Methods

We carried out a single-blind, within-subjects experimental study. Fourteen healthy young adults participated (9 female). Participants were reimbursed with course credit. Prior to experimental sessions, written informed consent was obtained from all participants. The experiment was carried out in accordance with the declaration of Helsinki and has been approved by the local ethics committee. Each participant underwent tVNS and sham stimulation on two separate days, at least 48 hours apart and at the same daytime. The order of tVNS and sham stimulation was randomized across participants.

TVNS was administered to the tragus of the left ear, and sham stimulation was administered to the left earlobe. In both conditions, electrical stimulation was delivered as rhythmic direct current pulses through conventional neurostimulation electrodes (Ambu Neuroline[31]), cut to a size of 4×4 mm and fixated to the skin at a center-to-center distance of 1 cm using Genuine Grass EC2 adhesive electrode cream (Natus Neurology[32]). The anode was mounted rostral from the cathode. Current was generated using a medical stimulation device (Digitimer DS7[33]) as square pulses of 200 µs duration, repeated at 25 Hz and with an on/off cycle of 30s/30s. Current intensity was set to 8 mA, if tolerated by the participants; else it was decreased stepwise until it was tolerable. Mean delivered current intensity was 5.5 mA (±1.9) in tVNS sessions and 4.4 mA (±1.7) in sham sessions. Electrical stimulation started 10 minutes prior to the PPI and measurement and continued throughout the measurement, which took ~18 minutes.

31 www.ambu.com

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33 www.digitimer.com

For PPI measurement, participants were seated comfortably in front of a 24” LCD screen. On the screen, a white fixation cross was presented centrally against a dark background. Participants were instructed to keep their gaze on the fixation cross throughout the experiment. Acoustic white noise was presented through headphones (Sennheiser HD 25[34]) at 60 dB (A). Startle stimuli were 50 ms noise bursts at 70 dB (A). Startle stimuli could be preceded by a prepulse, i.e., an attenuated noise burst presented at 64 dB (A). Inter-stimulus interval (ISI) between prepulse and startle stimulus could be 60 ms, 120 ms, or 200 ms. Fifteen trials of each ISI, 15 trials without prepulse and 15 prepulse-only trials were presented in pseudo-randomized order. Inter-trial interval was pseudo-randomly jittered between 8 and 22 s.

Startle responses were captured using EMG, recorded bipolarly from two electrodes placed below the right eye, over the orbicularis oculi muscle (Figure 16.). EMG signal was recorded at a sampling rate of 1000 Hz using a BrainAmp amplifier (BrainProducts[35]) with an online lowpass filter (cutoff 500 Hz).

Data Analysis was carried out using BrainVision Analyzer 2 (Brain Products). Raw EMG data were bandpass filtered (30 – 300 Hz) and segmented into single trials (±150 ms relative to the startle stimuli). The 150 ms prior to each stimulus were used for baseline correction. Baseline-corrected segments were rectified, i.e., converted to absolute values, and smoothed using a moving average filter (window length 30 ms). Segments with premature EMG responses, eyeblinks or other artifacts were excluded by visual inspection. Peaks were detected automatically and verified by visual inspection. PPI measurement and analysis were designed following recommendations in the literature (Blumenthal et al., 2005).

For statistical analysis, we first determined the presence of PPI univariately in the three prepulse conditions through paired t-tests comparing individual mean peak EMG responses from the three prepulse conditions (averaged over sham and tVNS sessions) with responses in the no-prepulse condition, respectively. Secondly, we examined effects of prepulse condition, stimulation, and their interaction on session-wise mean PPI in a repeated measures ANOVA. PPI was computed as percent change between

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[35] www.brainproducts.com

individual no-prepulse peak EMG responses and peak EMG responses in the three prepulse conditions, respectively.

Results

Average time-locked EMG data for the different conditions are shown in Figure 16.. Numerically, it can be seen that startle stimuli elicited an EMG response, peaking between 50 and 70 ms, and that this response was attenuated in trials with prepulses across the three ISIs.

Averaged over sham and tVNS sessions, peak EMG responses were significantly smaller in the 60ms prepulse (t_{13} = 5.6, p < .001), 120ms prepulse (t_{13} = 4.7, p < .001), and 200 ms prepulse (t_{13} = 3.5, p = .004) compared to the no-prepulse condition, i.e., PPI occurred across conditions (ISIs).

Comparing PPI between conditions and stimulations (Figure 17.), however, we found no significant main effects of condition ($F_{2,26}$ = 2.3, p = .121) and stimulation ($F_{1,13}$ = 0.1, p = .747), nor a condition×stimulation interaction ($F_{2,26}$ = 0.2, p = .852).

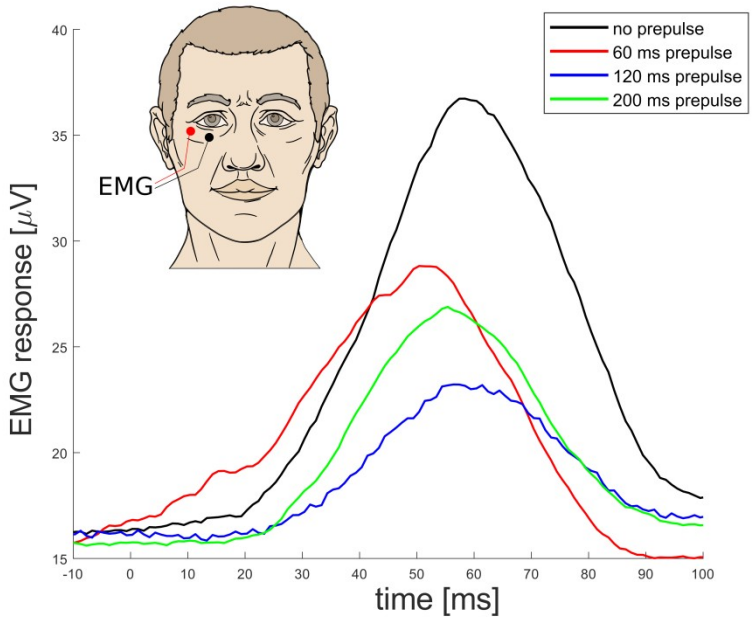

Figure 16. M. orbicularis oculi EMG responses to startle stimuli (at 0 ms). Responses are baseline corrected (baseline -150:0 ms) and averaged over sham and tVNS sessions. Human face image reprinted from Wikimedia Commons.

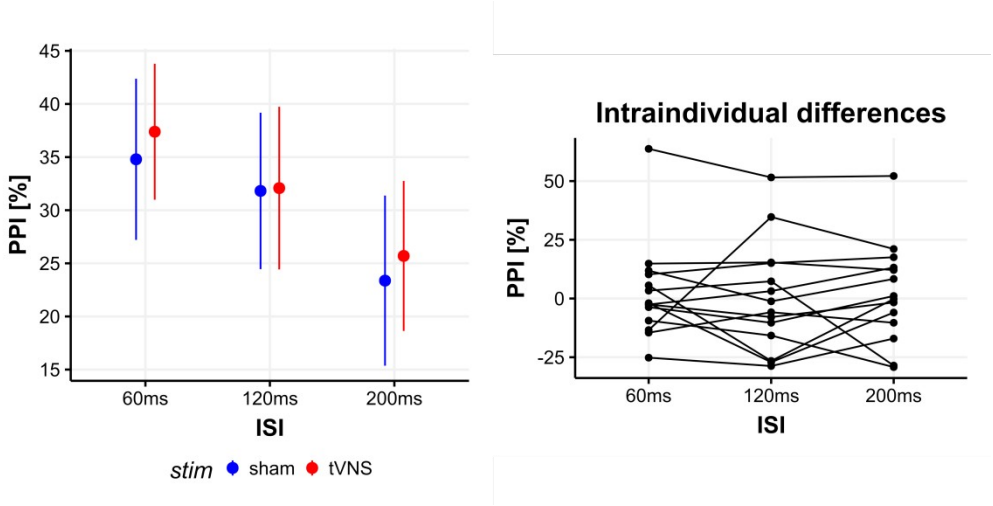

Figure 17. Mean ± SEM of PPI in the three trial conditions (ISI between prepulse and startle pulse 60 ms, 120 ms, and 200 ms) in sham and tVNS sessions. The right panel shows intraindividual (tVNS-sham) differences in PPI for the three ISIs and fourteen participants.

Discussion

We investigated effects of tVNS on prepulse inhibition of the startle (PPI), operationalized as attenuation of eyeblink responses to startle stimuli in the presence of a prepulse compared to startle responses without prepulses. We did not find evidence for PPI modulations through tVNS.

The neural mechanisms of PPI are complex, and it has not been linked to one neurotransmitter system in particular. Nonetheless, it was found to be sensitive to pharmacological manipulations of norepinephrine, GABA, and other neurotransmitters (Fendt et al., 2001; Geyer et al., 2001; Kumari et al., 2002; Phillips, Langley, et al., 2000; Yamashita et al., 2006), which rendered a modulation through tVNS plausible.

From a neuropsychological point of view, a modulation of PPI through tVNS would have been of interest, since PPI has been conceptualized as a preattentive mechanism, and relationships between PPI and measures of executive functions have been found (Bitsios & Giakoumaki, 2005; Dawson, Schell, Swerdlow, & Filion, 1997; Larrauri & Schmajuk, 2006; Oliveras et al., 2015).

As potential limitations should be noted that this study had a small sample size. Moreover, the duration of electrical stimulation was shorter than in the other studies described in this thesis, and tVNS was administered to the tragus rather than the cymba

conchae. However, the latter should not have a major influence on stimulation effects (Yakunina et al., 2018).

6.3 MEG brain oscillations as a tVNS biomarker

Abstract

Physiological and behavioral effects induced through transcutaneous vagus nerve stimulation (tVNS) are under scrutiny in a growing number of studies, yet its mechanisms of action remain poorly understood. One candidate mechanism is a modulation of γ-aminobutyric acid (GABA) transmission through tVNS. Two recent behavioral studies suggest that such a GABAergic effect might occur in a lateralized fashion, i.e., the GABA modulation might be stronger in the left than in the right brain hemisphere after tVNS applied to the left ear. Using magnetoencephalography (MEG), we will test for GABA-associated modulations in resting and event-related brain oscillations. Using source reconstruction, we will further test for a lateralization of effects.

Introduction

Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive brain stimulation technique that has received increasing attention in recent years. It has been introduced as a non-invasive alternative to direct or invasive vagus nerve stimulation (iVNS) (Ventureyra, 2000). Clinically, it is effective as an adjunct therapy for pharmacoresistant epilepsy (Bauer et al., 2016; He, Jing, Zhu, et al., 2013; Stefan et al., 2012) and pharmacoresistant depression (Fang et al., 2016; Trevizol et al., 2015). Furthermore, it has been suggested as a prospective treatment for a variety of conditions, including chronic headache (Barbanti et al., 2015; Magis et al., 2013), tinnitus (Lehtimäki et al., 2013), post-operative cognitive dysfunction (Xiong et al., 2009), cerebral ischemia (Lu et al., 2017), and Alzheimer's disease (Kaczmarczyk et al., 2018). Given this very broad range of potential applications of tVNS, a thorough understanding of its mechanisms of action is highly relevant and necessary for its future clinical investigation and therapy development.

As of now, no single mechanism of action for tVNS has been pinpointed. However, it is consistently found that the locus coeruleus-norepinephrine (LC-NE) system is activated through both iVNS and tVNS (Assenza et al., 2017; Badran, Dowdle, et al., 2018; Raedt et al., 2011). One of several other candidate mechanisms of action is an increase in γ-

aminobutyric acid (GABA) transmission in the brain (Ruffoli et al., 2011; Walker et al., 1999; Woodbury & Woodbury, 1991), mediated through the LC-NE system (Berridge & Waterhouse, 2003). In support of this, it has been found that $GABA_A$ receptor density was increased in patients after receiving long-term iVNS (Marrosu et al., 2003). Moreover, GABA concentration in the cerebrospinal fluid of patients receiving iVNS was increased (Ben-Menachem et al., 1995; Carpenter et al., 2004). The number of studies specifically investigating the relationship between tVNS and GABA transmission, however, is limited. Short-term (~1h) tVNS in healthy subjects modulated cortical excitability (Capone et al., 2015) as well as automatic motor inhibition (Keute, Ruhnau, Heinze, et al., 2018), both of which are highly correlated to GABA concentration in the motor cortex as measured by magnetic resonance spectroscopy (Boy, Evans, et al., 2010; Stagg et al., 2011).

Interestingly, both studies (Capone et al., 2015; Keute, Ruhnau, Heinze, et al., 2018) suggest a possible lateralization of the tVNS effect, in that GABA-associated parameters were modulated in the right, but not in the left brain hemisphere. Similarly, effects of iVNS on the electroencephalogram (EEG) spectrum have been found that were stronger in the right hemisphere (Marrosu et al., 2005). Since both iVNS and tVNS are almost exclusively administered to the left ear / vagus nerve, these findings are compatible with a selective or stronger GABAergic effect of t-/iVNS in the contralateral hemisphere. However, none of those studies explicitly tested for a lateralization of effects.

Brain oscillations as measured by EEG or magnetoencephalography (MEG) often have specific relationships to local GABA concentrations and can therefore be used as biomarkers: Pharmacological increases of systemic GABA levels are consistently associated to increases in beta power at rest (Greenblatt et al., 1989; Hall et al., 2009; Nutt et al., 2015; van Lier, Drinkenburg, van Eeten, & Coenen, 2004). Furthermore, GABA concentration in the motor cortex is related to peri-movement beta and gamma power modulations (Gaetz, Edgar, Wang, & Roberts, 2011; Muthukumaraswamy et al., 2013), and GABA concentration in the visual cortex is related to gamma power responses to visual stimulation (Edden, Muthukumaraswamy, Freeman, & Singh, 2009; Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009).

This study will use MEG to capture brain oscillations associated to GABA transmission. Using brain oscillations as a marker for GABA has several advantages: the combination of resting and event-related oscillations outlined above has a very specific relationship to GABA. MEG allows to record from the whole brain simultaneously at a good temporal and spatial resolution, and to spatially reconstruct sources of specific signals in the brain, which will be helpful to capture a possible lateralization of tVNS effects.

In fact, a recent study found that cervical tVNS increased beta and gamma power and decreased theta and alpha power (Lewine et al., 2018). Moreover, invasive stimulation of the nucleus of the solitary tract (NTS) in cats increased beta power (Martínez-Vargas, Valdés-Cruz, Magdaleno-Madrigal, Fernández-Mas, & Almazán-Alvarado, 2017). The NTS is one of the neural targets of vagus nerve stimulation (Clancy et al., 2013).

We hypothesize that tVNS will increase GABA concentration, leading to GABA-associated MEG alterations. Specifically, our first set of hypotheses relate to overall GABAergic modulation through tVNS:

H_1: resting-state beta power is increased during tVNS compared to sham.

H_{2A}: peri-movement beta desynchronization (PMBD) is stronger during tVNS compared to sham.

H_{2B}: post-movement beta rebound (PMBR) is weaker during tVNS compared to sham.

H_3: gamma power response to visual stimulation is stronger during tVNS.

Furthermore, we hypothesize that the effects from H_1 and H_2 are lateralized, i.e., stronger in the brain hemisphere contralateral to the stimulation.

H_4: The tVNS effect on resting-state beta power will be stronger in the right (contralateral) hemisphere.

H_{5A}: The tVNS effect on PMBD will be stronger in the right (contralateral) hemisphere for left-hand responses compared to PMBD in the left hemisphere for right-hand responses.

H_{5B}: The tVNS effect on PMBR will be stronger in the right (contralateral) hemisphere for left-hand responses compared to PMBR in the left hemisphere for right-hand responses.

Methods

General procedure

Upon arrival, written informed consent will be obtained from each participant. Participants will be reimbursed with money (8 €/hr) or course credit. Head landmarks and head shape will be digitized using a Polhemus Fastrak digitizer (Polhemus[36]). The stimulation electrodes will be attached (see below), and the participant will be seated inside the MEG device. The following procedure is sketched in Figure X: A 3-minute baseline MEG measurement will be carried out, with the instruction for the participant to relax, not to think about anything in particular, keep the eyes open and blink, cough, and move only during stimulation, as far as possible. Subsequently, electrical stimulation will be administered for 30 minutes with a 30s ON / 30s OFF cycle, during which the participant has no specific instruction. After pre-stimulation, resting MEG will be obtained for 3 minutes in total, with the same instruction as before. To avoid contamination of MEG data with artefacts from the electrical stimulation, only data from the OFF epochs will be analyzed (6 epochs of 30 s). Afterwards, six blocks (30s) of the motor task and six blocks of visual stimulation will be carried out. This procedure will be the same for sham and tVNS sessions, with the only difference being the stimulation site (cymba conchae / tVNS vs. scapha / sham). All experimental procedures will be carried out in accordance with the declaration of Helsinki and have been approved by the ethics committee of the medical faculty at the University of Magdeburg.

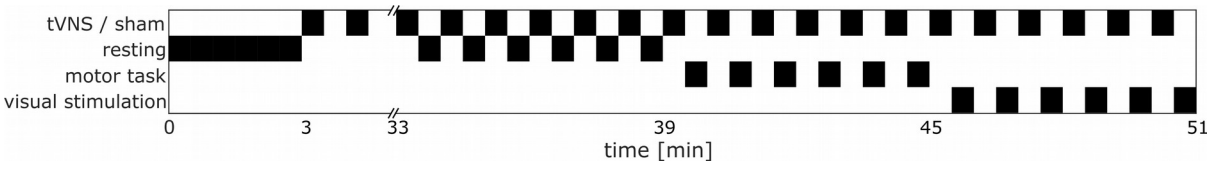

Figure 18. Experimental procedure.

[36] www.polhemus.com

Participants

The experiment will initially be carried out with 40 healthy young participants and will be increased, if necessary (see analysis plan below). Each participant will undergo sham and tVNS stimulation in pseudo-randomized order on separate days. Sham and tVNS measurements for each participant will be scheduled at least 48 hours apart and at the same daytime (± 1h). We will invite participants between 18 and 30 years of age, males and females in approximately equal proportions, who are free from any current or past neurological or psychiatric diseases and regular drug intake (both medical and recreational, except for oral contraceptives), have normal or corrected-to-normal vision and are eligible for tVNS, MEG and MRI (in particular, no cardiac pacemakers or metal implants in or close to the head).

Motor task

Peri-movement beta power will be assessed using a cued finger movement task. Participants will be instructed to press a button with their left or right index finger, according to the direction of an arrow displayed centrally on the screen (displayed in black on a grey background, width 1 degree, height 0.5 degree of visual angle). During each 30 s block, 4 left-pointing and 4 right-pointing arrows will be presented in pseudo-randomized order, with stimulus durations of 200 ms and a randomly jittered inter-stimulus interval between 3 and 3.5 s. A red fixation point will be visible on the center of the screen throughout the task to prevent eye movements.

Visual stimulation

Visual stimuli will be stationary, vertical circular gratings with a spatial frequency of 3 cycles per degree and maximum contrast. Throughout the experiment, a central fixation dot will be visible. The screen background will have the average luminance of the gratings. Stimuli will be presented centrally on the screen and subtend 2 degrees of visual angle. In each 30 s block, eight gratings will be presented for 1 s, followed by a jittered inter-stimulus interval between 2 and 2.5 s. This stimulus design is similar to the one used by Muthukumaraswamy et al. (2009).

Electrical stimulation

TVNS will be administered to the cymba conchae, sham stimulation to the scapha of the left ear. Two medical Ag/AgCl stimulation electrodes (4×4 mm) will be mounted on a piece of silicone at a center-to-center distance of 1 cm. The electrodes will be attached to the ear using a small amount of adhesive electrode cream (Natus Neurology[37]) and medical adhesive tape, if necessary. Direct current pulses will be delivered using a medical stimulation device (Digitimer DS7[38]). Current intensity will be set to 1 mA, delivered in 200 µs pulses at 25 Hz. Stimulation will be administered for 30 s, followed by 30 s break, etc. These parameters are within the range of standard parameters used in other tVNS studies (Badran, Dowdle, et al., 2018; Frangos et al., 2015).

MEG measurement and analysis

MEG will be recorded from 306 sensors (102 magnetometers and 204 planar gradiometers) from 102 head positions using a Neuromag Triux device (Elekta AB[39]) at a sampling rate of 1000 Hz and an online band-pass filter (0.01 - 330 Hz). Offline data analysis will be carried out using the FieldTrip toolbox (Oostenveld et al., 2011) in Matlab 2018 (MathWorks[40]). Bad sensors (high noise level or flat) will be identified by visual inspection, removed from the data and, for data visualization only, reconstructed using spline interpolation. Severely artifact-laden epochs will be excluded from further analysis, based on visual inspection. Ocular artifacts will be removed by means of independent component analysis (ICA). Data will be visually inspected again, and segments with remaining gross artifacts will be excluded. Participants will be excluded from further analyses if more than half of the epochs in the motor task or more than half of the visual stimulation epochs or half of the resting-state recording time have to be excluded, or if they have no clear PMBD, PMBR, or visual gamma response, based on visual inspection and running t-tests against baseline, in one or both sessions.

Subsequently, MEG data will be transformed to source space using linearly constrained minimum variance (LCMV) beamforming, resulting in source level epochs (Lithari, Sánchez-García, Ruhnau, & Weisz, 2016; Neuling et al., 2015). Briefly, individual

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structural magnetic resonance images will be aligned to the MEG space with the information from the head shapes. Then an equally spaced 1 cm grid in MNI space will be warped to the individual brain volume. Using this MNI space grid (~3000 voxels) allows for direct statistical comparisons of activity across participants. The aligned brain volumes will be further used to create single-sphere head models and lead field matrices (Nolte, 2003). Together with the head model, the lead field matrix and the average covariance matrix beamformer filters for each grid point will be calculated. These filters will subsequently be multiplied with the sensor level epochs resulting in source level epochs.

A time-frequency analysis of source level data will be carried out using Morlet wavelets. Center frequencies will be logarithmically spaced between 1 and 64 Hz in steps of 0.125 octaves at a frequency resolution $f/\sigma_f = 6$, moving along the signal in steps of 50 ms. Resulting power estimates will be baseline-normalized and converted to dB [$10*\log_{10}$ (Power / $\text{Power}_{\text{baseline}}$)]. For the resting-state measurement, the 3 minutes measurement prior to electrical stimulation will serve as baseline. For the motor task, pre-movement beta desynchronization (PMBD) and post-movement beta rebound (PMBR) will be assessed by subtracting $\log_{10}$-transformed source-space power in the contralateral motor cortex across the beta band (15-30 Hz) and over a time window between -1.25 – 0.5 s relative to the button press (for PMBD) or between 1 – 1.75 s (for PMBR) from time-averaged log-power over the entire trial (-1.25 – 1.75 s). For the visual stimulation, we will use a baseline of -1 – 0 s relative to stimulus onset and compare it to the presentation time of the stimuli (0 – 1 s). For the analysis of resting and movement-related beta power, we will average the baseline-corrected log-power values over beta frequencies (15 – 30 Hz), for the analysis of gamma power, we will average over gamma frequencies (30 – 60 Hz). For event-related data from the motor task and visual stimulation, we will additionally average over time bins and trials. To test for lateralization of tVNS effects, we will compute lateralization indices as differences between resting beta log-power in the left and right hemisphere, and between PMBD and PMBR to left- and right-hand movements in the contralateral motor cortex, respectively. Resulting session-wise values for resting beta power, PMBD, PMBR, visual gamma response, and lateralization indices will be compared between sham and tVNS sessions by means of paired-sample one tailed Bayesian t-tests using R and the BayesFactor package (Morey, Rouder, & Jamil, 2015). Based on

previous literature, we expect log_{10}-transformed spectral power values to have approximately normal distributions (Kiebel, Tallon-Baudry, & Friston, 2005), so that we will use Gaussian likelihood functions and weakly regularizing Gaussian priors.

Design analysis and interpretation plan

A recent study, though in a small sample, found that cervical tVNS increased beta and gamma power and decreased theta and alpha power (Lewine et al., 2018). This study reports, for the comparison between baseline-normalized beta power in the tVNS vs. sham condition, a t-value of 2.64, which, given a sample size of 8 subjects in a within-subjects design, corresponds to an effect size of $d_z \sim 0.93$. Effects of similar magnitude have been found for peri-movement beta oscillations 3h after administration of 15mg tiagabine ($d_z \sim 0.81$, Muthukumaraswamy et al., 2013), and for alpha power following transcranial alternating current stimulation ($d_z \sim 0.86$, Zaehle, Rach, & Herrmann, 2010). Given a possible publication bias, we have a more conservative expectation to find effect sizes $d_z \sim 0.5$ for all our hypotheses. A simulation-based Bayes factor design analysis (Schönbrodt & Wagenmakers, 2018) found that given $d_z = 0.5$ and $n = 40$, Bayes factors conclusively favored the working hypothesis ($BF > 6$) 76.5% of the time for the simulated data. If necessary, sample size will be increased until Bayes factors clearly favor either the null or working hypothesis for all hypotheses, up to a total sample size of 60 participants (120 experimental sessions), which we consider the maximum number of participants that is technically and economically feasible.

If all of hypotheses H_1-H_3 would be confirmed, we would interpret this as a confirmation for an overall increase in GABAergic activity induced through tVNS. Conversely, if all respective null hypotheses would be confirmed, we would conclude that tVNS has no effect on GABAergic activity in healthy individuals. If only some of the hypotheses were confirmed, we would conclude that tVNS has regionally or functionally selective effects on GABAergic activity. The strength of this conclusion would depend on whether or not tests for the non-confirmed hypotheses would have conclusive results (in favor of the respective null hypotheses).

Likewise, confirmation of hypotheses H_4-H_5 would lead us to the conclusion that GABAergic modulation through tVNS occurs in a lateralized fashion, and a partial confirmation to the conclusion that lateralization is functionally specific.

In case we reach the specified maximum sample size without obtaining conclusive results, we will run additional explorative analyses (e.g., cluster analyses) to check whether we can identify responder and non-responder subpopulations.

Pilot data acquisition and analysis

We used EEG data from 15 participants recorded during a subliminal response priming experiment with one sham and one tVNS session per participant (experiment reported in detail in chapter 2 and in Keute et al., 2018) to carry out preliminary analyses for movement-locked beta changes (PMBD / PMBR). Electroencephalogram (EEG) was recorded from four Ag/AgCl scalp electrodes (Fz, C3, C4, Pz), referenced to the right earlobe, at a sampling rate of 1 kHz using a BrainAmp amplifier and BrainVision Recorder software (BrainProducts[41]). EEG data were analyzed using FieldTrip (Oostenveld et al., 2011). Raw data were bandpass (0.3 – 40 Hz) and notch (48.5 – 51.5 Hz) filtered and cut into segments, time-locked to responses (-3500 – +3500 ms). Segments containing gross artifacts were excluded based on a ±80 µV threshold criterion. Only trials with compatible primes and correct responses were used for spectral analysis, which was carried out using Fast Fourier Transformation (FFT) over Hann-tapered time windows of 500 ms length that moved along the segments in steps of 100 ms. FFT was performed for frequencies from 2 to 36 Hz in steps of 2 Hz. For beta band analyses, we averaged over frequencies from 14 to 26 Hz. For the analysis of movement-related beta changes over the left (electrode C3) and right (electrode C4) hemisphere, we used data segments containing responses of the respective contralateral hand. Spectral power values were log-transformed, and beta log-power was baseline corrected to the inter-trial interval (-750 to -250 ms pre-prime).

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Pilot Data

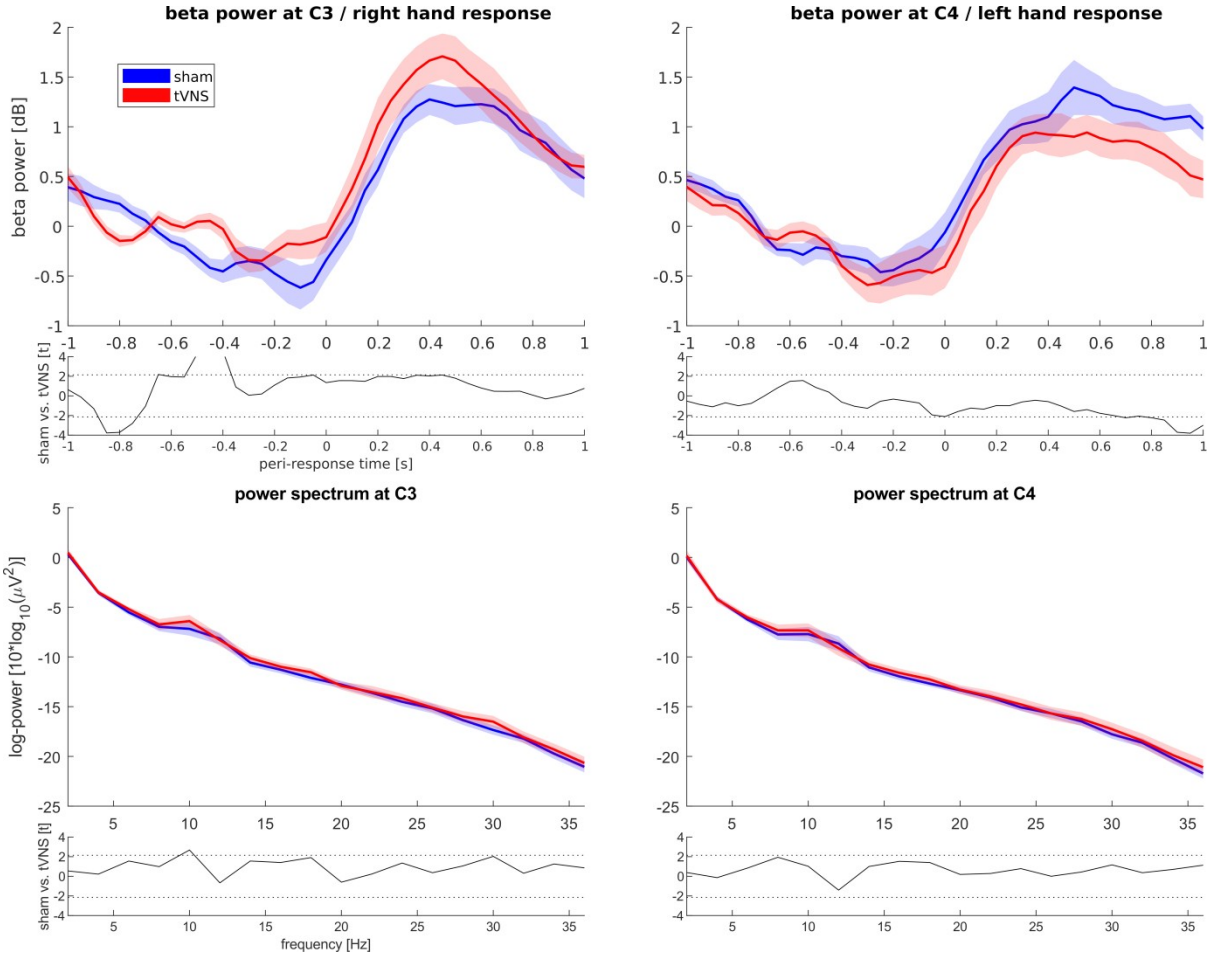

Figure 19. Upper row: Response-locked beta power, corrected to pre-prime baseline, for electrodes C3 / right hand responses (left) and C4 / left hand responses (right) from trials with compatible priming. Lower row: time-averaged power spectra, computed across response-locked epochs (right and left hand responses) for electrodes C3 (left) and C4 (right). Small panels below show paired-sample t-values comparing sham and tVNS, dashed lines indicate $t = \pm 2.14$, the threshold for uncorrected statistical significance in a two-tailed t-test at $\alpha = .05$ and df = 14.

The analysis of movement-related beta changes (Figure 19., upper row) revealed that there was a trend towards decreased PMBD and increased PMBR at electrode C4, i.e., contralateral to the stimulated ear, whereas this pattern tended to be reversed at electrode C3, i.e., ipsilateral to the stimulated ear. Even though inspection of t-value time courses (Figure 19., upper row, small panels) suggests that these trends would probably not survive rigorous statistical analysis, their direction is consistent with our hypotheses and a replication in a larger sample and with a more tailored experimental design might yield statistical approval of a lateralized effect of tVNS on peri-movement beta changes.

Inspection of full time-averaged power spectra of the response-locked data segments (Figure 19., lower row) revealed that power at beta frequencies was, numerically, slightly higher in tVNS compared to sham sessions, but the difference did not approach statistical significance at either electrode. However, these data were recorded during task execution, not during rest. A recent study using cervical tVNS (Lewine et al., 2018) did show increased beta power at rest during tVNS compared to sham, consistent with our hypothesis.

In sum, our preliminary analyses, together with a recent resting-state EEG study (Lewine et al., 2018), bear promise that data from the proposed MEG study will confirm the hypotheses outlined above and thus enable further development of tVNS biomarkers based on brain oscillations.

6.4 VSEP: another candidate biomarker

Introduction

Evoked potential measurement is an established diagnostic tool in neurology (Cruccu et al., 2008). Evoked potentials can be defined as the brain's response to an external stimulus, typically measured by EEG (Chiappa & Ropper, 1982). The family of evoked potentials includes, among others, auditory- and visual-evoked, and, as a relatively new member, vagus-sensory evoked potentials (VSEP). VSEP are fast deflections of the EEG signal in response to tVNS pulses comprising three deflections (P1, N1, P2, see Figure 20.) that occur within ~10 ms, measured at ipsilateral electrodes relative to the stimulation (Fallgatter et al., 2003). Based on the similarity between auditory evoked potentials and VSEP, particularly the short latencies, it is argued that the latter are a direct index of vagus nerve activity, or excitability of the vagus nerve projection areas (NTS and LC), rather than being generated by upstream brain areas (Fallgatter et al., 2003).

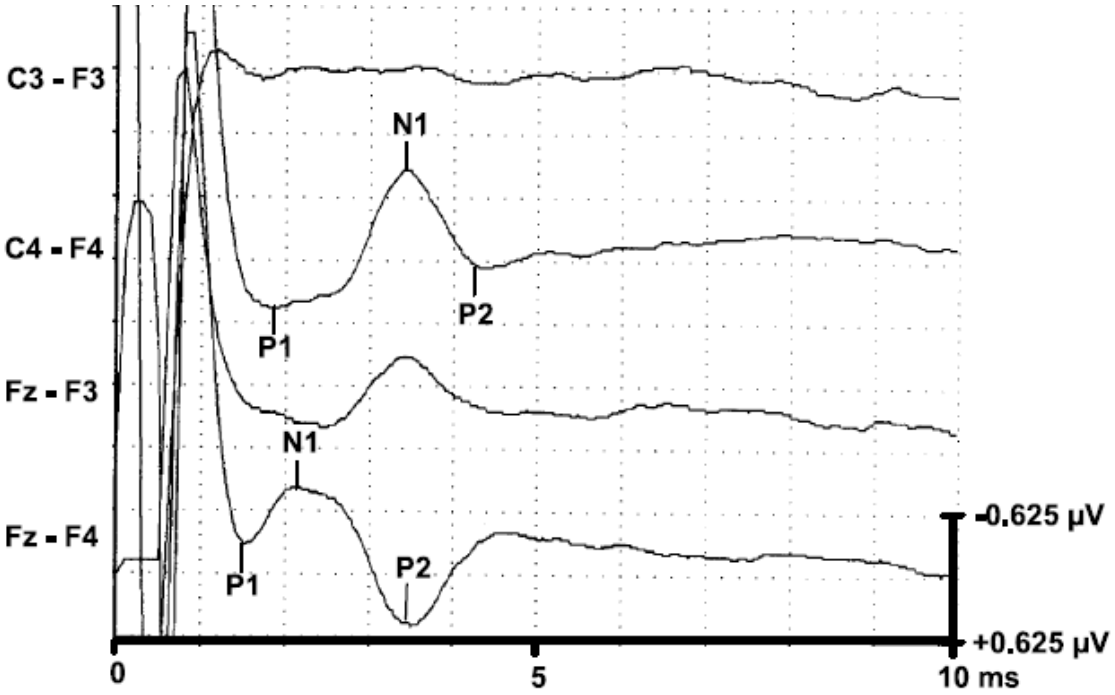

Figure 20. Exemplary VSEP. The three components constituting the VSEP (P1, N1, P2) can be clearly seen at electrodes C4-F4 and Fz-F4. Note that in this study, electrical stimulation was administered to the right ear, i.e., components are visible at ipsilateral electrodes. Reprinted from Fallgatter et al. (2003).

The latencies of VSEP components have been found to increase with healthy aging (Fallgatter, Ehlis, Ringel, & Herrmann, 2005), Alzheimer's disease (Polak et al., 2007), subjective memory impairment (Hagen et al., 2015), and Parkinson's disease (Polak et al., 2011). The aim of this additional pilot study was to replicate VSEP in healthy young adults and to explore relationships between VSEP and effects of tVNS on pupil size readouts, as reported in study 4.

Methods

VSEP were obtained from 12 healthy volunteers, who were a subset of the participants in study 4, in a separate session. Participants were seated comfortably in a dimly lit, air-conditioned room. They received money (8€/h) or course credit as a reimbursement. All procedures were carried out in accordance with the Declaration of Helsinki and approved by the ethics committee of the medical faculty at the University of Magdeburg.

Electrical stimulation was applied to the cymba conchae, while EEG was measured bipolarly from three pairs of scalp electrodes (Fz-F3, C3-F3 and C4-F4 according to the international 10-20-system). A saline-soaked band electrode placed around the head, directly above the ears, served as ground electrode. Participants received 200 stimulation pulses with alternating polarity, at a pulse width of 100 µs and 8mA current intensity, applied to the cymba conchae of the left ear. Inter-pulse interval was randomly jittered between 1 and 1.1 s. EEG was recorded at a sampling rate of 30 kHz with an online bandbass filter (0.3 to 7500 Hz) using a Neuroport amplifier (Blackrock Microsytems[42]). The measurement protocol was designed based on previous literature (Fallgatter et al., 2003) and in-house clinical experience. For offline data analysis, EEG data were notch filtered to remove line noise, and subsequently cut into segments of 100 ms length, time-locked to the peaks of stimulation pulses. Segments were z-normalized by subtracting the mean potential calculated across a pre-pulse baseline (-60 to -10 ms) and dividing by its standard deviation. Segments containing gross artifacts were excluded by visual inspection, based on intra-trial variance. Identification of VSEP components was based on visual inspection.

[42] www.blackrockmicro.com

Results

Visual inspection revealed that the overall data quality was highest, and unambiguous potential deflections were most clearly visible, at C3-F3. Averaged across participants, three deflections following stimulation pulses could be identified (N1-P1-N2 in Figure 21.). Their latencies (N1 ~1.7 ms, P1 ~4.4 ms, N2 ~5 ms) were comparable to previous reports (Fallgatter et al., 2005; Hagen et al., 2015), but their polarity was reversed, i.e., the first deflection following the pulse was negative.

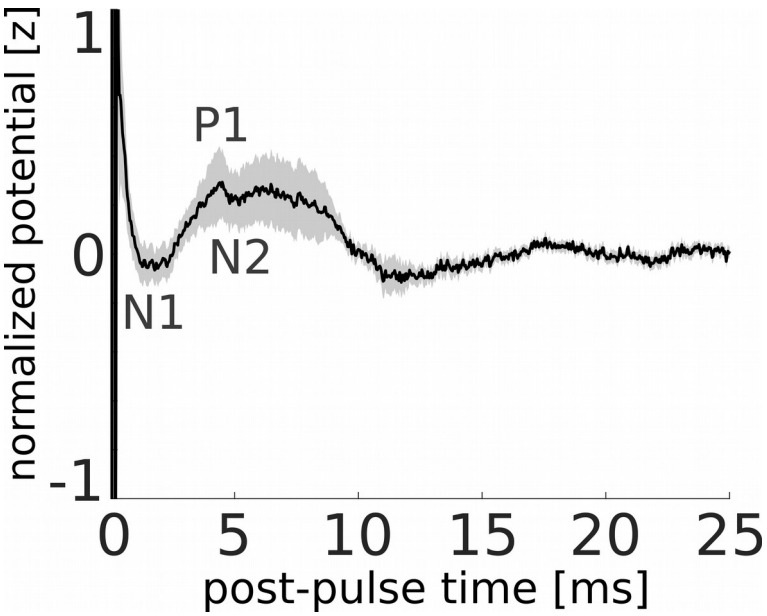

Figure 21. Grand average VSEP from electrode C3-F3. Mean ± standard error over subjects.

Inspection of individual VSEP (Figure 22.) confirmed that for 10 out of 12 subjects, VSEP components could be identified with reversed polarity (N1-P1-N2), whereas whereas for two subjects, only one positive deflection could be unambiguously identified (S2 and S10).

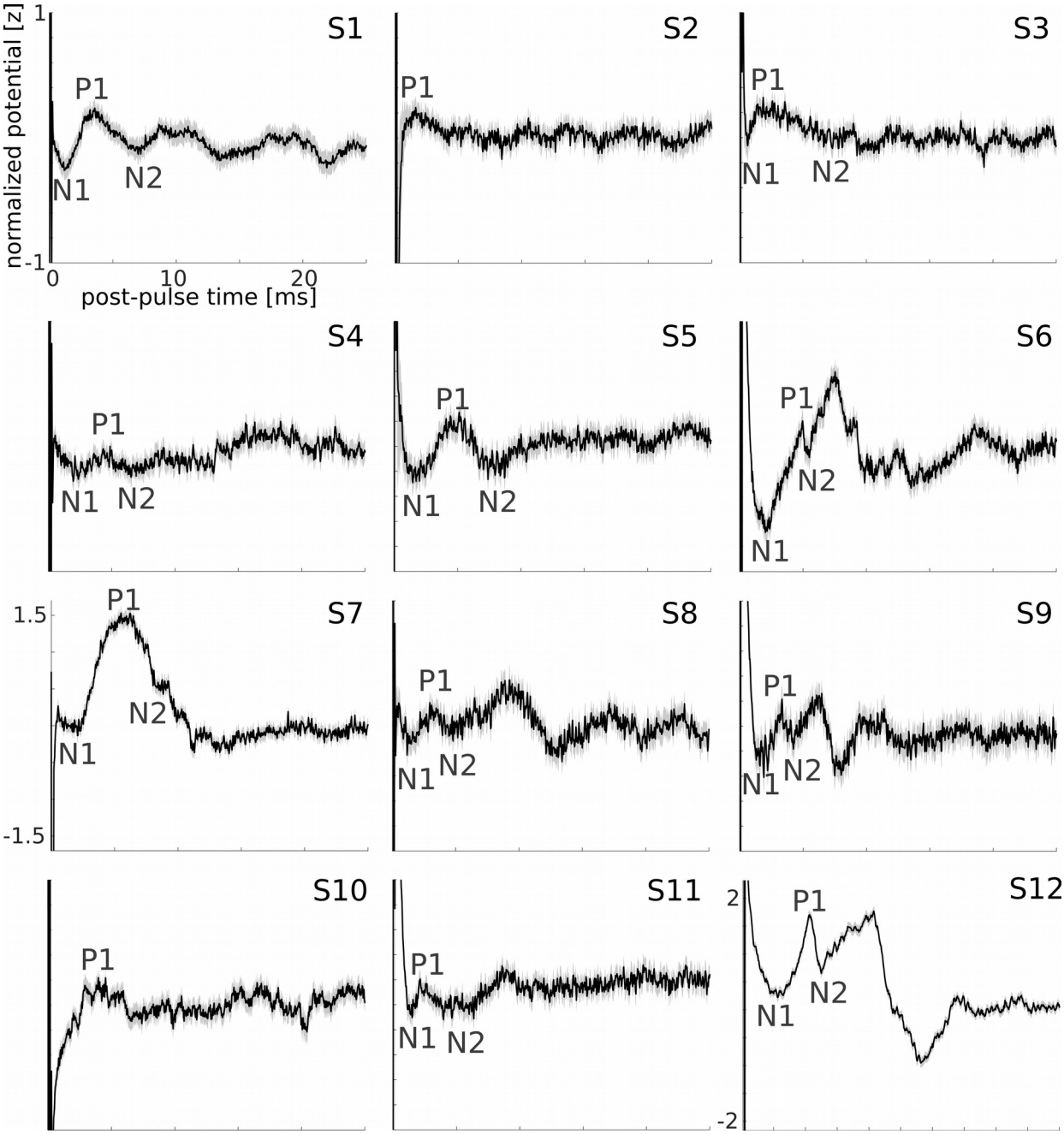

Figure 22. VSEP from 13 individual participants from channel C3-F3. Mean ± standard error over repeated measurements. Y-Axes range from z = -1 to z = 1 unless labeled otherwise.

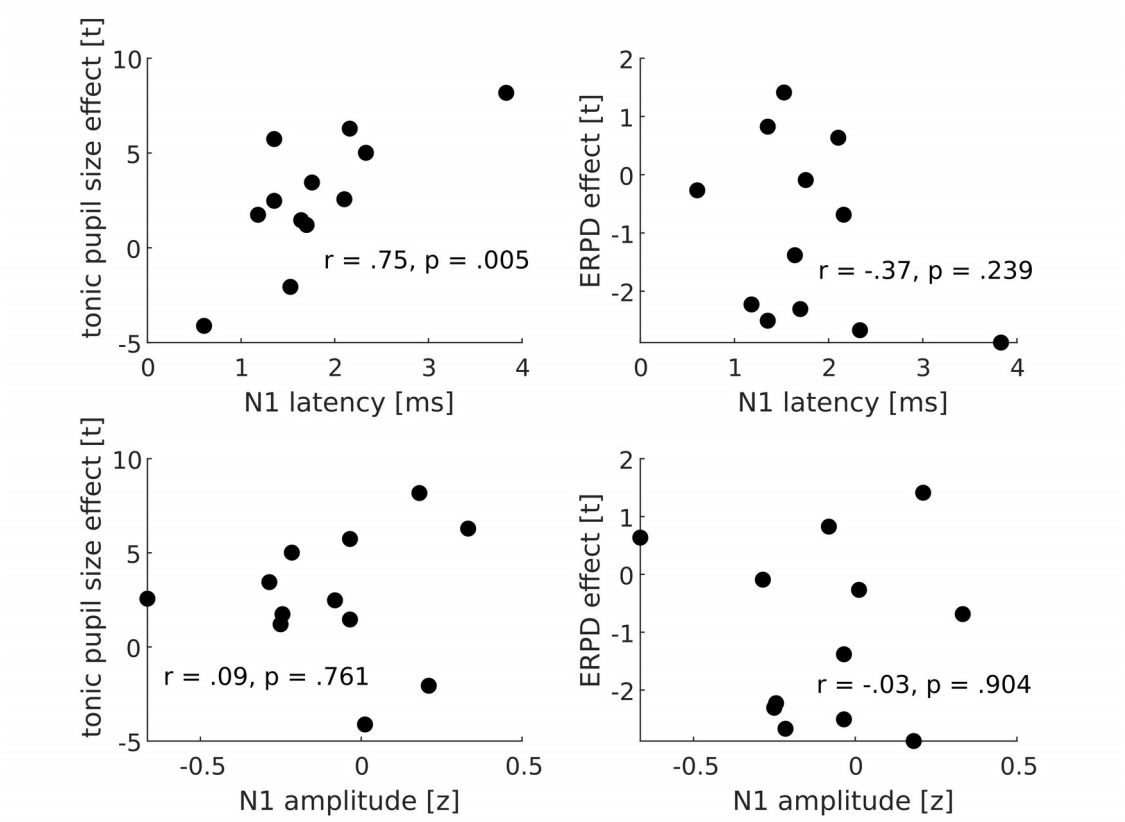

Figure 23. Relationships between VSEP and tVNS effects on pupil size measures. Note that the x-axis always indicates the latency or amplitude of the first post-pulse deflection, which is P1 rather than N1 for two subjects (S 2 and S10).

Relationships between the amplitude and latency of the first VSEP component (N1, or, for two subjects, P1) on the one hand and the t-values comparing pre-tVNS to on-tVNS pupil size or ERPD on the other hand are shown in Figure 23.. N1 latencies were positively correlated to the effect of tVNS on tonic pupil size. No other significant correlation between either VSEP component and either tVNS effect was found.

Discussion

We explored VSEP as an explanatory variable for tVNS effects on pupil size measures, as described in study 4. We had no clear initial hypothesis about the relationship between VSEP and tVNS effects on pupil size measures. If VSEP reflect the excitability of the NTS and LC through the vagus nerve, as hypothesized (Fallgatter et al., 2003), it is conceivable that subjects with stronger VSEP deflections, or shorter latencies, also show stronger effects of tVNS on pupil size readouts. However, the evidence for the hypothesis that VSEP components reflect vagus nerve activity or excitability of vagus projection areas is sparse and rather indirect. Our data do not provide compelling

evidence for such a relationship. With respect to N1 latency, we found a positive correlation with tVNS effect on tonic pupil size, i.e., slower N1 components were associated with a higher tVNS effect. This effect is difficult to interpret, since its direction is counterintuitive. One could speculate that slower VSEP components reflect a lower baseline activity of the vagus nerve or its projection areas, and that subjects with higher N1 latencies thus profit more from tVNS. However, our data are of highly preliminary character and do not allow for any strong conclusions. On the other hand, we have replicated VSEP measurement as described in the literature, albeit with reversed polarity. Considering that previous VSEP literature is very sparse and stems from only a small circle of authors, our findings are of value since they provide one of the first independent replications of VSEP. Provided that future research can further elucidate the open questions regarding the optimal measurement, neural origin, functional and clinical significance of VSEP, they may become established as a useful diagnostic tool in neurology, and potentially as a predictive biomarker for tVNS responsiveness.

7 General discussion

7.1 Summary

In this thesis, I have described several studies concerned with the *neuropsychology of tVNS*. All studies were carried out in healthy young adults, who received auricular tVNS and sham stimulation in within-subject experimental designs, the underlying goal being to assess the potential of tVNS to modulate neuropsychological functions and how this potential might be exploited in future research as well as clinical and therapeutic practice.

The first study investigated behavioral and electrophysiological consequences of tVNS on automatic inhibition in the motor cortex. Consistent with our hypothesis, we found a modulation of automatic motor inhibition and lateralized readiness potentials for left-hand responses and over the right motor cortex, which suggests a GABAergic modulation in the motor cortex induced by tVNS. This modulation was not found in the right motor cortex, which suggests a possible lateralization of this GABAergic modulation.

The second study focused on effects of tVNS on the dynamics of visual bistable perception, indexing GABAergic modulation in the visual system. We found no effect of tVNS on percept stability. The negative result was supported by Bayesian inference and Bayes factor model comparison.

The third study was not based on functional correlates of a particular neurotransmitter system, but had a more functional focus instead. We showed that tVNS enhanced global behavioral accuracy and reduced the cost of go/change conflicts on behavioral performance in a cued go-nogo-change task. Electrophysiologically, tVNS increased frontal midline theta activity during go/stop conflicts. We concluded that tVNS can interact with the neural mechanisms underlying conflict monitoring and executive control of action, and therefore its further investigation as a clinical and subclinical treatment for executive control deficits is promising.

The fourth study used pupillometry as a non-invasive marker of LC-NE activation. LC-NE activation is assumed to be a key mechanism of action of tVNS. This study aimed to provide direct physiological evidence for a modulation of LC-NE activity by tVNS, and

to assess the potential of pupillometry as a prospective biomarker for tVNS responsiveness and efficacy. Contrary to our hypotheses, we did not find any systematic effects of tVNS, neither on pupil size nor event-related pupil dilation during an auditory oddball task. The negative result was supported by model comparisons based on information criteria.

Chapter 6 contained three additional short reports. The first one described a pilot study assessing the effect of tVNS on prepulse inhibition (PPI) of the acoustic startle reflex. PPI occurred across sham and tVNS sessions, and no modulation by tVNS was found. The second short report described a study proposal. Since study 1 had supported GABAergic neuromodulation in the motor cortex, whereas study 2 did not imply GABAergic neuromodulation in the visual cortex, the proposed study aims to further eludicate the question of GABAergic neuromodulation induced by tVNS, differences between brain regions, and a possible lateralization of GABAergic neuromodulation in the motor cortex, using brain oscillations as measured by MEG. The third short report explored the potential of VSEP as a biomarker for tVNS effects on pupil size measures as reported in study 4. We could replicate similar VSEP as in previous literature, albeit with reversed polarity. We found that the latency of the first VSEP component was positively correlated with the tVNS effect on tonic pupil size in study 4, but concluded that a better fundamental understanding of VSEP is necessary before it might prospectively serve as a tVNS biomarker.

7.2 Integration of findings

The overarching research question of this thesis, and the range of neuropsychological functions investigated and methods applied in the above studies, are relatively broad, which is partly due to the fact that empirical knowledge about the mechanisms of action of tVNS is rather vague as of now – effects on central neurotransmission and on peripheral-autonomic nervous activity have been postulated and partially confirmed in empirical studies, as detailed in chapter 1, but a comprehensive, empirically founded model of tVNS mechanisms is pending.

In studies 1 and 2, my aim was to probe neuropsychological consequences of modulations in GABA transmission induced by tVNS. Results of study 1 supported a

GABAergic modulation in the motor cortex contralateral to the stimulated ear, whereas study 2 provided no support for GABAergic modulation in the visual cortex. Moreover, study 1 suggested a possible lateralization of GABAergic modulation in the motor cortex through tVNS, in line with a previous study (Capone et al., 2015).

In chapter 6.2, I described a pilot study that found no effect of tVNS on PPI. Functionally, PPI has some similarity with automatic motor inhibition, which was investigated in study 1: both processes can be conceptualized as noise suppression mechanisms that inhibit behavioral reactions to weak sensory stimuli (prepulses or masked primes, respectively), because these stimuli are classified as behaviorally irrelevant, which leads to inhibited reactions to subsequent, stronger and behaviorally relevant, stimuli (startle pulses or target stimuli, respectively) (Sumner & Husain, 2008; Swerdlow, Caine, Braff, & Geyer, 1992). We found no effect of tVNS on PPI, which indicates that the effects of tVNS on automatic motor inhibition do not result from a higher-level modulation of preattentive noise suppression mechanisms, and further supports the interpretation that the effects of tVNS on automatic motor inhibition reflect GABAergic neuromodulation in the motor cortex.

GABAergic effects of tVNS are presumably mediated through the ‘classical’ vagus nerve projection areas, NTS and LC (Jones, 1991; Toussay, Basu, Lacoste, & Hamel, 2013; Walker et al., 1999). A possible explanation for the discrepant results of studies 1 and 2 is that effects of tVNS on GABA transmission are different between the motor and visual cortex. This difference might be based on the respective anatomical connections between the NTS and LC on the one hand and the visual and motor cortex on the other hand. In fact, it has been shown that LC projections can have different physiological properties in different terminal regions (Chandler, Gao, & Waterhouse, 2014). LC projections can have a direct influence on spinal motor output (Fung, Manzoni, Chan, Pompeiano, & Barnes, 1991), whereas in the visual system, they have a role in cortical plasticity (Kasamatsu, 1991). Likewise, it is conceivable that tVNS-induced and LC-/NTS-mediated GABAergic neuromodulation manifests itself differently in the visual and motor cortex. The overall findings from studies 1 and 2 are not fully conclusive with respect to the question of whether tVNS causes GABAergic neuromodulation, and whether this neuromodulation occurs globally or regionally in the brain. Moreover, study 1 suggested a lateralized GABAergic neuromodulation, which

has been suggested before (Capone et al., 2015; Marrosu et al., 2005), but has not been demonstrated in a statistically sound way so far and thus warrants further investigation. These open questions were the starting point for the conceptualization of the research proposal contained in chapter 6.3. The proposed study will set out to clarify the open questions from studies 1 and 2 by using a particular combination of MEG readouts indicative of global, regional, and lateralized GABAergic neuromodulation. These questions, in particular the question of whether tVNS has lateralized effects, are of high relevance for therapeutic applications and might even account for part of the interindividual variability in clinical effects of tVNS – e. g., an epilepsy patient with an epileptic focus in the left brain hemisphere might be expected to profit less from left-ear tVNS, provided that tVNS effects are stronger in the contralateral (right) hemisphere.

Study 4 focused on effects of tVNS on the LC-NE system. No modulation of tonic and event-related pupil size readouts by tVNS was found. It is one of the basic assumptions on tVNS mechanisms that it increases activity in the LC-NE system, and there is converging neurochemical, neuroimaging, and behavioral evidence for this assumption. On the other hand, tonic and event-related pupil size have been shown to be a reliable non-invasive marker of tonic and phasic LC-NE activity. Therefore, the lack of a systematic modulation of tonic pupil and/or event-related pupil size by tVNS is surprising, and the missing link between tVNS and pupil size remains elusive. In the discussion of study 4, I speculated that interactions between LC-NE activation and cholinergic neuromodulation (both induced by tVNS) might account for the negative result. However, as in many previous tVNS studies, there was substantial interindividual variability, and on the within-subject level, some individuals showed highly significant pupil size increases as well as decreases under tVNS. Additional VSEP measurement for a subset of the participants in study 4 (chapter 6.4) suggested that VSEP latencies might be a predictor of an individuals pupillary reaction to tVNS, but the results are highly preliminary and should be interpreted with caution.

In their seminal opinion article, Van Leusden et al. (2015) proposed several behavioral experiments that might provide empirical support for modulations of the noradrenergic, cholinergic, and GABAergic neurotransmitter systems, respectively, through tVNS, and conversely, it is argued in the article that tVNS could be used prospectively to induce experimental neuromodulation and study the role of these neurotransmitters in behavior.

The basic paragidm underlying this thesis, especially studies 1, 2, 4, and the research proposal in chapter 6.3, is similar, in that these studies asked for behavioral and physiological effects of tVNS that are specifically associated to GABA (study 1 and 2) and NE (study 4) modulation. Study 2 has drawn direct inspiration from Van Leusden et al. (2015).

Four years after Van Leusden et al. (2015) asked 'tVNS – a new neuromodulation tool in healthy humans?', I am inclined to respond *probably no*, at least not in such a way that tVNS should be used to study the role of single neurotransmitters in behavior, as suggested in the opinion article. For instance, in a research context where the role of NE for response inhibition is under investigation, an experimental design employing targeted, selective pharmacological stimulation of NE transmission (as in Bari, Eagle, Mar, Robinson, & Robbins, 2009; Chamberlain et al., 2007) is likely to allow for stronger and more specific conclusions on the role of NE compared to experimental neuromodulation using tVNS, which only allows for conclusions on the joint role of NE, GABA, and ACh (as in Beste et al., 2016). Notwithstanding, using tVNS as a neuromodulation tool for research purposes has some obvious practical and ethical advantages over pharmacological neuromodulation, and for certain experimental paradigms, it can, in fact, be argued that effects of tVNS on behavioral and electrophysiologal readouts are likely mediated by one neurotransmitter in particular (Beste et al., 2016; Keute, Ruhnau, Heinze, et al., 2018). However, since recent findings have not always been fully compatible with the assumption that tVNS reliably and selectively increases transmission in these three neurotransmitter systems (e.g., study 4 in this thesis provides no evidence for NEergic neuromodulation), it must be stated that the mechanisms of action of tVNS are still not well understood. Hence, it is currently not advisable to draw strong conclusions from tVNS experiments on the role of a particular neurotransmitter for the behavior under investigation.

The situation is obviously different for studies that explicitly investigate the role of the *vagus nerve* in behavior, as in recent studies probing the polyvagal theory of emotion recognition (Colzato et al., 2017; Sellaro et al., 2018). In these studies, the underlying hypotheses do not focus on a particular neurotransmitter system, but on the vagus nerve itself, therefore, the problems outlined above do not apply and it appears legitimate to use tVNS as a neuromodulation tool. Nonetheless, it might still be advisable in such

studies to ensure consistent vagus nerve engagement across subjects, e.g., by obtaining VSEP or individual effects of tVNS on heart rate variability (Clancy et al., 2014).

In sum, the usefulness of tVNS as a tool for basic neuroscience (e.g., for studying behavioral correlates of NE or GABA transmission) is currently limited by the insufficient understanding of the central and peripheral nervous effects that tVNS entails, and more generally by the fact that tVNS is assumed to modulate several neurotransmitter systems, perhaps in a mutually interacting way. When functional roles of the vagus nerve are under investigation, the use of tVNS appears to be more appropriate. However, the dependence of tVNS effects on stimulation parameters and the determinants of interindividual differences in tVNS responsiveness are even less well understood than general tVNS mechanisms, which poses an additional limitation.

On the other hand, a number of studies have clearly shown that tVNS can ameliorate symptoms of several neurological and psychiatric diseases, and that it can enhance a range of cognitive-behavioral functions in clinical and healthy populations. Thus, in clinical contexts, where functional outcomes are more important than isolated effects on particular neurotransmitter systems, tVNS does bear promise as a neuromodulation tool. Along those lines, study 3 demonstrated the potential of tVNS to enhance aspects of executive control of action, which adds to previous evidence that tVNS is a promising candidate treatment for neuropsychological deficits. Likewise, study 1 also has some relevance to executive control of action and further corroborates the prospective clinical potential of tVNS, since automatic motor inhibition has been shown to interact with interference control (Boy, Husain, & Sumner, 2010).

7.3 Limitations

As detailed in the introduction, interindividual variability in stimulation responsiveness is a major challenge for tVNS research. I have argued that successful future investigation and therapeutic implementation of tVNS will depend on a better understanding of the determinants of this variability, and on the discovery of reliable biomarkers predictive of individual stimulation responsiveness, in order to separate responders from non-responders and to optimally tune stimulation parameters for responders. I have discussed three candidate biomarkers: pupillometry, VSEP, and

MEG brain oscillations. Study 4 failed to demonstrate any systematic modulation of pupil size and event-related pupillary responses through tVNS on the group level, and both other candidate biomarkers are still at a very early stage of investigation. As a consequence of this responder/non-responder problem, the effects of tVNS that have been demonstrated in studies 1 and 3 can only be interpreted on the group level, but for efficient further clinical and therapeutic implementation, it will be highly desirable to be able to predict individual responsiveness, and further research in this field is highly recommendable.

A more fundamental limitation of all experimental research in humans is the inherent data noisiness, which arises from the fact that humans are not laboratory animals raised and kept under controlled conditions, but have individual developmental trajectories, traits and dispositions, both physically and psychologically. Even though human freedom and individuality are very high goods in general, they can severely hamper interpretation of experimental results, especially with the usual small to medium sample sizes in human experimental research, since effects of an experimental manipulation may become fully masked by inter- and intraindividual 'random' fluctuations in behavior. It has often been argued in recent years that many – perhaps most – studies in experimental psychology and adjacent fields are underpowered, make inappropriate use of statistical methods, have low reproducibility, and have a high risk of producing false positive as well as false negative results (Christley, 2010; Higginson & Munafò, 2016; Ioannidis, 2005; Lohse, Buchanan, & Miller, 2016; Vadillo, Konstantinidis, & Shanks, 2016). Obviously, it is a challenging task to optimize scientific quality and rigor within given economic and practical constraints, and trade-offs cannot be avoided. All studies in this thesis had small to medium sample sizes. Theoretically, following the logic of classical null hypothesis significance testing, significant results (as obtained in studies 1 and 3) should provide sufficient evidence against the null hypothesis, and this evidence should be valid for all sample sizes, or even stronger for smaller samples (Wagenmakers, 2007), but the much-invoked replication crisis in experimental psychology speaks a different language, and many experimental results may simply not contain sufficient information to draw definite conclusions (Loken & Gelman, 2017). With this in mind, the findings presented in this thesis should be considered, like all empirical findings, as preliminary to a certain degree, open to be challenged by future findings, and certainly demanding independent replication.

7.4 Conclusion

The studies incorporated in this thesis suggest that tVNS is a promising therapeutic method for clinical treatment of neuropsychological deficits, but currently less so for basic research. However, many open questions remain, perhaps more than before, and further work is necessary with respect to the general mechanisms of action of tVNS, identification of biomarkers predictive of stimulation responsiveness, optimization, and perhaps personalization, of stimulation parameters, and clinical investigation in patient populations with neuropsychological deficits.

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Ehrenerklärung

Ich versichere hiermit, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; verwendete fremde und eigene Quellen sind als solche kenntlich gemacht.
Ich habe insbesondere nicht wissentlich:

- Ergebnisse erfunden oder widersprüchlich Ergebnisse verschwiegen,
- statistische Verfahren absichtlich missbraucht, um Daten in ungerechtfertigter Weise zu interpretieren,
- fremde Ergebnisse oder Veröffentlichungen plagiiert,
- fremde Forschungsergebnisse verzerrt wiedergegeben.

Mir ist bekannt, dass Verstöße gegen das Urheberrecht Unterlassungs- und Schadensersatzansprüche des Urhebers sowie eine strafrechtliche Ahndung durch die Strafverfolgungsbehörden begründen kann.
Ich erkläre mich damit einverstanden, dass die Arbeit ggf. mit Mitteln der elektronischen Datenverarbeitung auf Plagiate überprüft werden kann.
Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form als Dissertation eingereicht und ist als Ganzes auch noch nicht veröffentlicht.

Magdeburg, 24. Mai 2019

(Marius Keute)