THE IMMEDIATE EFFECTS OF VAGUS NERVE STIMULATION ON EXECUTIVE AND AFFECTIVE FUNCTIONS IN PATIENTS WITH EPILEPSY

Katri Holm
Syventävien opintojen kirjallinen työ
Tampereen yliopisto
Lääketieteen yksikkö
Käyttäytymisneurologian tutkimusyksikkö
Tampereen yliopistollinen sairaala
Joulukuu 2016

Tampereen yliopisto
Lääketieteen yksikkö
Käyttäytymisneurologian tutkimusyksikkö
Tampereen yliopistollinen sairaala

HOLM KATRI: VAGUSHERMOSTIMULAATION VÄLITTÖMÄT VAIKUTUKSET TOIMINNANOHJAUS- JA TUNNETOIMINTOIHIN EPILEPSIAA SAIRASTAVILLA

Kirjallinen työ, 14 s. ja alkuperäisartikkeli 19 s.
Ohjaaja: Dosentti Kaisa Hartikainen

Joulukuu 2016

Avainsanat: käyttäytymisneurotiede, kognitio, elektroenkefalogrammi, reaktioaikatesti, herätevaste, N1, frontaalinen alfa-asymmetria

Key words: behavioral neuroscience, cognition, electroencephalogram, reaction time test, event-related potential, N1, frontal alpha asymmetry

---

Vagushermostimulaatio on neuromodulatorinen hoitomuoto, jota käytetään vaikean epilepsian ja masennuksen hoidossa. Sen tiedetään vähentävän kohtauksien määrää epilepsiassa, mutta sen vaikutuksia kognitioon ei ole vielä kattavasti selvitetty. Tässä työssä tutkittiin vagushermostimulaation vaikutuksia tarkkaavaisuuteen, kognitioon ja tunteiden säätelyyn potilailla, jotka saavat vagushermostimulaatiohoitoa vaikeaan epilepsiaan. Työssä käytettiin tietokoneella tehtävää tunneärsykkeitä sisältävää reaktioaikatestiä mittaamaan koehenkilöiden (n=20) kognitiivista suoriutumista stimulaattorin ollessa päällä tai pois kytkettynä. Koehenkilöiltä mitattiin testin aikana aivosähkökäyrää (EEG), josta tutkittiin herätevasteita ja aivojen etuosien toiminnan epäsymmetriaa. EEG:sta tutkittiin erityisesti vagushermostimulaation vaikutusta näköinformaation prosessointiin liittyvän herätevasteen N1-komponentin amplitudiin ja aivosähkötoiminnan frontaalisen alfataajuuden epäsymmetriaan. Vagushermostimulaation ollessa päällä koehenkilöt tekivät testissä merkittävästi vähemmän virheitä ja aivojen herätevasteiden N1-komponenttien amplitudi kasvoi. Virheiden väheneminen viittaa työmuistin paranemiseen ja N1-komponentin amplitudin kasvu tarkkaavuuden lisääntymiseen stimulaation seurauksena. Lisäksi tutkimuksessa havaittiin, että koehenkilöiden reaktioajat pidentyivät ja aivojen etuosien toiminta oli epäsymmetrisempää stimulaation aikana, kun mukana oli negatiivinen uhkaan liittyvä tunneärsyke. Lisäksi vagushermostimulaation huomattiin muokkaavan aivojen reaktioita uhkaan. Tämä on ensimmäinen tutkimus, jossa kuvataan vagushermostimulaation välittömiä vaikutuksia aivojen fysiologiaan, tiedonkäsittelytoimintoihin ja tunnereaktioihin. Vagushermostimulaatiolla havaittiin olevan hyödyllisiä vaikutuksia kognitioon ja sen potentiaalia muissakin kliinisissä sovelluksissa tulee tutkia.

Tämän opinnäytteen alkuperäisuus on tarkastettu Turnitin OriginalityCheck -ohjelmalla Tampereen yliopiston laatujärjestelmän mukaisesti.

TABLE OF CONTENTS

1 Introduction 1
2 Vagus nerve stimulation 1
2.1 Vagus nerve and the effects of vagus nerve stimulation in central nervous system 1
2.2 Vagus nerve stimulation in clinical use 2
3 Executive functions and working memory 3
4 Attention and emotion 4
5 Methods 6
5.1 Executive reaction time test 6
5.2 Electroencephalogram 7
5.2.1 The principle of electroencephalogram 7
5.2.2 Event related potential 8
5.2.3 Frontal alpha asymmetry 9
Literature 9
Appendix 1. Original Research Article 15

1 INTRODUCTION

Neuromodulatory treatments for neurological and psychiatric disorders are becoming more popular due to the limitation of traditional therapy methods and the promising results of these treatments. Vagus nerve stimulation (VNS) is a safe neuromodulatory therapy used for refractory epilepsy and pharmacoresistant depression (Nemeroff et al. 2006; Vonck et al. 2014a). Despite the positive outcome in treating epilepsy and depression, the exact mechanisms by which VNS affects cognition, executive and affective functions in addition to neurophysiology are still unclear (Nemeroff et al., 2006; Vonck et al., 2014). For these reasons, it is essential to develop methods to assess and understand the effects of stimulation. In this study, the aim was to investigate the immediate effects of VNS on executive and affective functions in patients with epilepsy. The methods used include a computer based visual attention task with emotional distractors (i.e., the Executive reaction time (RT) test) and electroencephalogram (EEG). The hypothesis of this work is that VNS has immediate effects on cognitive or affective brain functions and this could be seen as a change in cognitive performance in the Executive RT test, emotional interference or physiological markers extracted from the EEG recorded during the Executive RT test.

2 VAGUS NERVE STIMULATION

2.1 Vagus nerve and the effects of vagus nerve stimulation in central nervous system

Vagus nerve (cranial nerve X) is a paired nerve that consists of both efferent and afferent (sensory, 65-80 %) nerve fibers (Foley & DuBois, 1937). The parasympathetic efferent fibers are unmyelinated and responsible for the autonomic regulation of heart and gastrointestinal organs but also larynx, esophagus, and trachea. There are anatomical and physiological differences between the right and the left vagus nerve. For example, the right nerve regulates heart rate by innervating the sinus node and the left innervates the atrioventricular node. By stimulating the right vagus nerve it is possible to cause bradycardia (Randall et al., 1988). The myelinated motor

efferent fibers innervate vocal cords and other voluntary laryngeal muscles. The sensory afferent fibers convey information from head, neck, thorax and abdomen to the nucleus tractus solitarii (NTS) in medulla. (Ben-Menachem, 2002)

The neurobiological mechanisms of how VNS therapy reduces seizure burden of epileptic patients are not fully understood. The current view is that by activating the norepinephrine system of locus coeruleus (LC) VNS helps to suppresses the seizures (Fornai et al., 2011; Krahl & Clark, 2012). The afferent cervical fibers of the vagus nerve innervate the NTS which in turn forwards information to several brain regions including parabrachial nucleus (PBN), LC, and dorsal raphe nucleus (DRN) (Nemeroff et al., 2006; Krahl & Clark, 2012). PBN relays information, for example, to hypothalamus, thalamus, and amygdala and thus the afferent fibers of vagus nerve affect limbic, pre-motor, and endocrine systems (Nemeroff et al., 2006) and LC and DRN play an important role in the decreasing the seizure burden during VNS therapy (Krahl & Clark, 2012).

2.2 Vagus nerve stimulation in clinical use

VNS is successfully used for treating refractory epilepsy (Penry & Dean, 1990; McGlone et al., 2008; Zeiler et al., 2015) and pharmacoresistant depression (Penry & Dean, 1990; Sackeim et al., 2001; McGlone et al., 2008). In Finland, VNS therapy is currently used only for treating patients with refractory epilepsy. The first VNS device in humans was implanted to a patient with epilepsy in 1988 (Penry & Dean, 1990). Positive effects on mood were reported after some time when several patients with epilepsy had been treated with VNS therapy (Elger et al., 2000; Klinkenberg et al., 2012) and after that VNS therapy was also applied to patients with refractory depression (Rush et al., 2000; Marangell et al., 2002). In Tampere University Hospital, the first VNS device was implanted in 2003 and until now about hundred patients have undergone the implantation surgery (personal communications with Jukka Peltola, 2016).

In VNS therapy, a stimulation device is implanted surgically. In the surgery, a helical bipolar lead with two electrodes is placed around the left cervical vagus nerve and a subcutaneous generator in the left upper chest (Cyberonics Inc., 2016). The VNS device is recommended to be turned on earliest 2 weeks after the surgery (Cyberonics Inc., 2016). There are different stimulation parameters that can be adjusted in the VNS device: output current (mA), signal frequency (Hz),

pulse width (μs), signal on time (s), and signal off time (s) (Cyberonics Inc., 2016). An external programming wand is used to adjust the parameters. The current is ramped up to the target value in one to two week intervals taking care of the tolerance of the patient (personal communications with Jukka Peltola, 2014).

VNS therapy can have side effects, acute or long-term effects. The acute complications of VNS implantation include infection, vocal-cord paresis, lower facial weakness, and rarely, asystole and bradycardia. The most common side effects due to the stimulation are stimulus-related coughing, throat pain, voice alterations and hoarseness. All of these side effects are typically mild and they are likely to diminish over time. (Ben-Menachem, 2002)

Cognitive and affective effects of VNS have mostly been positive or the changes have not been significant as recently reviewed by Vonck et al. (2014b). Furthermore, VNS has also been found to improve recognition memory similarly to arousal and thus inspired its application in treatment of Alzheimer's disease (Clark et al., 1999). Overall, it can be said that the knowledge on effects that VNS therapy have on cognition or emotion, especially on executive functions and attention-emotion interaction, is limited. VNS therapy has been also used for treatment-resistant depression since positive effects on mood during VNS therapy on patients with epilepsy were reported (Sackeim et al., 2001) and in 2005 VNS therapy was approved as a treatment for treatment-resistant depression by FDA. Despite the positive outcome in treating depression, the exact mechanisms by which VNS affect the mood are still unclear (Nemeroff et al., 2006; Vonck et al., 2014).

3 EXECUTIVE FUNCTIONS AND WORKING MEMORY

Executive functions are processes that are involved in controlling of our behavior, cognition and affective functions. They are a vital part of our everyday life since they enable planning of the tasks to be done and a fluent order for them so that we are able to do what we want or are required to do. Considering all this, it is clear that a decline in executive functions could make individual's daily life difficult. Anatomically the neural networks associated with executive

functions extend to the frontal lobe of the brain (Fuster, 2001; Alvarez & Emory, 2006). Executive functions include working memory, planning, focusing attention, shifting from a task to another, and emotional control.

As one of the important parts of executive functions, working memory is also a part of memory that is used to keep things in mind for a short period, from seconds to minutes. The maintaining time is just enough for the brain to use, process and react according to the information and, if necessary, to transfer it to the long-term memory deposit. The capacity of working memory is limited. Baddeley and Hitch (1974) have suggested a model for working memory that involves a central executive system which has two subsystems, the phonological loop for encoding acoustic information and the visuospatial sketch pad for encoding visual and visuospatial information. Based on lesion studies, the phonological loop has been anatomically mapped to the left supramarginal gyrus, whereas, the visuospatial sketchpad has been mapped to the parietal and occipital lobes (Gazzaniga et al., 2014). The visuospatial sketchpad involves both hemispheres but damage to the right side lead to more difficult working memory problems than the damage on left. Despite the mechanisms for memory, it is not possible to remember anything if you cannot focus your attention to it. Thus according to current understanding, attention is a requirement for other cognitive operations including working memory with attention and working memory as concepts intricately intertwined.

4 ATTENTION AND EMOTION

To be able to perform a task, allocating of an attention to it is needed. Attention makes it possible to focus on one stimulus, task, or though and to ignore irrelevant stimuli, tasks, and thoughts. The brain has a capacity to process all stimuli and thus dynamic selective focusing of attention is required (Desimone & Duncan, 1995). According to current theories, the attentional control mechanism can be divided in to goal guided (top-down) and stimulus guided (bottom-up) control (Corbetta & Shulman, 2002). The prefrontal cortex is responsible for the top-down control of attention which is voluntary (Shimamura, 2000). In voluntary top-down control of attention, the brain chooses to focus the attention to the task in hand, for example reading this thesis. The involuntary bottom-up mechanisms arising from limbic system, on the other hand, withdraw the

attention from the voluntary task to the stimulus i.e., shift from top-down control to bottom-up control. The stimulus can be for example a sound of a fire alarm or a rapid movement at the edge of the field of vision.

According to current understanding, emotions are valenced responses to external stimuli or intrinsic mental depiction and they involve changes in various response systems in the body, for example, behavioral or peripheral physiology (Ochsner & Gross, 2005). Emotions are separate from mood in a sense that they have usually identifiable objects or triggers (Ochsner & Gross, 2005). They are can be also automatic or learned responses to stimuli (Ochsner & Gross, 2005). The interaction of attention and emotion has been under widening investigation. It has been shown that emotional stimuli, especially negative threat-related stimuli, occupy attentional resources and interfere with the task performance although being irrelevant regarding the task (Hartikainen et al., 2000, 2010a, 2012b) and activate attentional networks (Mäki-Marttunen et al., 2014).

Our adaptive behavior is due to the flexible and delicate balance between the top-down and bottom-up control mechanisms. Our research group has previously shown that orbitofrontal cortex contributes to the appropriate balance between voluntary and involuntary attention allocation especially in context of emotional stimuli (Hartikainen et al., 2012a; Mäki-Marttunen et al., 2016). It is thought that depression could be due to imbalance between these mechanisms as the bottom-up mechanism dominates voluntary attention allocation and tasks become difficult. Amygdala is a part of the limbic system and its hyperactivity has been observed in patients with depression in the presence of negative or arousing visual stimuli (Jaworska et al., 2015) and it has been linked also to posttraumatic stress disorder (Koenigs & Grafman, 2009). There is some evidence that VNS might also affect the amygdala. Zobel et al. (2005) and Kraus et al. (2007) have suggested that VNS inhibits (transcutaneous VNS in later) activity of amygdala in humans by reducing blood flow and Lyubashina and Panteleev (2009) that it reduces the amygdala-cortical interaction in rats. Pena, Engineer and McIntyre (2013) have also shown that VNS reduces conditioned fear in rats.

5 METHODS

5.1 Executive reaction time test

Executive reaction time test (Executive RT test) is a computer-based visual attention Go-NoGo test in which the subject has to respond (Go) or refrain from responding (NoGo). The test used in this work include distractors, neutral and emotional. Traditional neuropsychological tests measure usually only one executive function at a time and thus at least subtle, but relevant in managing everyday life, impairments in executive functions are not detected. The executive RT test requires multiple executive functions, such as working memory, inhibition, focusing attention, shifting from task to another, and emotional control, to be engaged simultaneously. It is thus a powerful method to test executive functions and control of behavior, cognition and emotions. The test has been previously shown to detect subtle executive impairment after mild head injury (Hartikainen et al., 2010b), alterations in emotion-attention interaction due to deep brain stimulation in patients with epilepsy (Hartikainen et al., 2014; Sun et al., 2015), and improvement in cognitive flexibility after a heart surgery (Liimatainen et al., 2016).

The subjects sat in a sound-attenuated room one meter away from a computer screen and responded to visual stimuli according to instructions. The test was presented and the response data was collected using a computer program (Presentation, Neurobehavioral Systems, Inc.). Each trial in the test started with a triangle pointing up or down followed by a fixation cross and then a Go or NoGo signal each shown for 150 ms at the center of the screen (Figure 1. in Original research article). The orientation of the triangle was random. The Go/NoGo signal was presented indicating whether the subject should respond with a button press to the orientation of the previously presented triangle or withhold from responding. After the Go signal the subject had 1150 ± 150 ms to press a button on a special keyboard (Cedrus RB-830) with index finger if the triangle had been pointing down and a different button with middle finger if the triangle had been pointing up. The subjects used their right hand when responding except one subject, who was not able to use his right hand, used left hand. Half of the trials were Go trials and half were NoGo trials.

In the Executive RT test the subject can make three kind of errors: missed responses, incorrect button presses, and commission errors. In missed response, the subject does not press the button after the Go signal and it may indicate decrease in attention or problems with rule switching. Whereas when there is an incorrect button press, it may indicate problems with working memory as the subject remembers the orientation of the triangle incorrectly or difficulties in focusing attention to the task. In commission error, the subject presses a button after NoGo signal and this may indicate difficulties in refraining from answering, i.e., inhibition.

In addition to errors, there are evidence that variability in reaction times could relate to alternations in behavior. According to a recent study by Antonini et al. (2013), there is an association between observed attention and reaction time variability, i.e., more inattentive you are, more variability there is in reaction times.

5.2 Electroencephalogram

5.2.1 The principle of electroencephalogram

Electroencephalogram (EEG) presents electrical activity of the brain that is measured using electrodes placed on the scalp. EEG measures the voltage, i.e., it records a potential of the current to move between two electrodes (active and reference) and the recorded voltage is a sum of the synaptic activity of population of neurons on the cortex (Gazzaniga et al., 2014; Luck, 2005). EEG has a high temporal but poor spatial resolution. The overall activity of the brain changes according to the mental state (for example exited, relaxed, asleep, deep sleep, epileptic activity) of a person and this can be seen in EEG as differences in frequency and amplitude of EEG signal (Gazzaniga et al., 2014). EEG can be characterized by the frequency to different wave types: delta (1–3 Hz), theta (4–7 Hz), alpha (8–15 Hz), mu (7.5–12.5 Hz), SMR (12.5–15.5 Hz), beta (16–31 Hz), and gamma (32–100 Hz). In the current work, especially the alpha waves in the frontal area are of interest.

In EEG recordings, the electrodes are positioned according to general guidelines (International 10–20 system, The General Assembly of the International Federation of Clinical Neurophysiology) and the names of the electrodes reflect their location on the scalp. The name consists of one or two capital letters and a number or a small letter z. The capital letters F, C, T, and O represent frontal,

central, temporal and occipital lobes, respectively, of which the central lobe is not a neuroanatomical entity, but refers to the top central area of the skull. The electrodes on the right hemisphere are given even numbers, on the left hemisphere, they are given odd numbers, and in the middle, they are given a small letter z.

In this study, EEG was recorded with actiCAP having 64 Ag/AgCl electrodes (Brain Products GmbH, Germany). The electrodes in actiCAP are active electrodes that have integrated noise subtraction circuit to reduce the noise during the recording and to improve the signal to noise ratio. Electrodes also have integrated technology to measure the impedance and indicate it using a led (red, yellow, green). (Brain Products GmbH, 2016)

5.2.2 Event related potential

Event-related potential (ERP) is a subtle electrophysiological change evoked by a stimulus and it can be observed after averaging the post-stimulus EEG signal over a series of trials. The stimulus can be sensory, motor or cognitive event. In addition to studying the differences in ERPs evoked by different stimuli, ERPs can be used to study the conduction velocity in nervous system. First ERP studies have been performed already in 1930's (Davis et al., 1939; Davis, 1939) but ERPs became more popular in 1960's. (Luck, 2005)

ERPs have a stereotypic waveform in which different peaks are called components. They are named based on their direction (P = positive, N = negative) and order. Most commonly in the x-axis (potential), the negative values are plotted upward and positive values downward. Sometimes the number in the name of the component can refer, not to the order but, to the time elapsed after the stimulus, for example N100 (around 100 ms after stimulus) and P300 (around 300 ms after stimulus). (Luck, 2005)

The meaning of different ERP component have been studied extensively (Luck, 2005). The components can be linked to different cognitive properties, but it has to be noted that the properties depend on the modality of the stimulus. The early components are more dependent on the modality than the later components. For example, the auditory P1 and visual P1 have different neural basis but the corresponding P3s may share neural processes (Luck, 2005). Rather than the

stimulus modality the later components including P3 are dependent on the cognitive task involved. In this study, the amplitude of visual N1 was of interest. The increase in N1 amplitude is associated with increased attention to visual stimulus(Mangun & Hillyard, 1991; Luck & Ford, 1998). The parieto-occipital N1 amplitude has been closely linked with attention, where enhanced visual attention is associated with increase in N1 amplitude (Luck & Ford, 1998).

5.2.3 Frontal alpha asymmetry

Davidson et al. (1990; 1992) were among first ones to measure and show asymmetrical alpha power in frontal lobes and linked it to approach-withdrawal theory of emotional responses. Multiple studies using EEG (see, for example, Henriques (1990), Henriques (1991), Davidson (1998), Liao (2013), Gollan (2014)) have shown evidence that the activity of the frontal lobes is asymmetrical in patients with depression and thus strengthens the association between behavioral withdrawal and depression (reviewed in Jesulola et al. (2015)). In their studies on depression, they have found that frontal lobe activity is greater on right than left hemisphere. Alpha wave activity across the brain area of interest (i.e., alpha power) has been used as a measure for the activity on the area (Jesulola et al., 2015). The alpha power behaves inversely compared to the activity, i.e., the alpha power relatively increases in hypoactivity and decreases in hyperactivity (Jesulola et al., 2015). Quraan et al. (2014) have suggested that hemispheric asymmetry could be used as a marker for effectiveness of neuromodulatory treatment (deep brain stimulation) for depression.

LITERATURE

Alvarez, J.A. & Emory, E. (2006). Executive function and the frontal lobes: a meta-analytic review. *Neuropsychology review*. 16 (1). pp. 17–42.

Antonini, T.N., Narad, M.E., Langberg, J.M. & Epstein, J.N. (2013). Behavioral Correlates of Reaction Time Variability in Children With and Without ADHD. *Neuropsychology*. 27 (2). pp. 201–209.

Baddeley, A.D. & Hitch, G. (1974). Working memory. *The psychology of learning and motivation*. 8. pp. 47–89.

Ben-Menachem, E. (2002). Vagus-nerve stimulation for the treatment of epilepsy. *The Lancet Neurology*. 1 (8). pp. 477–482.

Brain Products GmbH (2016). *actiCAP - The third generation of active electrodes*. [Online]. 2016. Available from: http://www.brainproducts.com/productdetails.php?id=4. [Accessed: 26 September 2016].

Clark, K.B., Naritoku, D.K., Smith, D.C., Browning, R.A. & Jensen, R.A. (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nature Neuroscience*. 2 (1). pp. 94–98.

Corbetta, M. & Shulman, G.L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*. 3 (3). pp. 215–229.

Cyberonics Inc. (2016). *VNS Therapy® System Physician's Manual*.

Davidson, R.J. (1998). Anterior electrophysiological asymmetries, emotion, and depression: Conceptual and methodological conundrums. *Psychophysiology*. 35. pp. 607–614.

Davidson, R.J. (1992). Emotion and Affective Style: Hemispheric Substrates. *Psychological Science*. 3 (1). pp. 39–43.

Davidson, R.J., Saron, C.D., Senulis, J.A., Ekman, P. & Friesen, W. V (1990). Approach Withdrawal and Cerebral Asymmetry - Emotional Expression and Brain Physiology I. *Journal of Personality and Social Psychology*. 58 (2). pp. 330–341.

Davis, H., Davis, P.A., Loomis, A.L., Harvey, E.N. & Hobart, G. (1939). Electrical reactions of the human brain to auditory stimulation during sleep. *Journal of Neurophysiology*. 2 (6). pp. 500–514.

Davis, P.A. (1939). Effects of acoustic stimuli on the waking human brain. *Journal of Neurophysiology*. 2 (6). pp. 494–499.

Desimone, R. & Duncan, J. (1995). Neural mechanisms of selective visual attention. *Annual Reviews Neuroscience*. 18. pp. 193–222.

Elger, G., Hoppe, C., Falkai, P., Rush, A.J. & Elger, C.E. (2000). Vagus nerve stimulation is associated with mood improvements in epilepsy patients. *Epilepsy Research*. 42 (2–3). pp. 203–210.

Foley, J.O. & DuBois, F.S. (1937). Quantitative studies of the vagus nerve in the cat. I. The ratio of sensory to motor fibers. *Journal of Comparative neurology*. 67 (1). pp. 49–67.

Fornai, F., Ruffoli, R., Giorgi, F.S. & Paparelli, A. (2011). The role of locus coeruleus in the antiepileptic activity induced by vagus nerve stimulation. *European Journal of Neuroscience*. 33 (12). pp. 2169–2178.

Fuster, J.M. (2001). The Prefrontal Cortex — An Update : Time Is of the Essence. *Neuron*. 30. pp. 319–333.

Gazzaniga, M.S., Ivry, R.B. & Mangun, G.R. (2014). *Cognitive Neuroscience: The Biology of the Mind*. New York, NY, USA: W. W. Norton & Company.

Gollan, J.K., Hoxha, D., Chihade, D., Pflieger, M.E., Rosebrock, L. & Cacioppo, J. (2014). Frontal alpha EEG asymmetry before and after behavioral activation treatment for depression. *Biological Psychology*. 99 (1). pp. 198–208.

Hartikainen, K.M., Ogawa, K.H. & Knight, R.T. (2012a). Orbitofrontal cortex biases attention to emotional events. *Journal of Clinical and Experimental Neuropsychology*. 34 (6). pp. 588–597.

Hartikainen, K.M., Ogawa, K.H. & Knight, R.T. (2000). Transient interference of right hemispheric function due to automatic emotional processing. *Neuropsychologia*. 38 (12). pp. 1576–1580.

Hartikainen, K.M., Ogawa, K.H. & Knight, R.T. (2010a). Trees over forest: unpleasant stimuli compete for attention with global features. *Neuroreport*. 21 (5). pp. 344–348.

Hartikainen, K.M., Siiskonen, A.R. & Ogawa, K.H. (2012b). Threat interferes with response inhibition. *Neuroreport*. 23 (7). pp. 447–450.

Hartikainen, K.M., Sun, L., Polvivaara, M., Brause, M., Lehtimäki, K., Haapasalo, J., Möttönen, T., Väyrynen, K., Ogawa, K.H., Ohman, J. & Peltola, J. (2014). Immediate effects of deep brain stimulation of anterior thalamic nuclei on executive functions and emotion-attention interaction in humans. *Journal of Clinical and Experimental Neuropsychology*. 36 (5). pp. 540–550.

Hartikainen, K.M., Waljas, M., Isoviita, T., Dastidar, P., Liimatainen, S., Solbakk, A.-K., Ogawa, K.H., Soimakallio, S., Ylinen, A. & Ohman, J. (2010b). Persistent symptoms in mild to moderate traumatic brain injury associated with executive dysfunction. *Journal of Clinical and Experimental Neuropsychology*. 32 (7). pp. 767–774.

Henriques, J. & Davidson, R.J. (1990). Regional brain electrical asymmetries discriminate between previously depressed and health control subjects. *Journal of Abnormal Psychology*. 99 (1). pp. 22–31.

Henriques, J.B. & Davidson, R.J. (1991). Left frontal hypoactivation in depression. *Journal of Abnormal Psychology*. 100 (4). pp. 535–45.

Jaworska, N., Yang, X.-R., Knott, V. & MacQueen, G. (2015). A review of fMRI studies during visual emotive processing in major depressive disorder. *The World Journal of Biological Psychiatry*. 16 (7). pp. 448–471.

Jesulola, E., Sharpley, C.F., Bitsika, V., Agnew, L.L. & Wilson, P. (2015). Frontal alpha asymmetry as a pathway to behavioural withdrawal in depression: Research findings and issues. *Behavioural Brain Research*. 292. pp. 56–67.

Klinkenberg, S., Majoie, H.J., van der Heijden, M.M., Rijkers, K., Leenen, L. & Aldenkamp, A.P. (2012). Vagus nerve stimulation has a positive effect on mood in patients with refractory epilepsy. *Clinical Neurology and Neurosurgery*. 114 (4). pp. 336–340.

Koenigs, M. & Grafman, J. (2009). Posttraumatic stress disorder: the role of medial prefrontal cortex and amygdala. *Neuroscientist*. 15 (5). pp. 540–548.

Krahl, S. & Clark, K. (2012). Vagus nerve stimulation for epilepsy: A review of central mechanisms. *Surgical Neurology International*. 3 (5). pp. 255–259.

Kraus, T., Hosl, K., Kiess, O., Schanze, A., Kornhuber, J. & Forster, C. (2007). BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *Journal of Neural Transmission*. 114 (11). pp. 1485–1493.

Liao, Z., Zhou, H., Li, C., Zhou, J., Qin, Y., Feng, Y., Feng, L., Wang, G. & Zhong, N. (2013). *The Change of Resting EEG in Depressive Disorders*. In: Springer International Publishing, pp. 52–61.

Liimatainen, J., Peräkylä, J., Järvelä, K., Sisto, T., Yli-Hankala, A. & Hartikainen, K.M. (2016). Improved cognitive flexibility after aortic valve replacement surgery. *Interactive CardioVascular and Thoracic Surgery*. pp. 1-7.

Luck, S.J. (2005). *An Introduction to the Event-Related Potential Technique*. Cambridge, MA, USA: MIT Press.

Luck, S.J. & Ford, M.A. (1998). On the role of selective attention in visual perception. *Proceedings of the National Academy of Sciences of the United States of America*. 95 (3). p.pp. 825–830.
Lyubashina, O. & Panteleev, S. (2009). Effects of cervical vagus nerve stimulation on amygdala-evoked responses of the medial prefrontal cortex neurons in rat. *Neurosci Res*. 65 (1). pp. 122–125.

Mäki-Marttunen, V., Kuusinen, V., Peräkylä, J., Ogawa, K.H., Brause, M., Brander, A. & Hartikainen, K.M. (2016). Greater Attention to Task-Relevant Threat due to Orbitofrontal Lesion. *Journal of Neurotrauma*. pp. neu.2015.4390.

Mäki-Marttunen, V., Pickard, N., Solbakk, A.-K., Ogawa, K.H., Knight, R.T. & Hartikainen, K.M. (2014). Low attentional engagement makes attention network activity susceptible to emotional interference. *Neuroreport*. 25 (13). pp. 1038–43.

Mangun, G.R. & Hillyard, S.A. (1991). Modulations of Sensory-Evoked Brain Potentials Indicate Changes in Perceptual Processing During Visual-Spatial Priming Program in Cognitive Neuroscience Dartmouth Medical School. *Journal of Experimental Psychology: Human Perception and Performance*. 17 (4). pp. 1057–1074.

Marangell, L.B., Rush, A.J., George, M.S., Sackeim, H.A., Johnson, C.R., Husain, M.M., Nahas, Z. & Lisanby, S.H. (2002). Vagus nerve stimulation (VNS) for major depressive episodes: one year outcomes. *Biological psychiatry*. 51 (4). pp. 280–287.
McGlone, J., Valdivia, I., Penner, M., Williams, J., Sadler, R.M. & Clarke, D.B. (2008). Quality of life and memory after vagus nerve stimulator implantation for epilepsy. *The Canadian Journal of Neurological Sciences*. 35 (3). pp. 287–296.

Nemeroff, C.B., Mayberg, H.S., Krahl, S.E., McNamara, J., Frazer, A., Henry, T.R., George, M.S., Charney, D.S. & Brannan, S.K. (2006). VNS therapy in treatment-resistant depression: clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology*. 31 (7). pp. 1345–1355.

Ochsner, K.N. & Gross, J.J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences*. 9 (5). p.pp. 242–249.

Pena, D.F., Engineer, N.D. & McIntyre, C.K. (2013). Rapid remission of conditioned fear expression with extinction training paired with vagus nerve stimulation. *Biological Psychiatry*. 73 (11). pp. 1071–1077.

Penry, J.K. & Dean, J.C. (1990). Prevention of intractable partial seizures by intermittent vagal stimulation in humans: preliminary results. *Epilepsia*. 31 (s2). p.pp. S40–S43.
Quraan, M.A., Protzner, A.B., Daskalakis, Z.J., Giacobbe, P., Tang, C.W., Kennedy, S.H., Lozano, A.M. & McAndrews, M.P. (2014). EEG power asymmetry and functional connectivity as a marker of treatment effectiveness in DBS surgery for depression. *Neuropsychopharmacology*. 39 (5). pp. 1270–1281.

Randall, W.C., Ardell, J.L., O'Toole, M.F. & Wurster, R.D. (1988). Differential autonomic control of SAN and AVN regions of the canine heart: structure and function. *Progress in clinical and biological research*. 275. pp. 15–31.

Rush, A.J., George, M.S., Sackeim, H.A., Marangell, L.B., Husain, M.M., Giller, C., Nahas, Z., Haines, S., Simpson, R.K. & Goodman, R. (2000). Vagus nerve stimulation (VNS) for treatment-resistant depressions: a multicenter study. *Biological psychiatry*. 47 (4). pp. 276–286.

Sackeim, H.A., Keilp, J.G., Rush, A.J., George, M.S., Marangell, L.B., Dormer, J.S., Burt, T., Lisanby, S.H., Husain, M., Cullum, C.M., Oliver, N. & Zboyan, H. (2001). The effects of vagus nerve stimulation on cognitive performance in patients with treatment-resistant depression. *Neuropsychiatry, Neuropsychology, and Behavioral Neurology*. 14 (1). pp. 53–62.

Shimamura, A.P. (2000). Toward a cognitive neuroscience of metacognition. *Consciousness and Cognition*. 9 (2). pp. 313–316.

Sun, L., Peräkylä, J., Polvivaara, M., Öhman, J., Peltola, J., Lehtimäki, K., Huhtala, H. & Hartikainen, K.M. (2015). Human anterior thalamic nuclei are involved in emotion–attention interaction. *Neuropsychologia*. 78. pp. 88–94.

Vonck, K., Raedt, R., Naulaerts, J., De Vogelaere, F., Thiery, E., Van Roost, D., Aldenkamp, B., Miatton, M. & Boon, P. (2014). Vagus nerve stimulation. . .25 years later! What do we know about the effects on cognition? *Neuroscience and Biobehavioral Reviews*. 45. .pp. 63–71.
Zeiler, F.A., Zeiler, K.J., Teitelbaum, J., Gillman, L.M. & West, M. (2015). VNS for refractory status epilepticus. *Epilepsy research*. 112. pp. 100–113.

Zobel, A., Joe, A., Freymann, N., Clusmann, H., Schramm, J., Reinhardt, M., Biersack, H.J., Maier, W. & Broich, K. (2005). Changes in regional cerebral blood flow by therapeutic vagus nerve

stimulation in depression: an exploratory approach. *Psychiatry Research: Neuroimaging*. 139 (3). pp. 165–179.

APPENDIX 1. ORIGINAL RESEARCH ARTICLE

This is the authors accepted manuscript of an article published as the version of record in the Journal of Clinical and Experimental Neuropsychology 19 Feb 2017
http://dx.doi.org/10.1080/13803395.2017.1285869

VAGUS NERVE STIMULATION IMPROVES WORKING MEMORY PERFORMANCE

Lihua Sun[1,2], Jari Peräkylä[1,2], Katri Holm[1,2], Joonas Haapasalo[3], Kai Lehtimäki[3], Keith H. Ogawa[4], Jukka Peltola[5], Kaisa. M. Hartikainen[1,5]*

[1]Behavioral Neurology Research Unit, Tampere University Hospital, Tampere, Finland
[2]School of Medicine, University of Tampere, Tampere, Finland
[3]Department of Neuroscience and Rehabilitation, Unit of Neurosurgery, Tampere University Hospital, Tampere, Finland
[4]John Magaddino Neuroscience Laboratory, Saint Mary's College of California, Moraga, CA, USA
[5]Department of Neuroscience and Rehabilitation, Unit of Neurology, Tampere University Hospital, Tampere, Finland

Lihua Sun and Jari Peräkylä contribute equally.
*Correspondence to: Kaisa M Hartikainen. E-mail: kaisa.hartikainen@uta.fi, Tel: +358 3 31164385.

Key words: VNS, attention, cognition, executive functions, frontal alpha asymmetry

ABSTRACT

Vagus nerve stimulation is used for treating refractory epilepsy and major depression. While the impact of this treatment on seizures has been established, its impact on human cognition remains equivocal. The goal of this study is to elucidate the immediate effects of vagus nerve stimulation on attention, cognition and emotional reactivity in patients with epilepsy. Twenty patients (12 male and 8 female; 45 ± 13 years old) treated with VNS due to refractory epilepsy participated in the study. Subjects performed a computer-based test of executive functions embedded with emotional distractors while their brain activity was recorded with electroencephalography. Subjects' cognitive performance, early visual event related potential N1 and frontal alpha asymmetry were studied when cyclic vagus nerve stimulation was on and when it was off. We found that vagus nerve stimulation improved working memory performance as seen in reduced errors on a subtask that relied on working memory, OR = 0.63 (95% CI 0.47-0.85) and increased N1 amplitude [$F(1, 15) = 10.17$, $p = 0.006$]. In addition, vagus nerve stimulation resulted in longer reaction time [$F(1, 16) = 8.23$, $p = 0.019$] and greater frontal alpha asymmetry [$F(1, 16) = 11.79$, $p = 0.003$] in response to threat-related distractors. This is the first study to show immediate improvement in working memory performance in humans with clinically relevant vagus nerve stimulation. Furthermore, vagus nerve stimulation had immediate effects on emotional reactivity evidenced in behavior and brain physiology.

INTRODUCTION

Pharmacoresistant neurological and psychiatric disorders highlight the importance of novel neuromodulation treatments. Vagus nerve stimulation (VNS) is an effective and safe therapy (Vonck et al. 2014; Grimonprez, Raedt, Baeken, Boon & Vonck 2015a; Grimonprez et al. 2015b) and is reported to be successful in treating pharmacoresistant epilepsy (Penry & Dean 1990). In addition to reducing seizures, the reported improvements in mood (Elger, Hoppe, Falkai, Rush & Elger 2000; Klinkenberg et al. 2012) and verbal recognition memory following VNS stimulation have led to its use in treating other brain disorders, including its clinical use in depression (Rush et al. 2000; Marangell et al. 2002) and its experimental use in treating Alzheimer's disease (Sjogren et al. 2002). While there is robust evidence for the therapeutic effect of VNS in reducing seizures (Ben-Menachem et al. 1994), the evidence for VNS's impact on cognition remains equivocal along with several methodological limitations (Dodrill & Morris 2001; Sackeim et al. 2001a; Sjogren et al. 2002; Merrill et al. 2006; McGlone et al. 2008; Klinkenberg et al. 2012). Patients treated with VNS due to refractory epilepsy frequently have compromised cognitive functions due to epilepsy or antiepileptic drugs, the type of epilepsy and etiology vary, they may have covert seizures and double blinded studies may not be possible due sensation of VNS stimulation. To that end, there is a need for further studies to better understand potential cognitive and affective effects of VNS treatment.

Previously reported beneficial effects of VNS on human cognition and emotion are thought to arise from increased levels of norepinephrine (NE) (Vonck et al. 2014; Grimonprez et al. 2015a; Grimonprez et al. 2015b). VNS innervates the nucleus tractus solitaries (Kalia & Sullivan 1982) which is connected to the locus coeruleus (LC) (Aston-Jones et al. 1991; Van Bockstaele, Peoples & Telegan 1999), the principal site for the brain's synthesis of NE (Aston-Jones & Cohen 2005). However, studies of the chronic effects of VNS provide contradictory evidence for its influence on mood (Sackeim et al. 2001b; McGlone et al. 2008) and cognition (Sjogren et al. 2002; Merrill et al. 2006) with either improvement or no change. Chronic effects of VNS are typically confounded by several factors influencing emotion and cognition including medications, seizure frequency, etc. Improved cognitive function due to VNS has been found in very specific situations, thus limiting the generalizability of the findings. For example improved verbal recognition memory has been

shown (Clark, Naritoku, Smith, Browning & Jensen 1999) when low intensity VNS was delivered during memory consolidation phase (Clark, Krahl, Smith & Jensen 1995).

In this study, we investigated the immediate effects of VNS on human executive functions by comparing the cognitive performance when cyclic VNS stimulation is administered and when it is not. The comparison within subjects allows uncovering the immediate and direct effects of VNS on human cognition. Subjects performed an experimental computer based visual attention task with emotional distractors, i.e. the Executive - Reaction Time (RT) test (Figure 1a), while having their electroencephalogram (EEG) recorded. The task is designed to simultaneously engage several cognitive control functions including working memory, response inhibition and emotional control. The Executive-RT test has been shown to be a sensitive method in revealing alteration in executive function performance and emotion-attention interaction due to neuromodulation (Hartikainen et al. 2014; Sun et al. 2015; Sun et al. 2016), brain injury (Mäki-Marttunen V. et al. 2015; Mäki-Marttunen Verónica et al. 2016) and cardiac surgery (Liimatainen et al. 2016).

In addition to behavioral measures we used measures derived from EEG to assess the impact of VNS on cognitive and affective brain functions. We examined the impact of VNS on early visual evoked potential, i.e. N1. The parieto-occipital N1 amplitude has been closely linked with attention, where enhanced visual attention is associated with increase in N1 amplitude (Mangun & Hillyard 1991; Luck & Ford 1998). Moreover, we also studied the effect of VNS on threat induced frontal alpha asymmetry. Relatively increased right frontal activity, as indicated by increased frontal alpha asymmetry has been associated with vigilance to threat (Perez-Edgar, Kujawa, Nelson, Cole & Zapp 2013).

In summary, we expected that if VNS has immediate effects on cognitive or emotional brain functions, these would be reflected in cognitive performance, emotional interference or frontal alpha asymmetry. Furthermore, comparing N1 amplitude to targets when VNS is on to when it is off allows evaluating impact of VNS on attentional processes.

MATERIALS AND METHODS

Subjects

Twenty patients (12 male and 8 female; 45 ± 13 years old) treated with VNS due to refractory epilepsy participated in this study, Table 1. VNS Therapy® System (Cyberonics, Inc.) was implanted by neurosurgeons at the Tampere University hospital. The implanted VNS device consists of a helical bipolar electrode surrounding the left cervical vagus nerve and a programmable pulse generator at the upper left chest. The therapeutic goal of VNS is to control seizure frequency and improve general well-being. Stimulation parameters used for clinical treatment are adjusted and optimized by neurologists from the hospital.

To rule out subjects with severe depression, subjects filled in Beck Depression Inventory (BDI). Three subjects were excluded from the data analysis due to poor performance (total error rate over 15%). All patients provided their written consent for participation. The study was approved by the regional ethical committee of Tampere University Hospital, Tampere, Finland, and conducted in accordance with the guidelines set forth in the Declaration of Helsinki governing the treatment of human subjects.

Table 1. Medical information of the subjects.

Patient ID	Age (y) at Diagnosis	Types of Epilepsy	Duration of Stimulation (months)	BDI score	Medication
V01	25	Multifocal	6	5	Levetiracetam, Oxcarbazepine, Zonisamide
V02	22	Temporal	5	15	Escitalopram, Lamotrigine, Lacosamide, Zonisamide
V03	17	Frontal	100	0	Levetiracetam, Oxcarbazepine, Lacosamide, Zonisamide
V04*	3	Multifocal	104	8	Valproic Acid, Vigabatrin, Topiramate, Olanzapine
V05	9	Parietal	82	14	Lamotrigine, Zonisamide,
V06	5	Temporal	4	11	Escitalopram, Clobazam, Carbamazepine, Lacosamide
V07*	25	Multifocal	99	0	Quetiapine, Lamotrigine, Mirtazapine, Quetiapine, Lacosamide, Zonisamide
V08	1	Multifocal	108	0	Perampanel, Lacosamide
V09	8	Temporal	4	6	Valproic Acid, Levetiracetam, Lamotrigine, Zonisamide
V10	1	Temporal	52	4	Pregabalin, Lacosamide, Eslicarbazepine acetate
V11[e]	19	Temporal	86	7	Carbamazepine, Zonisamide
V12*	2	Multifocal	109	19	Gabapentin, Lacosamide
V13	16	Multifocal	51	17	Clobazam, Lamotrigine, Zonisamide
V14	13	Fronto-temporal	61	4	Carbamazepine, Lacosamide, Pregabalin, Perampanel
V15	9	Multifocal	38	10	Topiramate, Valproic Acid, Clobazam
V16	18	Fronto-temporal	4	0	Carbamazepine, Clobazam
V17	46	Temporal	63	4	Oxcarbazepine
V18	20	Fronto-parietal	2	18	Lamotrigine, Valproic Acid, Perampanel, Clobazam
V19	27	Multifocal	5	10	Levetiracetam, Oxcarbazepine
V20	50	Unknown	130	16	Oxcarbazepine, Clonazepam, Levetiracetam, Gabitril

* = subjects excluded in all data analysis; [e] = excluded in the event-related potential data analysis; BDI = Beck Depression Inventory.

Experimental design

The Executive-RT test

Subjects performed a computer-based Executive-RT test (Figure 1A) as described in our previous studies (Hartikainen et al. 2014; Sun et al. 2015). The participants had to store the orientation of the triangle in their working memory and press the corresponding button after the Go signal while withholding from responding after a NoGo signal. The Go or NoGo signals were alternated so that in half of the trials a green traffic light was a Go signal and in half of the trials red light was a Go signal.

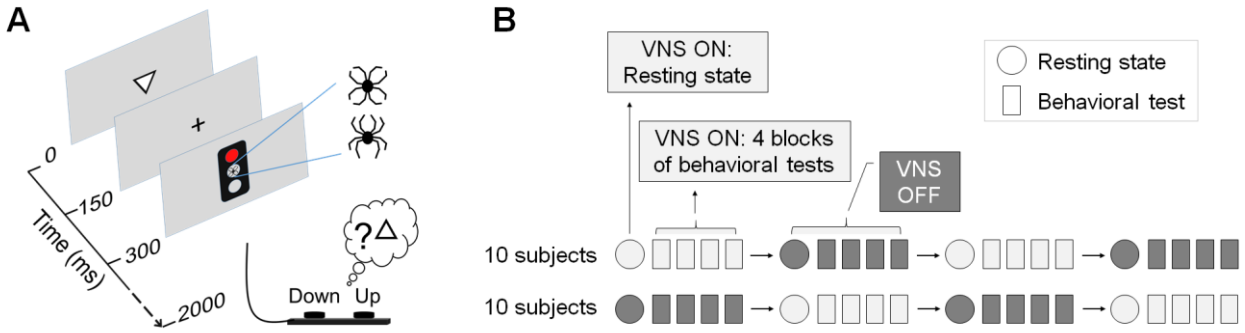

Figure 1. The experimental design. A) The Executive-RT test. At the onset of each trial a triangle was presented pointing either up or down, and relying on their working memory subjects needed to report the orientation of the previously presented triangle by pressing one of two buttons in case of a Go-signal, the traffic light, presented at 300 ms. The color of the traffic light was a Go or a NoGo signal indicating weather subject was supposed to respond or withhold from responding. In the middle of the traffic light an irrelevant emotional or emotionally neutral line-drawing was presented. This distractor was either a spider-like shape conveying negative threat-related information or an emotionally neutral non-threatening figure as a control. The elements composing the distractors were identical to one another and only the configuration of the figure differed. This allows for efficient control of low-level visual attributes such as color, brightness, contrast, etc. (Vuilleumier & Schwartz 2001) The duration of one trial was two seconds. B). The stimulation protocol. VNS status (ON and OFF) was counter balanced, with 10 subjects beginning the task with cyclic VNS ON and the remaining 10 beginning the task with VNS OFF. Each VNS status started with a four-minute resting state and then four blocks of behavioral testing, where each block of test contained 64 two-second trials.

Within the test, orientations of the triangles, sequence of Go/NoGo signals and types of distractors were all randomized. The behavioral test was presented and data collected with Presentation software (Neurobehavioral System, Inc. Berkeley, CA, USA). The patients were required to respond as fast and accurately as possible and only RTs of correct response were included in the analysis. Behavioral outcome of the Executive-RT test includes reaction times to

different stimuli and three error types, i.e. incorrect button presses (in Go trials), misses (no button press in Go trials) and commission errors (any button press in NoGo trials). In general, incorrect button presses in Go trials reflect lapses in working memory performance, a miss signifying a failure to initiate a response within given time and commission error in NoGo trials a failure in response inhibition.

The stimulation protocol

During the experiment, VNS ON refers to VNS cycling with stimulation on for 30 seconds and off for 48 seconds, ensuring two duty cycles with stimulation in each ON block of behavioral testing. When stimulation was turned ON the output current was set to 1.5-1.75 mA depending on the subjects' tolerance. If the subjects' clinical tolerance was higher than 1.75 mA, we use 1.75 mA. If their clinical tolerance was lower than 1.75 mA, we used their clinically used current. This approach, i.e. the current is the same to subject's clinical setting or less, ensured that subjects would not have inconvenience during the experiment which could affect their performance. When VNS was OFF the current was set to 0 mA. The stimulation frequency was 30 Hz and pulse width 250 μs. Every time when VNS stimulator status was changed there was a resting period before the test was continued to allow for sensory habituation (Figure 1B). In light of the potential sensory reactions due to VNS, the study is not eligible for blind design.

Analysis of EEG

EEG was recorded during the Executive-RT test with actiCAP Ag/AgCl electrodes and the 64-channel QuickAmp amplifier (Brain Products GmbH, Gilching, Germany) and digitized at 500 Hz sampling rate. Impedance of all electrodes was kept below 5 kΩ during the recording. Offline EEG data was analyzed with Brain Vision Analyzer2 software (Brain Products GmbH, Germany). Initial processing of EEG data included down sampling to 250 Hz and ocular movement correction using the ICA (Independent Component Analysis) ocular correction function, where one or two ICA components representing ocular movement artifact were removed.

In the analysis of parietal-occipital N1 potential, EEG signal was re-referenced to linked earlobe reference. EEG signal was band-pass filtered at 0.1-30 Hz and segmented into 1000 ms segments starting 200 ms before the onset of each trial. Segments were baseline-corrected and then subjected for artifact rejection where any segment with amplitude exceeding ± 80 μV was rejected. The remaining segments were averaged to yield the ERP (event-related potential)

waveform. N1 was defined as the negative peak detected between 150 and 250 ms after trial onset. Peak amplitude of N1 component was exported for statistical analysis. For the analysis of N1 amplitude, one more subject was excluded due to epileptiform activity leading to excessive artifacts and unidentifiable ERPs.

In the analysis of frontal alpha asymmetry, EEG signal was re-referenced to Cz electrode. After band-pass filtering at 3-30 Hz, EEG signal was segmented into 2000 ms segments starting from the onset of each trial. Then the segments were subjected for artifact rejection where any segment with amplitude exceeding ± 80 μV was rejected. The remaining segments were applied Fast Fourier transform (FFT) to calculate the power spectrum ($\mu V^2/Hz$) which was averaged. Finally, the alpha (8-13 Hz) power was analyzed at EEG electrodes F3 and F4 typically used for assessing effects related to affect and motivation (Davidson 1995). Alpha power was log-transformed and the asymmetry was calculated by subtracting the log-transformed alpha power at F4 by those at F3.

Statistical analysis

RTs, ERPs and the frontal alpha asymmetry were analyzed using repeated measure ANOVA. In the analysis of reaction time, VNS status (ON vs. OFF) and emotional valance (negative vs. neutral) were used as factors. In the analysis of N1 amplitude and the frontal alpha asymmetry, VNS status, emotional valence, and response types (Go vs. NoGo) were used as factors. The parieto-occipital region of interest covered electrodes P1, Pz, P2, PO1, POz, PO2, O1, Oz and O2. Interaction effect was followed by further post hoc analysis.

All data analyzed with repeated measure ANOVA were checked for normality and transformed if necessary. RTs were skewed to the right and thus log-transformed. N1 amplitudes were normally distributed and did not need transformation. Frontal alpha asymmetry data were not normally distributed and were transformed by subtracting personal mean from each data point, thus shifting the personal mean of all subjects to zero.

Errors were analyzed using generalized mixed effects logistic regression model. Separate models were made for each error type predicting probability to make an error of a given type using subject, VNS status and emotional valence as predictors. Subject was a random effect, while VNS

status and emotional valence were fixed effects. Trial outcomes were dichotomized into either "error" or "other" classes. In the incorrect button press model "error" class included incorrect button presses and "other" class included correct and missing button presses. In the missing response model "error" included missing button presses and "other" class included correct and incorrect button presses. In the commission error model "error" class included any button presses during NoGo trials and "other" class included no response cases which were correct responses in NoGo trials.

All statistical analysis was done using R statistics (version 3.1.1., the R-foundation for Statistical Computing). Repeated measure ANOVA was done using ez package (version 4.2-2) and regression analysis using lme4 package (version 1.1-10).

RESULTS

VNS, working memory and visual attention

Subjects were required to hold the orientation of the triangle in working memory and indicate the orientation of the triangle by pressing the correct button after a Go signal. Analysis of incorrect button presses revealed a main effect of VNS status, where cyclic VNS ON reduced the probability of making such errors, OR = 0.63 (95% CI 0.47-0.85) (Figure 2A). In addition to improved cognitive performance, increase in N1 event-related brain potential amplitude to targets over the parieto-occipital region was observed, F (1, 15) = 10.17, p = 0.006, η^2_G = 0.01 (Figure 2B & 2C). VNS status had no effect on other error types. Emotional valence of the distractor had no effect on any errors.

VNS and emotional reactivity

Analysis of RTs revealed an interaction between VNS status and emotional valence, F(1, 16) = 5.15, p = 0.04. Post hoc analysis revealed that cyclic VNS ON led to increased RTs only when there were negative threat-related distractors, F(1, 16) = 8.23, p = 0.01, η^2_G = 0.004 (Figure 3A). VNS status had no effect on RTs in the context of neutral distractors, F(1, 16) = 0.48, p = 0.50.

We also investigated the impact of VNS on the task-related frontal alpha asymmetry in the context of negative threat-related and neutral non-threat related distractor. There was a main effect of VNS status, F(1, 16) = 7.37, p = 0.02, η^2_G = 0.17, where VNS increased frontal alpha asymmetry

(cyclic VNS ON -0.082 ± 0.33, VNS OFF -0.055 ± 0.35). There was also an interaction between VNS status and emotional valence, F(1, 16) = 7.13, p = 0.02. Post hoc analysis revealed that cyclic VNS ON increased frontal alpha asymmetry only when there were negative threat-related distractors, F(1, 16) = 11.79, p = 0.003, η^2_G = 0.35. (Figure 3B). VNS status did not affect frontal alpha asymmetry with neutral non-threatening distractors, F(1, 16) = 0.54, p = 0.47.

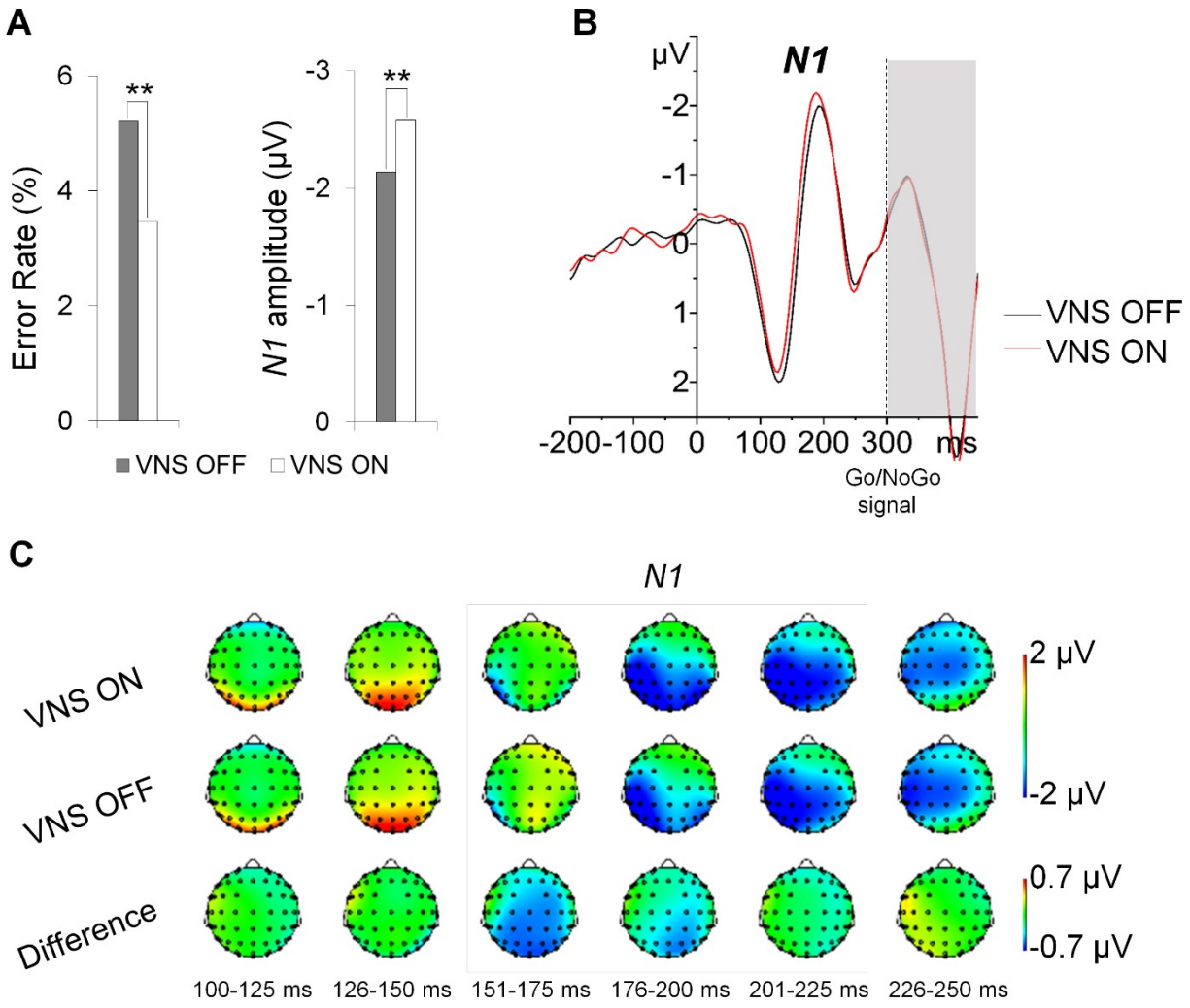

Figure 2. VNS improved working memory performance and enhanced visual attention. A) When cyclic VNS was ON, subjects made fewer errors in a subtask that depended on working memory performance, i.e. in responding weather previously presented triangle was up or down. Also, VNS increased parieto-occipital N1 amplitude. B) Grand average ERPs over the parieto-occipital brain region (covering electrodes P1, Pz, P2, PO1, POz, PO2, O1, Oz and O2). C) VNS Difference waveform VNS ON - VNS OFF illustrates the topography of the increased negativity during N1 time window (150-250 ms) due to VNS. ** = p < 0.01.

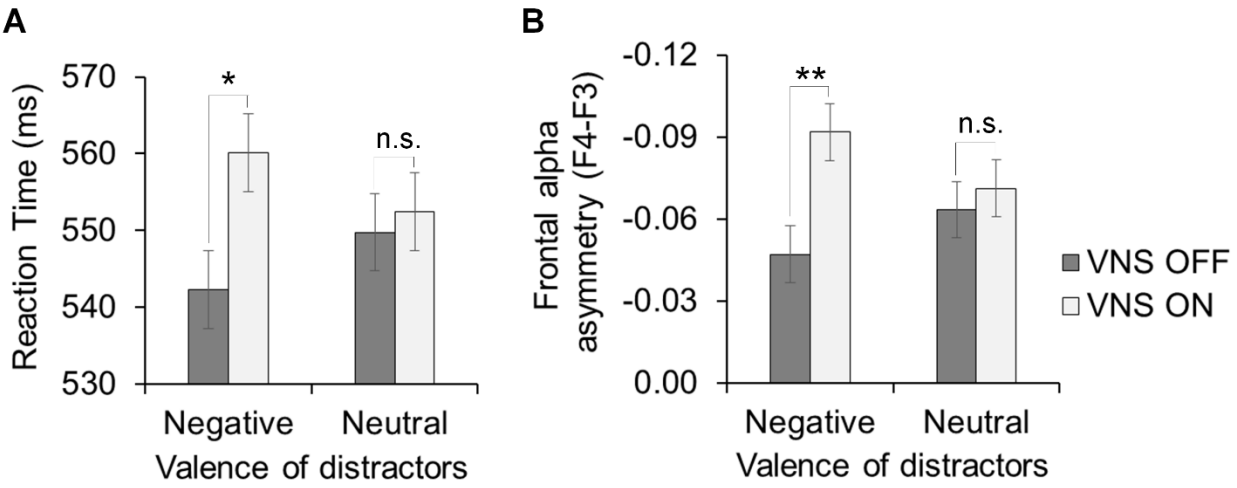

Figure 3. VNS increased threat-related behavioral and brain responses. A) In trials with emotionally negative threat-related distractors, VNS increased reaction times compared to when VNS was turned off. VNS status had no effect on reaction times in trials with neutral distractors. B) VNS increased frontal alpha asymmetry when there were emotionally negative threat-related distractors but not when there were neutral distractors. Error bar indicates Fisher's Least Significant Difference.

DISCUSSION

To our knowledge this is the first study to show immediate improvement of working memory performance with VNS stimulation in humans. Subjects made less errors on a subtask that relied on working memory when cyclic VNS was turned on in comparison to when it was turned off. Improved working memory performance lay the foundation for better cognitive performance in general. The current results provide evidence for the beneficial cognitive effects of VNS in treatment of epilepsy patients whose cognitive performance may be otherwise compromised due to epilepsy or the antiepileptic drugs. However, the patients analyzed in the current study were able to perform a rather demanding cognitive task, tapping into attention and executive function, at a high level indicating relatively intact cognition. Three of the subjects with poor performance suggesting compromised cognitive functioning were excluded from the analysis. Thus, with results obtained from patients with good cognitive performance, these results are probably not limited to only patients with epilepsy but may be generalizable to other subjects as well.

VNS increased early visual N1 amplitude similar to what is seen with increased level of attention (Mangun & Hillyard 1991; Luck & Ford 1998). Our findings suggest that attentional mechanisms might contribute to improved working memory performance due to VNS. Attention allows for

selecting information for further processing while working memory allows for information to be kept in an accessible state. Attention and working memory are interacting constructs and tightly intertwined, attention providing the basis for selecting what information will be encoded in working memory (Awh, Vogel & Oh 2006). Along with this electrophysiological attention-related brain response, the performance of the subjects improved in a task where subjects were supposed to indicate the orientation of a previously presented triangle by a corresponding button press. Greater N1 in response to triangles, whose orientation was maintained in working memory, suggests deeper processing and better fidelity of information encoding into working memory. In other words, improved selective attention allows for better working memory performance. General level of attention and performance remained unchanged with no other performance measures showing impact of VNS such as reaction times, missing responses or commission errors. With improved general attention or higher arousal levels, one might expect speeded reaction times or overall improvement in performance. However, specific improvement of working memory performance along with electrophysiological marker suggesting greater attention to targets encoded into working memory was observed due to VNS.

Besides the cognitive modulation of VNS, emotional effects were observed. When VNS was on, task-irrelevant threat-related distractors slowed reaction times and increased frontal alpha asymmetry in comparison to when stimulation was turned off. There seemed to be an increased vigilance to threat-related stimuli as an immediate effect of VNS stimulation. Weather emotional distractors have an impact on performance and on brain responses depends on several factors including task and subject-related factors (Hartikainen, Ogawa, Soltani & Knight 2007; Hartikainen, Siiskonen & Ogawa 2012; Mäki-Marttunen V. et al. 2014). Subject-related factors include mood with depression and anxiety typically increasing attention allocation to threat (MacLeod & Mathews 1988; Dalgleish & Watts 1990; Bishop 2008). Increased attention allocation to threat is also seen in patient groups with predisposition to depression such as mild head injury (Mäki-Marttunen V. et al. 2015), patients with orbitofrontal injury (Mäki-Marttunen Verónica et al. 2016) and epilepsy patients treated with deep brain stimulation (Hartikainen et al. 2014; Sun et al. 2015). In the current study fourteen subjects had no or minimal depression and six subjects had mild depression. Thus, as most subject did not suffer from depression it is unlikely to have a significant impact on the current findings. Furthermore, when comparing immediate effects of cyclic VNS stimulation, the mood can be controlled for as it is likely to remain relatively stable over

the short time periods of stimulation on and off and thus should cancel out in within subject design.

The increased vigilance to threat as seen in greater impact of threat-related distractors on behavior and brain responses may seem paradoxical to the use of VNS in treatment of depression. Greater attention to threat is a hallmark of anxiety (Kindt & Van Den Hout 2001). Meanwhile, both depression and anxiety are linked with dysregulation of NE (Goddard et al. 2010) and VNS is thought to modulate NE levels in the brain (Roosevelt, Smith, Clough, Jensen & Browning 2006). NE is known to have both anxiolytic and anxiogenic effects depending on several factors including the time course (Goddard et al. 2010). Thus, the time course of NE release and VNS stimulation, whether short term or chronic, is likely to be critical on the neuromodulatory impact of VNS on emotional responses and mood. While the relationship between the immediate effects of VNS observed in the current study and the mechanism of VNS alleviating depression remain speculatory, the observed effects may provide objective biomarkers of VNS's effect on emotion system that could be used in future studies linking effects of VNS on emotional processes and mood.

The current study shows that VNS has instant and direct effects on human cognitive and affective brain functions. These immediate effects on human working memory performance and brain's affective responses are probably linked to increased brain level of NE due to VNS (Vonck et al. 2014). It has been previously shown that VNS stimulation activates neurons in the LC and increases NE levels in neocortex, hippocampus, amygdala and other parts of the brain with efferent projections from LC (Hassert, Miyashita & Williams 2004; Raedt et al. 2011). According to the adaptive gain theory by Aston-Jones et al (2005), LC is normally driven by the utility assessment function processed in the orbitofrontal cortex and the anterior cingulate cortex which have direct connections to LC. The outcome of the utility assessment drives phasic firing of LC neurons increasing its instantaneous norepinephrine production, thus improving task performance (Aston-Jones & Cohen 2005). In rats' brains, high-density VNS (1 mA) leads to transient increase of NE in both cortical and limbic brain areas in comparison to the baseline NE level, i.e. the level of NE when VNS was off (Roosevelt et al. 2006). Increased NE level in hippocampus is reported to facilitate long-term potentiation which facilitates memory formation (O'Dell, Connor, Guglietta & Nguyen 2015). NE is also implicated in arousal related emotional memory and working memory

functions (Chamberlain, Muller, Blackwell, Robbins & Sahakian 2006). In line with our current findings of VNS improving working memory, moderate levels of NE may improve cognitive functions dependent on prefrontal networks such as working memory (Chamberlain et al. 2006). Increased NE level has been linked with increase in the amygdala activation while processing emotional pictures (Chamberlain et al. 2006; van Stegeren 2008). Increased amygdala activation may be one of the mechanisms of increased vigilance to threat due to VNS as observed in the current study.

Compared to previous studies, the current study on immediate effects of VNS on human executive functions holds methodological merits. Firstly, immediate comparison between stimulation ON and OFF allowed for controlling potential confounding factors including chronic effects of medication or alterations in seizure burden. Therefore, any observed difference can be attributed to the immediate and direct effect of VNS on cognitive and affective brain functions. Secondly, we used a relatively sensitive behavioral task, i.e. the Executive-RT test, which mimics everyday situations and engages several executive functions including working memory and emotional control (Hartikainen et al. 2010). Combination of EEG measurement and a computer based cognitive test with rapid presentation of stimuli along with challenging task allows for good control over general level of attention making it feasible to repeat the test over several cycles of stimulation on and off, thus providing a sensitive and reliable method for assessing the immediate effects of neuromodulation on brain functions (Hartikainen et al. 2014; Sun et al. 2015; Sun et al. 2016). Third, using clinically relevant VNS parameters in the current study extends the impact of the current findings beyond theoretical interest. In contrary, previous findings reporting the immediate beneficial effects of VNS on cognition used relatively lower current not commonly used in clinical treatment (Clark et al. 1999) or timing of VNS was linked to a specific phase of cognitive task, for example memory consolidation phase, which in real life setting is not feasible (Clark et al. 1995). Furthermore, although subjects were not completely blinded to VNS settings, any modulatory effect between ON and OFF conditions reflects the real-life effects of VNS. With vulnerable cognitive functions in these patients and with other treatments such as antiepileptic drugs frequently associated with compromised cognitive functions, evidence for positive effect of VNS on cognition is of significant clinical importance. It is also noteworthy, that these cognitive benefits are immediate to the stimulation and can be dissociated from the long-term chronic effects depending on multiple factors and often linked with plasticity.

In conclusion, we found that VNS has immediate and direct beneficial effects on human cognition. The use of clinically relevant VNS settings in this study extends the impact of these findings beyond theoretical interest. VNS increased early visual brain responses similar to enhanced attention and improved working memory performance. In addition to showing immediate beneficial effects of VNS on cognition in epilepsy patients, whose cognition may be slightly compromised due to antiepileptic drugs or the brain pathology related to epilepsy, beneficial effect of VNS on cognition might not be limited to patients with epilepsy. To that end, these findings call for future research on the potential benefit of VNS on cognitive enhancement or as a clinical intervention in cognitive dysfunction or attentional deficits in other patient groups.

ACKNOWLEDGEMENT

This research was supported by the Academy of Finland and the Competitive Research Fund of Pirkanmaa Hospital District. The authors declare no competing financial interests.

REFERENCES

Aston-Jones, G. & J. D. Cohen (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual review of neuroscience 28*, 403-450. DOI: 10.1146/annurev.neuro.28.061604.135709.

Aston-Jones, G., M. T. Shipley, G. Chouvet, M. Ennis, E. van Bockstaele, V. Pieribone, . . . et al. (1991). Afferent regulation of locus coeruleus neurons: anatomy, physiology and pharmacology. *Progress in brain research 88*, 47-75. http://www.ncbi.nlm.nih.gov/pubmed/1687622.

Awh, E., E. K. Vogel & S. H. Oh (2006). Interactions between attention and working memory. *Neuroscience 139(1)*, 201-208. DOI: 10.1016/j.neuroscience.2005.08.023.

Ben-Menachem, E., R. Manon-Espaillat, R. Ristanovic, B. J. Wilder, H. Stefan, W. Mirza, . . . J. F. Wernicke (1994). Vagus nerve stimulation for treatment of partial seizures: 1. A controlled study of effect on seizures. First International Vagus Nerve Stimulation Study Group. *Epilepsia 35(3)*, 616-626. http://www.ncbi.nlm.nih.gov/pubmed/8026408.

Bishop, S. J. (2008). Neural mechanisms underlying selective attention to threat. *Annals of the New York Academy of Sciences 1129*, 141-152. DOI: 10.1196/annals.1417.016.

Chamberlain, S. R., U. Muller, A. D. Blackwell, T. W. Robbins & B. J. Sahakian (2006). Noradrenergic modulation of working memory and emotional memory in humans. *Psychopharmacology (Berl) 188(4)*, 397-407. DOI: 10.1007/s00213-006-0391-6.

Clark, K. B., S. E. Krahl, D. C. Smith & R. A. Jensen (1995). Post-training unilateral vagal stimulation enhances retention performance in the rat. *Neurobiol Learn Mem 63(3)*, 213-216. DOI: 10.1006/nlme.1995.1024.

Clark, K. B., D. K. Naritoku, D. C. Smith, R. A. Browning & R. A. Jensen (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nature neuroscience 2(1)*, 94-98. DOI: 10.1038/4600.

Dalgleish, T. & F. N. Watts (1990). Biases of attention and memory in disorders of anxiety and depression. *Clinical Psychology Review 10(5)*, 589-604.

Davidson, R. J. (1995). Cerebral asymmetry, emotion, and affective style. Brain asymmetry, Canmbridge, MA: MIT**:** 361-387.

Dodrill, C. B. & G. L. Morris (2001). Effects of Vagal Nerve Stimulation on Cognition and Quality of Life in Epilepsy. *Epilepsy & behavior : E&B 2(1)*, 46-53. DOI: 10.1006/ebeh.2000.0148.

Elger, G., C. Hoppe, P. Falkai, A. J. Rush & C. E. Elger (2000). Vagus nerve stimulation is associated with mood improvements in epilepsy patients. *Epilepsy research 42(2-3)*, 203-210. http://www.ncbi.nlm.nih.gov/pubmed/11074193.

Goddard, A. W., S. G. Ball, J. Martinez, M. J. Robinson, C. R. Yang, J. M. Russell & A. Shekhar (2010). Current perspectives of the roles of the central norepinephrine system in anxiety and depression. *Depression and anxiety 27(4)*, 339-350. DOI: 10.1002/da.20642.

Grimonprez, A., R. Raedt, C. Baeken, P. Boon & K. Vonck (2015a). The antidepressant mechanism of action of vagus nerve stimulation: Evidence from preclinical studies. *Neuroscience and biobehavioral reviews 56*, 26-34. DOI: 10.1016/j.neubiorev.2015.06.019.

Grimonprez, A., R. Raedt, J. Portelli, I. Dauwe, L. E. Larsen, C. Bouckaert, . . . K. Vonck (2015b). The antidepressant-like effect of vagus nerve stimulation is mediated through the locus coeruleus. *Journal of psychiatric research 68*, 1-7. DOI: 10.1016/j.jpsychires.2015.05.002.

Hartikainen, K. M., K. H. Ogawa, M. Soltani & R. T. Knight (2007). Emotionally arousing stimuli compete for attention with left hemispace. *Neuroreport 18(18)*, 1929-1933. DOI: 10.1097/WNR.0b013e3282f1ca18.

Hartikainen, K. M., A. R. Siiskonen & K. H. Ogawa (2012). Threat interferes with response inhibition. *Neuroreport 23(7)*, 447-450. DOI: 10.1097/WNR.0b013e3283531e74.

Hartikainen, K. M., L. Sun, M. Polvivaara, M. Brause, K. Lehtimäki, J. Haapasalo, . . . J. Peltola (2014). Immediate effects of deep brain stimulation of anterior thalamic nuclei on executive functions and emotion-attention interaction in humans. *Journal of clinical and experimental neuropsychology 36(5)*, 540-550. DOI: 10.1080/13803395.2014.913554.

Hartikainen, K. M., M. Waljas, T. Isoviita, P. Dastidar, S. Liimatainen, A. K. Solbakk, . . . J. Ohman (2010). Persistent symptoms in mild to moderate traumatic brain injury associated with executive dysfunction. *Journal of clinical and experimental neuropsychology 32(7)*, 767-774. DOI: 10.1080/13803390903521000.

Hassert, D. L., T. Miyashita & C. L. Williams (2004). The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. *Behav Neurosci 118(1)*, 79-88. DOI: 10.1037/0735-7044.118.1.79.

Kalia, M. & J. M. Sullivan (1982). Brainstem projections of sensory and motor components of the vagus nerve in the rat. *The Journal of comparative neurology 211(3)*, 248-265. DOI: 10.1002/cne.902110304.

Kindt, M. & M. Van Den Hout (2001). Selective attention and anxiety: A perspective on developmental issues and the causal status. *Journal of Psychopathology and Behavioral Assessment 23(3)*, 193-202.

Klinkenberg, S., H. J. Majoie, M. M. van der Heijden, K. Rijkers, L. Leenen & A. P. Aldenkamp (2012). Vagus nerve stimulation has a positive effect on mood in patients with refractory epilepsy. *Clin Neurol Neurosurg 114(4)*, 336-340. DOI: 10.1016/j.clineuro.2011.11.016.

Liimatainen, J., J. Perakyla, K. Jarvela, T. Sisto, A. Yli-Hankala & K. M. Hartikainen (2016). Improved cognitive flexibility after aortic valve replacement surgery. *Interact Cardiovasc Thorac Surg*. DOI: 10.1093/icvts/ivw170.

Luck, S. J. & M. A. Ford (1998). On the role of selective attention in visual perception. *Proceedings of the National Academy of Sciences of the United States of America 95(3)*, 825-830. http://www.ncbi.nlm.nih.gov/pubmed/9448247.

MacLeod, C. & A. Mathews (1988). Anxiety and the allocation of attention to threat. *The Quarterly journal of experimental psychology 40(4)*, 653-670.

Mangun, G. R. & S. A. Hillyard (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *Journal of experimental psychology. Human perception and performance 17(4)*, 1057-1074. http://www.ncbi.nlm.nih.gov/pubmed/1837297.

Marangell, L. B., A. J. Rush, M. S. George, H. A. Sackeim, C. R. Johnson, M. M. Husain, . . . S. H. Lisanby (2002). Vagus nerve stimulation (VNS) for major depressive episodes: one year outcomes. *Biological psychiatry 51(4)*, 280-287. http://www.ncbi.nlm.nih.gov/pubmed/11958778.

McGlone, J., I. Valdivia, M. Penner, J. Williams, R. M. Sadler & D. B. Clarke (2008). Quality of life and memory after vagus nerve stimulator implantation for epilepsy. *Can J Neurol Sci 35(3)*, 287-296. http://www.ncbi.nlm.nih.gov/pubmed/18714795.

Merrill, C. A., M. A. Jonsson, L. Minthon, H. Ejnell, C. s. S. H, K. Blennow, . . . M. J. Sjogren (2006). Vagus nerve stimulation in patients with Alzheimer's disease: Additional follow-up results of a pilot study through 1 year. *J Clin Psychiatry 67(8)*, 1171-1178. http://www.ncbi.nlm.nih.gov/pubmed/16965193.

Mäki-Marttunen, V., V. Kuusinen, M. Brause, J. Peräkylä, M. Polvivaara, R. Dos Santos Ribeiro, . . . K. M. Hartikainen (2015). Enhanced attention capture by emotional stimuli in mild traumatic brain injury. *J Neurotrauma 32(4)*, 272-279. DOI: 10.1089/neu.2014.3557.

Mäki-Marttunen, V., V. Kuusinen, J. perakyla, K. Ogawa, M. Brause, A. Brander & K. Hartikainen (2016). Greater attention to task-relevant threat due to orbitofrontal lesion. *Journal of Neurotrauma In press*.

Mäki-Marttunen, V., N. Pickard, A. K. Solbakk, K. H. Ogawa, R. T. Knight & K. M. Hartikainen (2014). Low attentional engagement makes attention network activity susceptible to emotional interference. *Neuroreport 25(13)*, 1038-1043. DOI: 10.1097/WNR.0000000000000223.

O'Dell, T. J., S. A. Connor, R. Guglietta & P. V. Nguyen (2015). beta-Adrenergic receptor signaling and modulation of long-term potentiation in the mammalian hippocampus. *Learn Mem 22(9)*, 461-471. DOI: 10.1101/lm.031088.113.

Penry, J. K. & J. C. Dean (1990). Prevention of intractable partial seizures by intermittent vagal stimulation in humans: preliminary results. *Epilepsia 31 Suppl 2*, S40-43. http://www.ncbi.nlm.nih.gov/pubmed/2121469.

Perez-Edgar, K., A. Kujawa, S. K. Nelson, C. Cole & D. J. Zapp (2013). The relation between electroencephalogram asymmetry and attention biases to threat at baseline and under stress. *Brain Cogn 82(3)*, 337-343. DOI: 10.1016/j.bandc.2013.05.009.

Raedt, R., R. Clinckers, L. Mollet, K. Vonck, R. El Tahry, T. Wyckhuys, . . . A. Meurs (2011). Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *J Neurochem 117(3)*, 461-469. DOI: 10.1111/j.1471-4159.2011.07214.x.

Roosevelt, R. W., D. C. Smith, R. W. Clough, R. A. Jensen & R. A. Browning (2006). Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. *Brain Res 1119(1)*, 124-132. DOI: 10.1016/j.brainres.2006.08.048.

Rush, A. J., M. S. George, H. A. Sackeim, L. B. Marangell, M. M. Husain, C. Giller, . . . R. Goodman (2000). Vagus nerve stimulation (VNS) for treatment-resistant depressions: a multicenter study. *Biological psychiatry 47(4)*, 276-286. http://www.ncbi.nlm.nih.gov/pubmed/10686262.

Sackeim, H. A., J. G. Keilp, A. J. Rush, M. S. George, L. B. Marangell, J. S. Dormer, . . . H. Zboyan (2001a). The effects of vagus nerve stimulation on cognitive performance in patients with treatment-resistant depression. *Neuropsychiatry, neuropsychology, and behavioral neurology 14(1)*, 53-62. http://www.ncbi.nlm.nih.gov/pubmed/11234909.

Sackeim, H. A., A. J. Rush, M. S. George, L. B. Marangell, M. M. Husain, Z. Nahas, . . . R. R. Goodman (2001b). Vagus nerve stimulation (VNS) for treatment-resistant depression: efficacy, side effects, and predictors of outcome. *Neuropsychopharmacology 25(5)*, 713-728. DOI: 10.1016/S0893-133X(01)00271-8.

Sjogren, M. J., P. T. Hellstrom, M. A. Jonsson, M. Runnerstam, H. C. Silander & E. Ben-Menachem (2002). Cognition-enhancing effect of vagus nerve stimulation in patients with Alzheimer's disease: a pilot study. *J Clin Psychiatry 63(11)*, 972-980. http://www.ncbi.nlm.nih.gov/pubmed/12444809.

Sun, L., J. Perakyla, M. Polvivaara, J. Öhman, J. Peltola, K. Lehtimäki, . . . K. M. Hartikainen (2015). Human anterior thalamic nuclei are involved in emotion-attention interaction. *Neuropsychologia 78*, 88-94. DOI: 10.1016/j.neuropsychologia.2015.10.001.

Sun, L., J. Peräkylä, A. Kovalainen, K. H. Ogawa, P. J. Karhunen & K. M. Hartikainen (2016). Human Brain Reacts to Transcranial Extraocular Light. *PLoS One 11(2)*, e0149525. DOI: 10.1371/journal.pone.0149525.

Van Bockstaele, E. J., J. Peoples & P. Telegan (1999). Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain: evidence for a monosynaptic pathway. *The Journal of comparative neurology 412(3)*, 410-428. http://www.ncbi.nlm.nih.gov/pubmed/10441230.

van Stegeren, A. H. (2008). The role of the noradrenergic system in emotional memory. *Acta psychologica 127(3)*, 532-541. DOI: 10.1016/j.actpsy.2007.10.004.

Vonck, K., R. Raedt, J. Naulaerts, F. De Vogelaere, E. Thiery, D. Van Roost, . . . P. Boon (2014). Vagus nerve stimulation...25 years later! What do we know about the effects on cognition? *Neuroscience and biobehavioral reviews 45*, 63-71. DOI: 10.1016/j.neubiorev.2014.05.005.

Vuilleumier, P. & S. Schwartz (2001). Beware and be aware: capture of spatial attention by fear-related stimuli in neglect. *Neuroreport 12(6)*, 1119-1122. http://www.ncbi.nlm.nih.gov/pubmed/11338176.