Neural engineering solutions for the management of epilepsy

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Neural engineering solutions for the management of epilepsy

PhD Thesis by

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Preface

The work presented here was carried out during my employment as a PhD fellow (December, 2006 to August , 2010) at the Center for Sensory-Motor Interaction, Aalborg University, Denmark. The work was supported by The Danish Advanced Technology Foundation and Aalborg University, and would not have been possible without the guidance, help and support of the following peoples, to which I would like to express my gratitude:

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To the technicians at Aalborg Biomedical Laboratory: Ole Sørensen, Torben Madsen and Jens Sørensen, for their generous help and support during my many days at the animal lab.

Finally to my wife, Nete and my two children, Aya and Tristan, for their patience and support.

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English summary

With a prevalence of around 1 %, epilepsy is one of the most common neural disorders in the world. About 30 % of the patients do not achieve satisfactory seizure control by means of conventional treatments such as anti-epileptic drugs (AED) and surgery. Electrical stimulation of the vagus nerve (VNS) may be an option for treating these refractory patients, however, only about half of the patients respond to this treatment and few become totally seizure free. Thus improvements and new methods are needed for the treatment of refractory epilepsy. The current work investigated two neural engineering methods, which may improve the management of uncontrolled seizures.

Studies show that VNS initiated prior to or during the early start of seizures may alleviate, shorten or even prevent seizures from occurring. In order to realize that kind of treatment, early seizure detection is needed. The current study investigated if seizures can be detected early and monitored using neural recordings (ENG) from the vagus nerves. Our results show that a specific cardiac-related firing pattern could be derived from the vagus-ENG (VENG) recordings in a rat model. These firing patterns changed more than 80 s prior to and during seizures, thus indicating that seizure activity may be detected early and monitored using the VENG. The detection method may be suitable especially for controlling VNS, as stimulation and seizure detection could be performed using the same vagus nerve electrode. However, further studies are needed to evaluate if these (pre-)ictal changes are also present in other animal models and in humans.

Seizure inhibition via electrical stimulation may not be exclusively related to the vagus nerve, thus stimulation of other neural structures and peripheral nerves have been shown to induce seizure inhibition too. We investigated if dorsal epidural spinal cord stimulation (SCS), which is a well-known treatment for chronic pain, could be used to modulate seizure susceptibility. Our results showed that 4 Hz SCS induced a clear pro-convulsive effect, whereas 54 Hz SCS caused a statistically non-significant trend towards an anti-convulsive effect. Thus, seizure susceptibility may clearly be modulated by means of SCS, however, further research is needed to evaluate if stimulation parameters can be optimized to produce clinical relevant seizure inhibition.

Danish summary

Med en prævalens på omkring 1 % er epilepsi en af de mest udbredte neurologiske lidelser i verden. Omkring 30 % af patienterne opnår ikke en tilfredsstillende anfalds kontrol via konventionelle behandlinger såsom anti-epileptisk medicin eller operation. Elektrisk stimulation af vagus nerven (VNS) kan være en behandlingsmulighed for disse svært-behandlbare patienter, men kun omkring halvdelen har gavn af behandlingen og kun få bliver helt anfaldsfri. Så der er stadig et behov for forbedringer og ny metoder i behandlingen af svært-behandelbar epilepsi. Dette arbejde undersøger to medikotekniske metoder som kan forbedre håndteringen af ukontrollerbare anfald.

Studier viser at hvis VNS startes før eller i starten af anfald kan dette lindre, afkorte eller helt modvirke at anfaldene opstår. For at muliggøre denne behandlingsform er det nødvendigt at anfald detekteres tidligt. Dette studie undersøgte om anfald kan detekteres tidligt og monitoreres ud fra målinger fra vagus nerven (VENG). Vores resultater fra en rotte model viser at et specielt hjerte relateret aktivitets mønster kunne udledes fra VENG. Disse mønstre ændredes mere end 80 s forud for samt under anfald, hvilket indikerer at anfald kan detekteres tidligt og monitoreres ved brug af VENG. Detektions metoden er specielt velegnet til kontrol af VNS, da stimulation og anfaldsdetektionen kan udføres ved hjælp af den samme vagus nerve elektrode. Dog er yderligere studier nødvendige for at fastslå om disse (præ-)iktale ændringer er tilstede i andre dyremodeller og i mennesker.

Inhibition af anfald via elektrisk stimulation er måske ikke eksklusiv relateret til vagus nerven, således har stimuleringen af andre neurale strukture og perifere nerver også vist sig at kunne inhibere anfald. Vi undersøgte om epidural dorsal stimulation af rygmarven, hvilket er en kendt behandling mod kronisk smerte, kunne påvirkede sårbarheden over for anfald. Vores resultater viser at 4 Hz SCS resulterede i en klar pro-konvulsiv effekt, hvorimod 54 Hz SCS resulterede i en trend mod en anti-konvulsiv effekt. Sårbarheden over for anfald kan således klar moduleres via SCS, dog er flere studier er nødvendige for at evaluere om stimulations parametrene kan optimeres til at inducere en klinisk relevant anfalds inhibition.

1. Introduction

Epilepsy covers a wide range of disorders characterized by a chronic increased seizures predisposition and consequently the occurrence of recurrent unprovoked seizures. The seizures are transient events of abnormal neural synchronization in the brain, which may affect consciousness and be associated with a wide range of motor symptoms (Engel, 2007, Fisher, 2005). Epilepsy occurs as an interplay between both genetic predispositions and exposure to risk factors such as infections, head trauma, tumors and stroke (Banerjee and Hauser, 2007). With an estimated prevalence of around 1 %, epilepsy is one of the most common neurologic disorders in the world (Banerjee and Hauser, 2007, Forsgren et al., 2005, Hauser, 1996, World Health Organization, 2005).

Current Treatments

The preferential treatment for epilepsy is administration of antiepileptic drugs (AED), but only about 2/3 of patients become seizure free by this means, whereas the others are refractory to medication (Kwan, 2004). Since the 1980s the development of new drugs has resulted in a two-fold increase of the number of available AEDs. However, this abundance of new drugs has primarily helped in reducing the adverse effects of the treatment while the fraction of medical refractory patients has remained rather constant (Arzimanoglou et al., 2010, Stacey, 2008).

Up to half of the medical refractory patients may be candidates for resective surgery, which may provide long time seizure freedom in more than half of the operated patients. However, it seems that this treatment is little used in practice (Beleza, 2009, Engel et al., 2008, Engel, 2003, smith et al., 1989). Improvements in diagnostic and surgical techniques continue to increase the fraction of patients, which may potentially be treated by surgery, but in some patients no seizure focus can be defined or it may be located in structures of essential functional importance and therefore surgery is not an option (Duchowny et al., 2008, Jobst, 2009). Patients, who cannot be adequately treated with the described conventional means, are candidates for vagus nerve (VN) stimulation (VNS) (Beleza, 2009). Numerous clinical trials have shown that this therapy is able to reduce seizure frequency in otherwise intractable epilepsy patients by more than 50 % in around half of the treated patients, whom, however, rarely become seizure free (De Herdt et al., 2007, DeGiorgio et al., 2000, Labar, 2004, Morris and Mueller, 1999).

Hence, in spite of the wide range of epilepsy treatments currently available, some patients continue to experience inadequate seizure control, which increases the risks of injuries, anxiety, stigmatization, social isolation, unemployment and mortality (Baker, 1997, Elliott et al., 2005). Thus, the need for improving existing treatments and to explore novel approaches persists.

Project goals

The current work addressed two different areas, which both may facilitate the management of epileptic seizures. First, if seizure could be predicted this would render possible a range of new interventions aimed to both prevent seizures from occurring and to enable countermeasures to minimize the impact of seizures. Research within this area has mainly been focused on predicting seizures based on bioelectric signals recorded from the brain. In this project, a novel approach for seizure prediction based on VN activity was investigated, as such an approach would be suitable for controlling on-demand VNS. This area is introduced in the following section and articles describing this work constitute chapters 2 and 3.

Second, the ability to inhibit seizures may not be exclusively related to the VN, but stimulation of other neural structures may be effective in the treatment of epilepsy too. Research within this area has mainly focused on direct stimulation of various brain structures. In the current work, the effect of spinal cord stimulation (SCS) on seizure susceptibility is evaluated. SCS is an established treatment for chronic pain and peripheral ischemia but received little attention with respect to epilepsy. The background for this work is described in section 1.2 and an article describing the work done constitutes chapter 4.

In Chapter 5, the main findings of this work are discussed and conclusions are given.

Seizure prediction based on vagus nerve activity

Seizure prediction

Prediction of seizures would provide several advantages for epilepsy patients with uncontrolled seizures. If seizures could be predicted prior to their electroencephalographic (EEG) and clinical onset, treatment procedures could automatically be initiated to prevent them and also, the patient and healthcare personal could take countermeasures to minimize the impact of pending seizures.

Considering such benefits, it is obvious why seizure prediction has been an area for research for more than three decades. Nearly all the work done in this area has been aimed at predicting seizures based on recordings derived from intracranial EEG (Mormann et al., 2007). Although the initial results of those studies seemed promising (Litt, 2002), they looked less encouraging after the availability of larger data sets and improved evaluation methods (Mormann et al., 2007). No EEG-based prediction method is yet available for treatment of epilepsy.

Seizures are, however, not manifest in the EEG exclusively: autonomic symptoms such as pupil dilatation, skin blushing, respiratory changes and cardiac changes may also occur in relation to seizures (Delamont, 1999, Devinsky, 2004, Freeman and Schachter, 1995, Sevcencu and Struijk, 2010, Terndrup et al., 1999, Terndrup et al., 1999). Such autonomic changes may provide a source of information to assess the ictal state of a patient without the need of intracranial electrodes. The limited work which has been done within this area has focused on the discovery of seizure precursors based on changes in the pre-ictal cardiac control, as they can be derived from the easily obtainable electrocardiogram (ECG). For

example, Novak et al. used heart rate variability (HRV) analysis and showed that the parasympathetic activity peaks 30 s prior to the onset of complex partial seizures and that sympathetic activity peaks at seizure onset. The initial changes observed in that study occurred up to two minutes prior to seizure onset (Novak et al., 1999). Similarly, Kerem et al. showed that seizures could be predicted by 30 s to 10 min prior to onset using phase space analysis of the RR-interval in epilepsy patients and in a rat model (Kerem and Geva, 2005). However, still no ECG based methods for seizure predication are available for clinical applications.

On-demand treatments

Automated seizure prediction, or at least early detection, could enable on-demand treatment to be performed primarily during periods with particular need for seizure inhibition, thus optimizing the efficiency and minimizing the adverse effects of a given treatment. Several forms of treatments could be initiated when a seizure is predicted or detected. One such treatment could be administration of AED directly into the epileptic focus, as this has been shown to be effective in animal models (Eder, 1997, Stein, 2000). Another experimental treatment to arrest ictal activity could be the cooling of the neural tissues which constitutes the epileptic focus (Rothman, 2009). However, the area which so far has received most attention as an on-demand epilepsy treatment is electrical neurostimulation. One such implantable on-demand neurostimulator system (Neuropace responsive neurostimulator) has been developed and is being tested in clinical trials. The system comprises a stimulator implanted in the scull and two intracranial leads through which ictal events are detected and stimulation is performed, see Fig 1.1. Inter-ictal and ictal bursts of brain activity are detected using very simple algorithms and electrical stimulation is performed in response (Skarpaas and Morrell, 2009, Sun et al., 2008). Long term follow up studies suggest an approximately 50 % seizure reduction in 50 % of patients receiving this treatment (Talan, 2010).

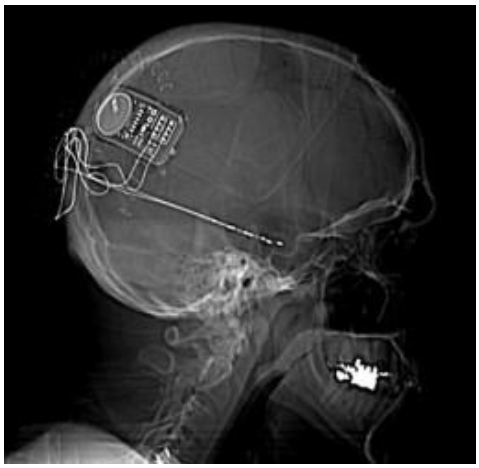

Fig 1.1 Neuropace system implanted in a patient (University of Rochester medical center, 2007).

In contrast with the above mentioned treatments, which are still experimental, VNS is already a widely used therapy in epilepsy, Fig. 1.2. The VN stimulator can also be triggered manually to stimulate prior to or during seizures. VNS is provided by a stimulator implanted in a sub-clavicular pocket and connected via leads tunneled under the skin to a cuff electrode mounted on the left VN. The treatment consists of scheduled stimulation typically for 30 s every 5 min and the induced seizure inhibiting effect tends to slowly increase over the first couple of years of treatment (Morris and Mueller, 1999). VNS was approved for the treatment of epilepsy in the mid-90s in both the EU and the USA. Since then, more than 60.000 patients have received an implant and nearly 10.000 stimulators are currently sold each year (Cyberonics, 2010).

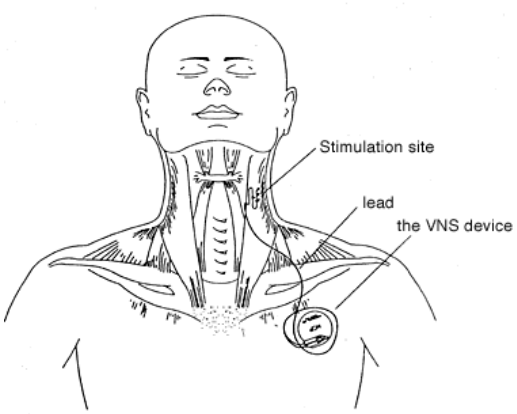

Fig. 1.2 VN stimulator is implanted in the a chest pocket and connected to a vagus nerve electrode in the neck. (The royal childrens's Hospital Melburn, 2009)

In addition to this conventional scheduled VNS, stimulation may also be triggered manually by sweeping a wrist-worn magnet over the implanted stimulator. The magnet-triggered stimulation lasts typically for 30 s to 60 s and is usually stronger than scheduled stimulation (Boon et al., 2001, Tatum, 2009). This form of control enables patients or caregivers to manually initiate on-demand stimulation on the suspicion of an impending seizure or during a seizure. Studies have shown that about half of VNS treated patients reported a benefit from this kind of on-demand stimulation, meaning that seizures are fully prevented, shortened or alleviated (Boon et al., 2001, Morris, 2003). These studies have, however, relied on the ability of patients and caregivers to predict or detect seizures and further to judge if seizure was prevented or shortened as a result of triggering VNS. These parameters may be difficult to assess objectively and also the task of triggering the stimulator might influence the outcome (Ricci et al., 1972). Indeed, in a group of patients where the stimulator was programmed not to stimulate when triggered with the magnet, a relatively large percentage (40.7 %) of patients reported a benefit compared to a group which received stimulation when using the magnet (52.5 %) (Morris, 2003). A recently developed system designed to trigger an implanted VN stimulator in response to external detection of seizures based on EEG and heart rate changes in VNS patients, may provide a better indication on how effective on-demand VNS could be (Shoeb et al., 2009). However, due to the very limited evaluation of this system in patients, results are still inconclusive.

In addition to studies in patients, several animal experiments also indicate an immediate seizure inhibitory effect of VNS. Thus, studies on experimentally induced seizures in rats and dogs have shown that VNS could abolish or shorten seizures, especially when initiated prior to or during the start of the seizures

(McLachlan, 1993, Takaya et al., 1996, Woodbury and Woodbury, 1990, Zabara, 1992). Given the immediate seizure inhibiting effect of VNS, the current scheduled form of VNS treatment might be improved by stimulating the VN on-demand, that is, prior to and during seizures. Although some patients already do this via the magnet-triggered stimulation method, many are incapable of triggering the device, either because they cannot operate the magnet, or they do not have any pre-ictal symptoms (i.e., prodrome or auras) warning them about oncoming seizures (Boon et al., 2001, Haut, 2007, Morris, 2003, Rajna et al., 1997). Thus, if an automated seizure prediction or detection method could be used for on-demand VNS, the efficiency of this treatment may increase. If such prediction methods are based on EEG recordings, additional cranial leads and electrodes would be required, which would increase the complexity of the surgery and increase the risk of complications. This could be avoided if the information required for providing on-demand VNS could be retrieved at the site of the implanted VN stimulator. Various methods have been suggested for the detection or the prediction of seizures, either to be used exclusively or in addition to EEG recordings. These methods include the use of: electrocardiogram (ECG), electromyogram (EMG), acceleration (accelerometers in implant) and respiratory activity (Adkins et al., 1999, Giftakis and Torgerson, 2010, Giftaktis and Graves, 2007, Lockard et al., 1994). To our knowledge, still no clinical systems are available based on these methods.

The first goal of this study was to develop a new method for the detection or the prediction of seizures based on the VN electroneurogram (VENG). As elaborated above, this would be most suitable for on-demand VNS since it is possible to record with the same VN electrode that is already used for stimulation and such an approach would thus not increase the complexity of the implantation procedure. Chapter 2 and 3 describe the work that was done within this area.

The effect of spinal cord stimulation on seizure susceptibility

Currently the only widely used form of electrical neurostimulation treatment of epilepsy is VNS. However, the ability to induce seizure inhibition by electrical stimulation may not be related to the VN exclusively. Considerable research has evaluated the effect of stimulating several other neural structures. Some approaches have directly targeted epileptic foci (Osorio, 2005, Skarpaas and Morrell, 2009), whereas others have focused on structures which may contain critical parts of the neural circuits involved in ictal activity (Mirski and Fisher, 1994, Mirski et al., 1997). The latter include the thalamus, where especially the effect of anterior thalamic stimulation has been evaluated (Fisher, 2010, Jobst, 2009, Mirski and Fisher, 1994). Although these intracranial neural stimulation treatments seem to be effective, the reduction in seizure frequency is typically similar to that reported with VNS (Ben-Menachem, 2002, Fisher, 2010, Talan, 2010), which does not require intracranial surgery. Until intracranial treatments show significantly better outcomes regarding seizure inhibition to justify the surgical complexity of the methods, it seems unlikely that these treatments will become equal alternatives to VNS.

The mechanisms of action of VNS are not fully known, but its antiepileptic effect seems to be mediated by activation of the afferent/sensory fibers in the VN (McLachlan, 1993, Zabara, 1992). Interestingly, other forms of sensory stimulation have been reported to prevent seizures. W. R. Gowers described in 1885 how tactile (touch), olfactory (smell) and gustatory (taste) stimulation could abolish pending seizures in his patients (Gowers, reprinted 1964).Later studies have consistently shown that auditory stimulation could abort absence seizures in humans as well (Rajna and Lona, 1989) and that tail warming and VNS were equally effective in reducing inter-ictal spikes in rats (McLachlan, 1993). More recently, DeGiorgio et al. reported that scheduled trigeminal nerve stimulation provided via cutaneous electrodes reduced seizure frequency by 59 % in medical refractory patients after 12 months of treatment (DeGiorgio, 2006, DeGiorgio, 2009). In contrast, photo, auditory and tactile stimulation have also been shown to promote and even to induce seizures (Forster and Madow, 1950, Harding and Jeavons, 1994, Manning and Uhlrich, 2009). It thus seems that sensory stimulation can exert both anti-convulsive and pro-convulsive effects.

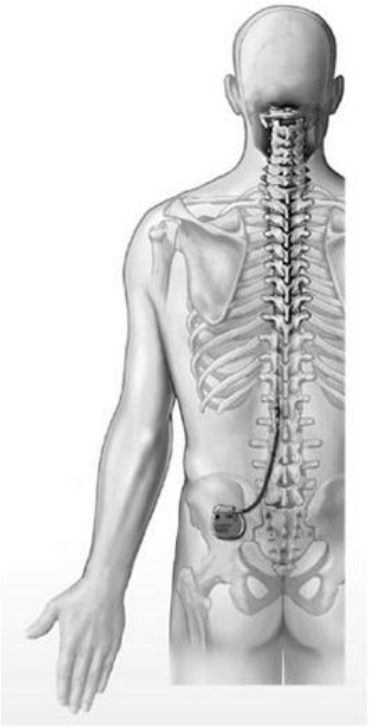

Fig. 1.3 Illustration of a patient with an implanted spinal cord stimulator (Capitol Spine & Pain Centers, 2010).

Considering the richness of sensory fibres running through the dorsal spinal cord columns (e.g. fasiculus gracilis and fasiculus cuneatus), spinal cord stimulation (SCS) might also be a way to modulate and inhibit seizure activity without the use of intracranial electrodes, see Fig. 1.3. While SCS has been shown to be a safe and effective method for the treatment of chronic neuropathic pain, angina pectoris and peripheral and cerebral ischemia (Broseta et al., 1986, Cameron, 2004, Murphy and Giles, 1987), the applicability of this therapy in epilepsy has received little attention. However, the few reported indirect results on potential antiepileptic effects of SCS are encouraging. Thus, a study in patients treated for pain showed that SCS significantly decreased the glutamergic mediated cortical excitability and also tended to increase Gamma Aminobutyric Acid (GABA) mediated inhibition (Schlaier et al., 2007). Interestingly the later increase in GABA mediated inhibition was also shown in epilepsy patients treated with VNS (Di Lazzaro, 2004). In addition, when Waltz et al. evaluated SCS as a treatment for spasticity, two patients with concomitant refractory epilepsy became seizure free while receiving this therapy (Waltz et al., 1981). The only study we found to directly investigate SCS as a treatment for epilepsy suggests that SCS may decrease seizure susceptibility, as the frequency of inter-ictal spikes in a rat epilepsy model was reduced by these means (Ozcelik et al., 2007).

The second objective of this project was to investigate alternative forms of neurostimulation for the treatment of epilepsy. Therefore, and based on the rationale elaborated above, the effect of SCS on seizure susceptibility has been evaluated in an experimental study in rats. This work is described in chapter 4.

2. Ictal and peri-ictal changes in cervical vagus nerve activity associated with cardiac effects

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3. Early seizure detection in rats based on vagus nerve activity

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4. The effect of spinal cord stimulation on seizure susceptibility

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5. Discussion, conclusion and perspectives

In spite of continuous advances within the development of new AEDs and surgical abilities, there still is a need for new treatments of epilepsy in general and refractory epilepsy in particular. The goal of this work was to investigate new means for improving the management of epilepsy. Two areas were investigated, the monitoring and early detection of ictal activity based on activity of the VN, and the effect of SCS on seizure susceptibility.

The experiments related to monitoring and early detection of seizures were carried out in an acute rat model of epileptic seizures, where seizures were induced by intravenous infusion of a PTZ solution (chapter 2 & 3). The results showed that the left and right VN exhibit distinct cardiac related firing patterns in normal conditions (i.e. in control animals and prior to administration of the epileptic agent PTZ in the epilepsy group, Fig. 2.3 & 2.4) and that this pattern is altered prior to and during convulsive seizures. Cardiac-related changes in the VENG were associated with increased parasympathetic cardiac tone. Interestingly, a cardiac-related VENG component which apparently originates from the efferent vagal activity persisted/increased in the VENG during seizures in contrast to other components. The cardiac-related VENG activity remained altered during the immediate post-ictal period and tended to return to baseline activity during the late post-ictal interval, about three minutes after the seizure offset (Fig. 2.2 to 2.4). When using the activity derived from the left VENG, it was possible to predict convulsive seizures more than 80 s prior to their onset.

The effect of SCS on seizure susceptibility was evaluated in a rat model where multiple seizures were induced by 10 min infusion of a PTZ solution (chapter 4). Dorsal SCS performed at a frequency in the typical range used for SCS treatment (54 Hz), did not significantly change any of the parameters used for quantifying seizure susceptibility. However, a small trend towards decreased seizure susceptibility was induced by 54 Hz SCS. In contrast, 4 Hz SCS induced 4 Hz SW-like ECoG activity and significantly increased seizure susceptibility.

Discussion

Seizure prediction based on vagus nerve activity

To enable on-demand treatment, seizures must be detected and, ideally, predicted. Baseline, pre-ictal, ictal and post-ictal VN activity as well as activity in controls were studied to explore possibilities of using VENG to derive information about the ictal status of patients.

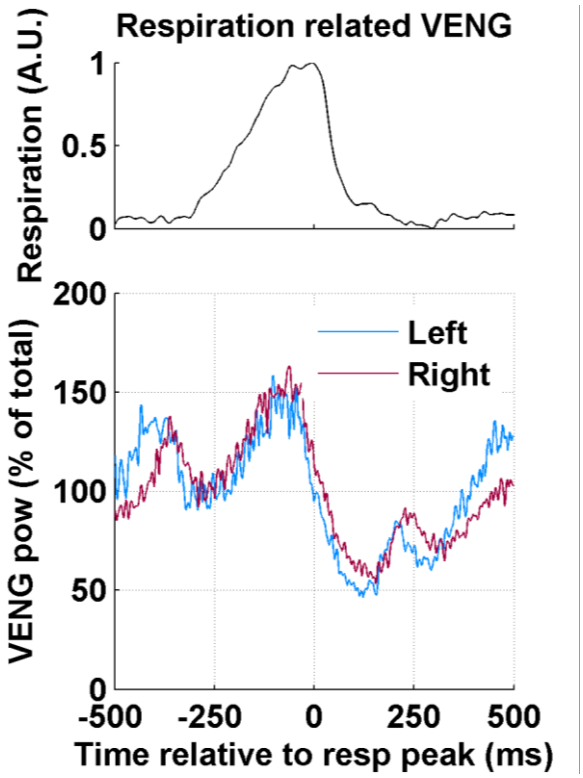

Fig 5.1 Respiration related VENG power based on 1 min of baseline recording in a control rat. Increases in VENG power is seen prior to and during respiration activity. The lowest drops reach around 50 % indicating that around half of the activity recorded is related to respiration.

Our results showed that the general vagal activity increased significantly during tonic-clonic seizures in the PTZ-rat model. This indicates that seizures could be detected in this model by monitoring the general level of the VENG. However, the increase in the general VENG power occurred simultaneous with the expansion of the thorax during convulsive seizures (KRN 1, Fig 4) and therefore this parameter may largely reflect ictal manifestations in the respiratory system. Indeed, the cervical vagus nerve contains efferent fibers innervating the larynx and afferent fibers originating from pulmonary stretch receptors. Since all those fibers fire in relation to respiration, the respiratory-related VENG power may constitute 40 - 60 % of the total VENG activity (unpublished findings, see 5.1). Therefore, changes in the respiratory pattern can cause marked changes in the general VENG power. Consistent with this observation, Paydarfar and Terndrup reported that the activity of efferent respiratory nerves, including the recurrent laryngeal branch of VN, becomes irregular and increases during seizures in cats and pigs (Paydarfar et al., 1991, Terndrup et al., 1999). As respiration can be controlled voluntarily and thus may be irregular, e.g. during talking, and as the changes in VENG power to a large extent reflect respiratory changes, this simple feature may not be specific enough for the prediction/detection and monitoring of seizures.

In contrast to respiration, cardiac function, although subjected to changes according to metabolic demands, is under very limited voluntary control and thus might provide more robust features. Interestingly, studies have shown that the cardiac control and functions may be affected by seizures, not only during the ictal period but also pre-ictally (Kerem and Geva, 2005, Novak et al., 1999, Sevcencu and

Struijk, 2010). An indirect way to access the cardiac control is to analyze the variations in the RR-intervals of the ECG (heart rate variability (HRV) analysis), which can provide an indirect estimate of the parasympathetic and sympathetic cardiac tones (Malik, 1996, Novak et al., 1999). Indeed, parameters derived from the RR-intervals have been shown to change prior to, during and following seizures (Delamont, 1999, Kerem and Geva, 2005, Novak et al., 1999). However, still no such methods are available for controlling on-demand treatment. Given that efferent and afferent cardiac fibers are present in the VN (Schultz, 2005), it may be expected that information concerning the cardiac parasympathetic control and the cardiovascular functions can be derived from the VENG. However, signals related to respiratory activity and various other types of visceral activities are also conducted through the vagus nerve, which hinders a direct assessment of cardiac control and functions.

In an attempt to access cardiac-related VENG (CrVENG) selectively, R-peak triggered averaging of the VENG activity was performed. This processing method emphasized the activity of those VN fibers that are active in phase with the cardiac cycle relative to other VN signals, In this way, the morphology of a CrVENG profile can be assessed and information regarding cardiac control can be extracted.

Our results showed that the left and the right VN exhibit different characteristic firing patterns in relation to the cardiac cycle (Fig 2.3 & 2.4). These differences may be due to functional asymmetry of the left vs. right vagus nerve (Ardell and Randall, 1986). The firing patterns could be divided into several components, C1 - C4. Based on the timing of the CrVENG components and on their correlation with parameters derived from the ECG, and based on the physiology of the VN, the physiological origin of the CrVENG components was suggested as follows. Activity following the P-wave and prior to the R-peak may be related to atrial contraction and ventricular filling, whereas activity following the R-peak may be related to the systolic increases in ventricular, coronary and aortic pressure. On the other hand, recordings in vagotomized rats showed that vagal firing around 50 ms after the R-peak is related to efferent parasympathetic activity, which indicates that the origin of at least parts of C3 is central. This analysis does not exclude that other CrVENG components are caused by parasympathetic control too. Further research is needed to verify these hypotheses and elucidate the physiological substrates of the CrVENG components.

The CrVENG of both nerves changed significantly during seizures. These changes were manifest as a decrease in VENG activity prior to the R-peak and a persistence or increase in the CrVENG components occurring after the R-peak. Post-ictally, the ictal firing patterns tended to return towards the pre-ictal firing patterns. No significant changes were seen prior to convulsive seizures when analyzing the VENG during a fixed 20 s interval just prior to convulsive seizure onset (Fig. 2.4). However, when the CrVENG analysis included the whole pre-ictal period in individual animals, detection of seizures was possible more than 80 s prior to the onset of the convulsive seizures (Chapter 3 & (Nielsen et al., 2008b)).

The feature configurations which were successfully used in the early detection of seizures (see "X" in Fig. 3.3) indicated a reduction in CrVENG activity around the R-peak (e.g. C1 in the left nerve, Fig. 2.3) relative to activity prior to and after the R-peak. This tendency was related to a general decrease in the phasic components of the CrVENG profile prior to seizures (Fig. 3.2c). A similar pattern, although more marked, was seen during seizure where the VENG activity occurring during the R-peak was reduced and activity following the R-peak actually tended to be increased (Fig. 2.4). These changes in the VN firing pattern probably reflect ictal-mediated changes in the parasympathetic cardiac control (i.e. C3 expansion in time and amplitude) and changes in cardiovascular functions. Using a pig epilepsy model Terndrup et al. showed that the firing of respiratory motor nerves becomes irregular during seizures (Terndrup et al., 1999). If a similar effect occurs in the cardiac control during seizures (e.g. due to ictal effects on vagal cardiac motor neurons), this might result in a reduction of the amplitude of the phasic CrVENG components and flattening of this profile, consequently.

There are some limitations to the presented study. Seizure prediction and detection methods have nearly always been based on human data, where e.g. preoperative assessment of patients subjected to epilepsy surgery may provide intracranial EEG recordings (Mormann et al., 2007, University Freiburg and the University Hospital Freiburg,). In comparison, VENGs from humans are not available and – considering the invasiveness of the methods, acquisition of such recordings during inter-ictal and ictal stages in epilepsy patients cannot be justified before clear indications of their usefulness have been provided. Thus, our study has been limited to data from an animal model. Our animals were anaesthetized, a state which clearly differs from the normal awake state in which a seizure detection/prediction application typically will be used. Due to this limitation it was not possible to evaluate if the CrVENG changes we observed were specific marker for seizures or if such changes occur due to normal fluctuations in cardiac function as well. Moreover, the systemic administration of PTZ involves a risk of direct effects of this drug on vagus nerve nuclei or the heart, which are not related to seizures. However, we do however not believe this has been the case in our study as the observed cardiac effects were clearly related to the ictal activity (Fig. 2.2). Thus, although the PTZ concentration remained constant throughout the recordings due to the long half-life of PTZ (2 hours in rats (Ramzan and Levy, 1985)), all the acquired parameters reverted during the post-ictal periods. Nevertheless, extrapolating the results from chemical induced seizures in animals to chronic epilepsy in human patients should be made with caution, as the mechanism supporting the seizure onset in these two situations may differ (Fisher, 1989). In addition, the different types of seizures and even the individual seizures in one and the same patient may also result in different cardiac manifestations (Sevcencu and Struijk, 2010).

Finally, to ensure similar pre-ictal conditions, the analysis for early detection of seizures (chapter 3) was restricted to only one pre-ictal period starting from baseline (prior to PTZ administration). Thus, the optimization of the detection method could only be performed across rats and not within each individual

animal. In addition, optimization and detection was performed on the same set of recordings and no dedicated test data was used. However, the results showed that a range of similar feature settings would enable the early detection of seizures, and therefore we do not believe that the lack of the test data has significantly affected the conclusions drawn from this study. Meanwhile, it is obvious that further research is needed to investigate if the observed pre-ictal effects would also occur in other animal models of seizures and if the identified features can be optimized to individual subjects (see "Further research").

The effect of spinal cord stimulation on seizure susceptibility

Various findings have shown that sensory stimulation affect seizure susceptibility (DeGiorgio, 2009, Forster and Madow, 1950, Gowers, reprinted 1964, Harding and Jeavons, 1994, Manning and Uhlrich, 2009, McLachlan, 1993, Rajna and Lona, 1989). These observations and the fact that the dorsal spinal cord columns are abundant in sensory fibres, suggest that SCS may also interfere with seizure susceptibility and thus be used for the treatment of epilepsy. SCS has been shown to be a safe and well tolerated treatment of pain and peripheral ischemia, thus the therapy could easily be extended to epilepsy patients too. As SCS has received little attention within the area of epilepsy, we aimed to investigate if seizure susceptibility could be modulated by SCS. For evaluating the effect of SCS on seizure susceptibility, the PTZ seizure model that we developed in anesthetized rats seems very suitable. Hence, various types of epilepsy treatments have been tested in the PTZ models including VNS, trigeminal nerve stimulation, anterior thalamic stimulation and antiepileptic drugs therapies. The results of those studies have later been shown to extrapolate well to patients (Ben-Menachem, 2002, DeGiorgio, 2009, Fanselow et al., 2000, Mirski et al., 1997, White, 1995, Woodbury and Woodbury, 1990)

In the present work, the animals were randomly separated in three experimental groups. One group acted as controls and did not receive any stimulation. A second group received SCS at a frequency, similar to the frequency of typical spike-wave discharges in absence seizures (4 Hz), which was considered as potentially pro-convulsive. Finally, a third group received 54 Hz SCS, which is similar to the frequency typically used in SCS treatments in patients (Gao et al., 2010). The latter frequency was hypothesised to be anti-convulsive as reduced cortical excitability was demonstrated in SCS patients treated for pain (Schlaier et al., 2007).

The results of this study showed that 4 Hz stimulation induced large spike-wave like ECoG complexes in a 1:1 manner, even prior to the administration of the pro-convulsive drug, PTZ. This relation was interrupted only during convulsive seizures characterized by around 10 Hz cortical spikes and during the post-ictal periods where ECoG suppression occurred. In a study on cats, Gutnick et al. observed a phenomenon which may explain the absence of cortical effects of 4 Hz SCS during convulsive seizures. These authors reported that experimentally induced cortical spikes cause an inhibition of the afferent thalamic transmission for up to approximately 200 ms following the cortical spikes (Gutnick and Prince, 1974). This indicates that the ictal cortical spikes occurring at frequencies above 5 Hz may progressively

inhibit the supraspinal effects of SCS by blocking their thalamic transmission, which suggests that seizure susceptibility may be modulated by SCS only pre-ictally. When the convulsive cortical activity is already present in the form of high frequency discharges, the central effects of SCS may thus be hampered.
Low frequency stimulation at supra motor threshold has been suggested as a mean for correct positioning of the spinal cord electrode during implantation. In addition, low frequency (4 – 25 Hz) SCS seems to be effective in the treatment of pain in humans (33) and hyperalgesia in rats (34). As our results indicate that such low frequencies may induce a pro-convulsive effect, further research should clarify if this may also be an issue in humans e.g. by evaluating if cortical synchronization occurs when using low frequency for the positioning of the stimulation electrode.

In contrast to our results, Ozcelik et al. indicated that low frequency SCS at 2 Hz reduced seizure susceptibility, as the frequency of cortical spikes were decreased by this means in a rat model (Ozcelik et al., 2007). The discrepancy between those and our results may be related to the different stimulation intensity, i.e. up to 0.1 mA used by Ozelik et al. and approximately 0.3 mA applied in our study. Thus, the higher currents that we used for SCS may have been able to activate enough dorsal column fibres to induce a potentially pro-convulsive thalamocortical synchronisation, whereas the 0.1 mA pulses used by Ozelik et al may have been too weak to cause a similar effect, but strong enough to interfere with the spontaneous spiking activity. This explanation is in accordance with the results of Hamani et al., who observed that the effect of the anterior thalamic stimulation depends on the stimulation intensity and is anti-convulsive, when using 0.5 mA pulses and pro-convulsive when increasing the stimulation current to 1.0 mA (Hamani et al., 2008).
In our study, 54 Hz SCS caused a small evoked cortical potential at the onset of stimulation, but no further changes in the ECoG activity. Stimulation at this frequency resulted in decreased seizure susceptibility in all evaluated parameters and the only animal to display only one seizure was from this group. However, these effects were not statistically significant.
The pro-convulsive effect induced by 4 Hz stimulation clearly shows that seizure susceptibility can be modulated by SCS. In addition, although below statistical and clinical significance levels, the anti-convulsive effects induced by 54 Hz stimulation suggest that SCS may have the ability to reduce seizure susceptibility. Thus, further research is needed to evaluate if the stimulation parameters can be optimized to induce a statistically significant and clinically relevant reduction in seizure susceptibility. As our experiments and the reports of others (e.g. Hamani et al., 2008) indicate that the effect of SCS on seizures may depend on both frequency and intensity of stimulation, both parameters should be analysed in future studies.

Conclusion

In the first part of this study, a specific cardiac-related firing pattern could be derived from VENG recordings in anesthetized rats. As these firing patterns change prior to and during seizures, the VENG may provide a source of information for prediction, detection and monitoring of seizures. Such VN-based methods may be especially suitable for controlling VNS in epilepsy patients, as stimulation and recording can be performed with one and the same electrode. Further research is needed to investigate if the method is applicable in awake animals and with human size VNs before such methods can be evaluated in humans.

The second part of this study showed that low frequency SCS significantly increases seizure susceptibility, whereas 54 Hz SCS resulted in a trend towards inhibition of seizures. Thus, SCS is clearly capable of modulating seizure susceptibility. Further research is needed to investigate if stimulation parameters can be optimized to inhibit seizures.

Perspectives

Both the areas investigated in the presented work require more research before clinical applications can be derived.

Seizure prediction based on vagus nerve activity

Seizure detection/prediction based on VENG analysis, should ideally be investigated further in a chronic epilepsy model with VN and cardiorespiratory characteristics similar to those of humans during awake and unrestrained conditions. However, this would require a lot of resources in the development of a (partly) implantable system for recording VENG. Thus, a more incremental approach could be to transfer the methods established in rats to an acute pig model, which is closer to humans regarding the anatomy of the cardiorespiratory system and the VN. To enable feature optimization in individual animals and proper evaluation of the applied seizure prediction/detection method (e.g. by comparing against a random predictor (Andrzejak, 2003)), longer recordings and several seizure are required from each animal. Another step could be to record VENG in patients during VNS implantation (e.g. via the stimulation electrode or a hook electrode), to investigate if a CrVENG profile can be derived from the human VNs during baseline / inter-ictal state.

The general idea of predicting/detecting seizures based on VENG is that information retrieved close to the implanted stimulator device could be used for enabling on-demand VNS treatment. Our results show that the CrVENG carries information which might be used for prediction and detection of seizures. However, seizure prediction/detection should not rely on one feature only. Information derived from multiple features may be combined to increase sensitivity and specificity. Some of these additional features might also be derived from the VENG, such as the VENG energy. However, features may also be derived from other types of inputs available at the site of the implant such as: ECG, EMG, Respiration (nerve recording

/ impedance), device movement (accelerometers), device orientation (accelerometers) ect. (Feldwisch-Drentrup, 2010, Giftaktis and Graves, 2007, Lockard et al., 1994). Information derived from accelerometers may e.g. help to differentiate if cardiac or respiratory changes are due to physical activity or ictal activity.

The required sensitivity and specificity will depend on different aspects, such as the consequences of a false detected seizure and missed seizures (Litt, 2003). The consequences of initiating VNS due to a false seizure prediction does not seem to have serious adverse effects, given that epilepsy patients currently receive scheduled stimulation. In fact, it may be advantages to provide a level of scheduled VNS in addition to on-demand stimulation, to ensure the induction of the slowly (over several months) accumulating seizure inhibition. However, if patients associate the onset of stimulation with an increased risk of having an imminent seizure, too many false predictions may cause unnecessary anxiety.

One of the challenges for a VENG based seizure detection method is to record the low amplitude nerve signal (μV) in the presences movement and noise from the surrounding muscle and heart activity (mV) (Hoffer and Kallesøe, 2001). In addition, chronic implanted cuff electrodes may be subject to ingrowth of connective tissue which may over time shield the electrode contacts from the nerve fibers, thus decreasing the signal amplitude. The use of synchronized averaging of the VENG signal makes the developed CrVENG-based detection method more robust to such deterioration of the VENG. However, further research should access VENG under awake conditions and also investigate the possibility of recording VN activity over longer periods of time to evaluate the effect of encapsulation on VENG signal to noise ratio, and the ability to predict/detect seizures under such conditions.

Seizure prediction/detection methods based on VENG will require further animal tests in both acute and chronic animal experiments before they can be evaluated in human patients. In addition, the hardware: a cuff electrode capable of both recording and stimulating, and an implantable device capable of acquiring VENG, signal processing and stimulation will need to be developed. Thus, if further research proves VENG based prediction/detection methods are effective, it may still take 5-10 years before clinical applications can be available.

The effect of spinal cord stimulation on seizure susceptibility

Regarding the further assessment of the effect of SCS on seizure susceptibility, a range of stimulation parameters needs to be evaluated. A method might be to evaluate the ability of different stimulation parameters to attenuate spike-wave activity (McLachlan, 1993, Ozcelik et al. 2007) in a PTZ model. Although this is an indirect method of evaluating the effect of SCS on seizure susceptibility, it enables the evaluation of many different stimulation parameters in each animal. The stimulation parameters most effective in inducing cortical desynchronization could later be tested in their ability to inhibit actual seizures in a cross over study (Takaya et al., 1996). In addition to animal studies, the effect of SCS in e.g.

pain patients with concomitant epilepsy may be accessed in a systematic way. However, it may be a difficult to find a sufficient number of such patients and variations between patients regarding epilepsy disorders and the SCS paradigm applied (spinal level of stimulation, duration of stimulation per day, stimulation frequency, stimulation current ect.) may also prove such a study problematic.

Given that SCS paradigms to inhibit seizures are developed, it will be of great benefit for the acceptance of the treatment that SCS is already widely used for other disorders. Thus, the technology and clinical expertise is already present within this area. A problem for many types of epilepsy treatments and in particular those involving invasive surgery, is that eventually a treatment will only be beneficially for a fraction of the treated patients e.g. only around half of VNS treated patients will become responders (>50% seizure reduction) (De Herdt et al., 2007, DeGiorgio et al., 2000, Labar, 2004, Morris and Mueller, 1999). If non-responders could be excluded prior to implantation, they would not need to be exposed to the risk of surgery, adverse effects of the treatment and also cost could be reduced. An approach is to evaluate the effect of the treatment in patients in a minimal invasive way prior to the actual implantation. This may be possible with dorsal SCS, as the effect of stimulation can be tested with transcutaneous electrodes and an external stimulator before implanting a permanent spinal electrode and stimulator (Kunnumpurath et al., 2009, North, 2007).

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