UNIVERSITEIT GENT

Innovative neurophysiological mechanisms and technologies for VNS in refractory epilepsy

Riëm El Tahry

Thesis submitted in fulfillment of the requirements for the degree of Doctor in Medical Science

27/09/2011

Promotor: Prof. Dr. Paul Boon

Reference center for Refractory Epilepsy
Laboratory for Clinical and Experimental neurophysiology

Universitair Ziekenhuis Gent

Ghent University Hospital Department of Neurology. Director: Prof. Dr. Paul Boon

Promotor:	Prof. Dr. Paul Boon
Examination Board	Prof. E. Ben-Menachem
	Prof. Dr. R. Van Coster
	Prof. Dr. E. Schmedding
	Prof. Dr.K. Van Rijckevorsel
	Prof. Dr.J. Caemaert
	Prof. Dr.E. Thiery
	Prof. Dr.P. Santens

Promotor:	Prof. Dr. Paul Boon
Reading Board	Prof. E. Ben-Menachem
	Prof. Dr.K. Van Rijckevorsel
	Prof. Dr.J. Caemaert
	Prof. Dr.E. Thiery

Table of Contents

List of abbreviations

AED	Anti epileptic drug
EA	Ethanolamine
CAP	Compound Action Potential
CBF	Cerbral Blood flow
CBZ	Carbamazepine
Ch	Channel
CLZ	Clonazepam
CPS	Complex partial seizure
CSF	Cerebro spinal fluid
EP	Evoked Potential
fMRI	Functional Magnetic Resonance Imaging
HN	Hering nerve
IL	Interleukine
ILAE	International League Against Epilepsy
LEV	Levetiracetam
LCAMP	Larynx compound muscle action potential
LTG	Lamotrigin
MAO	Mechanism of action
MRI	Magnetic Resonance Imaging
NTS	Nucleus Tractus Solitarius
PEA	Phospho ethanolamine
PET	Positron emission tomography
PHT	Phenytoïn

SIGN	Scottish Intercollegiate Guideline Network
SG	Secondary generalisation
SPECT	Single photon emission computed tomography
SPS	Simple partial seizure
SUDEP	Sudden Unexpected Death Epilepsy Patient
tDCS	Transcranial Direct Current Stimulation
TNS	Trigeminal nerve stimulation
TMP	Trans membrane potential
VPA	Valproic acid
VNS	Vagus nerve stimulation
VSEP	Vagal somatosensory evoked potential

CHAPTER 1

CHAPTER 1: RATIONALE, RESEARCH QUESTIONS AND OUTLINE OF THE THESIS

1.1 Rationale

VNS is widely recognized as an adjunctive treatment for refractory epilepsy and medical indications for VNS are currently expanding. Nevertheless, understanding the mechanism of action (MAO) of VNS remains for a large part unsolved. Moreover, clinical response to VNS is variable and unpredictable. In clinical practice, VNS candidates are those patients that run a complete pre-surgical evaluation and for which finally no surgery can be offered. There are currently no predictive factors in the process of identification of VNS patients. Identifying predictive factors might help to select good candidates much earlier in the pre-surgical evaluation. In addition, better comprehension of the mechanism of action of VNS, could lead to more appropriate choice of stimulation parameters. Worthwhile noticing is the fact that one third of patients treated with VNS do experience a >50% seizure reduction and some patients become even seizure free (1, 2) which compares favourably to the option of adding a third or fourth anti epileptic drug. Of course, we should consider that VNS therapy implicates a large cost (3), all the more reason why the mechanism of action of VNS should be investigated more profoundly, as understanding of the therapy will ultimately lead to a better use of it. Globally, VNS is considered to be very cost efficient and advancements in VNS technologies are still expanding.

Research on the mechanism of action of VNS consists of two main parts. First, electrical stimulation and activation of the vagus nerve is essential, as adequate activation of the vagus nerve itself is necessary to induce any upstream effects in the brain (4,5). Surprisingly, electrophysiological data of the vagus nerve in humans and animals are scarce and existing data were insufficiently integrated in practical use of VNS in epilepsy. A second important branch of research consists in unravelling the different effects of VNS in the brain. Anatomically, the vagus nerve has wide and broad connections with several structures that play an important role in epilepsy, such as for example amygdala, hippocampus and thalamus. Effects of VNS in the brain can be examined in experimental animal models, through intra and extracellular recordings of different projection sites (6,7), by non invasive imaging techniques (8-10) or with post-mortem immune histochemical stainings.

The main goal of this thesis is to identify and characterize a parameter that reflects activation of the vagus nerve, which eventually could be used as a biological marker for stimulation. This might be of a clinical importance, as parameters of stimulation could therefore be derived from objective patient specific information.

For this purpose, research on Compound Action Potentials (CAPs) of the vagus nerve in animals and humans was performed. For animal use a custom made spiral cuff electrode was designed to stimulate and record stimulation induced vagal activity. In humans, a new vagus nerve stimulator, the ADNS-300 provided by Neurotech, was implanted in three patients and postoperative recordings of vagal nerve compound action potentials in humans were described for the first time.

1.2 RESEARCH QUESTIONS

The following questions were raised:

Animals:

1. Is the implantation of a combined stimulation and recording spiral cuff electrode around the left cervical vagus nerve in rats feasible?

2. Can we identify a parameter that reflects adequate stimulation of the vagus nerve using our new electrode and applying parameters that are commonly used in clinical practice?

3. If yes, what is the nature of this physiological parameter: does recorded activity correspond to a compound action potential of the vagus nerve?

4. Is it possible to record earlier described VNS activation parameters in a chronic way?

5. Do the characteristics of this VNS-induced stimulation parameter remain stable over time?

6. How does the impedance of stimulation electrodes contact evolve over time?

7. Is it possible to record in all implanted rats a VNS activation parameter immediately after surgery?

8. Is it possible to record a VNS-induced evoked potential in one of the vagal projection sites in the rat brain using stainless steel depth electrodes?

9. What could hypothetically improve recording of VNS induced evoked potentials in the rat brain?

Humans:

1. What are the technical advancements and improvements of the Cyberonics VNS model 103 and 104 (Demipulse) for treatment of refractory epilepsy?

2. What are the main differences between ADNS-300 and Demipulse VNS systems for refractory epilepsy?

3. What was the patient outcome of the first pilot trial with the ADNS VNS device?

4. Could vagal nerve CAPs be recorded and what were the characteristics of the recorded signals?

5. During which time period could vagal nerve CAPs be recorded?

6. Can dose-response curves of vagal CAP be recorded? Is it possible to distinguish different fiber bundles of the vagal nerve CAP by applying higher charge values?

7. What are technical and practical aspects of the ADNS that need to be improved?

1.3. Outline of the thesis

Chapter1: Describes the rationale and research questions

Chapter 2: Provides a general introduction on epilepsy and gives an overview of existing anti epileptic drug treatments and highlights the pre-surgical evaluation for medically refractory epilepsy. In addition, a short overview of different neurostimulation techniques for refractory epilepsy are listed and briefly explained. Finally, a subchapter is dedicated to VNS, in which history, clinical efficacy, acute and long term side effects and possible research fields are summarized **Chapter 3:** Describes the theoretical background and is divided in two sections.

The first section gives a comprehensive overview of the electrophysiology of extracellular stimulation of nerves. In this context, extracellular recordings and the recording of the Compound Action Potential are defined. In addition, the effects of extracellular stimulation on neural tissue are briefly explained. The first section ends with an illustration of the different kinds of stimulation pulses that can be used for extracellular stimulation.

The second section of this chapter gives an overview of the different parameters in the literature for electrical activation of the vagus nerve. Markers of stimulation range from non invasive human scalp recordings of vagal evoked potentials to more invasive depth electrode recordings of vagal evoked potentials in experimental animal studies. In addition, the effects of VNS on other commonly used evoked potentials in clinical practice are reviewed. Finally, effects of VNS in the brain visualized through different neuro-imaging techniques are summarized and the possible relationships to seizure outcome are highlighted.

Chapter 4: Manuscript: “Evolution in VNS therapy for refractory epilepsy, experience with Demipulse devices at Ghent University Hospital”

Chapter 5: Manuscript: “A novel implantable vagus nerve stimulation system (ADNS-300) for combined stimulation and recording of the vagus nerve: pilot trial at Ghent University Hospital”

Chapter 6: Manuscript: “Chronic electrophysiological properties of human vagus nerve recorded with the ADNS-300”

Chapter 7: Manuscript: “Implantation of self sizing cuff electrode around the vagus nerve in experimental rats for repeated assessment of larynx muscle compound action potentials in rodents”

Reference List

1. Vonck K, Thadani V, Gilbert K et al. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience. Journal of Clinical Neurophysiology 2004;21[4]:283-289.

2. De Herdt V, Vonck K, Verschelde H et al. Vagus nerve stimulation for refractory epilepsy: Pooled results from epilepsy centres in Belgium. Epilepsia 2006;47:205-6.

3. Boon P, Vonck K, D'Have M, O'Connor S, Vandekerckhove T. Vagus nerve stimulation is a cost-beneficial treatment for refractory epilepsy. Epilepsia 1999;40:89.

4. Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. Epilepsia 31 1990 (Suppl 2):S7-19.

5. Woodbury JW, Woodbury DM. Vagal stimulation reduces the severity of maximal electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording. Pacing Clin Electrophysiol 1991;14[1]:94-107.

6. Zagon A, Kemeny AA. Slow hyperpolarization in cortical neurons: a possible mechanism behind vagus nerve simulation therapy for refractory epilepsy? Epilepsia 2000;41[11], 1382-1389.

7. Groves DA, Bowman EM, Brown VJ. Recordings from the rat locus coeruleus during acute vagal nerve stimulation in the anaesthetised rat. Neurosci Lett 2005;379:174-9.

8. Chae JH, Nahas Z, Lomarev M et al. A review of functional neuroimaging studies of vagus nerve stimulation (VNS). J Psychiatr Res 2003;37:443-55.

9. Henry TR, Bakay RA, Votaw JR et al. Brain blood flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: I. Acute effects at high and low levels of stimulation. Epilepsia 1998;39[9]:983-990.

10. Henry TR, Bakay RA, Pennell PB, Epstein CM, Votaw JR. Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. prolonged effects at high and low levels of stimulation. Epilepsia 2004;45[9]:1064-1070.

11. Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119:124-32.

12. Raedt R, Clinckers R, Mollet L et al. Anti-epileptic effects of vagus nerve stimulation in the focal pilocarpine model. European Journal of Neurology 2009;16:141.

13. Cunningham JT, Mifflin SW, Gould GG, Frazer A. Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by Vagal nerve stimulation. Neuropsychopharmacology 2008;33:1884-95.

CHAPTER 2

CHAPTER 2: INTRODUCTION

2.1 Epilepsy: epidemiology, definition, classification

Epilepsy is a chronic neurological disorder that is characterized by recurrent epileptic seizures. It has a prevalence of 0.5 to 1% of the population and appears as frequently in men as in women. The highest rate is observed in the first week of life; thereafter incidence is lowest during adulthood and increases again in the elderly due to a higher prevalence of cerebro-vascular disorders (1,2).

The traditional definition of 'epilepsy' as more than one unprovoked seizure episode seems to be changing as the International League against Epilepsy (ILAE) has proposed to adapt the definition to 'at least one seizure and evidence of an enduring predisposition for further seizures' (3). In this way multiple epileptic seizures due to multiple different causes in the same patient would not be considered as epilepsy. However, a single seizure due to an enduring epileptogenic abnormality would indicate epilepsy. As inevitably always is the case with definitions, there is a certain grey area in which every neurologist decides individually upon further treatment and follow-up (4). Neurologists must determine the cause of the seizure or define the type of epilepsy and if possible establish a syndromic diagnosis by means of clinical examination, EEG, video-EEG monitoring and neuro-imaging. Such distinctions are important for an optimal management of the patient.

Epileptic seizures are classified in two categories based on ictal semiology and electroencephalographic (EEG) characteristics (5). Table 1 gives an overview of classification of seizures.

1. *Focal epileptic seizures* are conceptualized as originating within a network limited to part of one hemisphere. They maybe discretely localized or more distributed, but for every seizure type, ictal onset is consistent for recurrent seizures. In addition, it is possible that several different networks are involved, leading to several seizures types in one patient. Depending on regions involved, clinical symptoms include motor, somato-sensory, visual, gustatory, auditory and or autonomic phenomena (6).

2. *Generalized epileptic seizures* originate at one point within, and rapidly engage bilaterally distributed networks. Although individual seizure onsets can be lateralized, the location and lateralization are not consistent from one seizure to another. In this sense, generalized seizures can be asymmetric. Pattern and rate of propagation of seizure activity determine the sequence of signs and symptoms that develop throughout the course of the seizure.

Table 1: Classification of seizure types

I. Focal seizures

1. Without impairment of consciousness or awareness

   - ✓ With observable motor or autonomic components (This roughly corresponds to earlier concept of 'simple' partial seizure)
   - ✓ Involving subjective sensory or psychic phenomena only. (This corresponds to the concept of an aura)

2. With impairment of consciousness or awareness (This roughly corresponds to the complex partial seizure). Can be accompanied by motor, sensory and autonomic events)

3. Evolving to a bilateral, convulsive seizure (involving tonic, clonic or tonic-clonic components). This expression replaces the term secondarily generalized seizure.

II. Generalized seizures

1. Tonic clonic
2. Absence (Typical, Atypical, Absence with special features (Myoclonic absence, Eyelid Myoclonia))
3. Myoclonic (Myoclonic, Myoclonic atonic, Myoclonic tonic)
4. Clonic
5. Tonic
6. Atonic

III. Unknown

After defining the type of seizure, recognition of aetiology and syndrome diagnosis is the second step in the general work-up.

Previous classifications used to subdivide epilepsies into epilepsies without an identifiable lesion or aetiology and presumed hereditary predisposition ('idiopathic'), epilepsies with known aetiology ('symptomatic') and finally epilepsies in which aetiology is suspected but not proved ('cryptogenic'). These terms are nowadays replaced by 'genetic', 'structural/metabolic' and of 'unknown cause' (5).

Further subdivisions are based on the basis of the *age onset of specific electro-clinical syndromes*. In addition to the electro-clinical syndromes with strong developmental and genetic components, *distinctive constellations are made on the basis of specific lesions or other causes*. They are diagnostically meaningful forms of epilepsy and have particular implications for clinical treatment, particularly for epilepsy surgery. These include for example mesial temporal lobe epilepsy with hippocampal sclerosis or hypothalamic hamartoma with gelastic seizures. In these disorders age at presentation is not a defining feature. The next group includes *epilepsies that are secondary to specific structural or metabolic lesions or conditions*, such as for example malformations of cortical development, tumours, infections or trauma. Finally, these epilepsies that in the past were called cryptogenic are now being referred to as being of '*unknown' cause* (5).

2.2 Anti-epileptic drug (AED) treatment

Anti-epileptic drugs are the mainstay of the treatment of epilepsy, and although their number has expanded exponentially, current principles governing drug therapy haven't changed spectacularly over time.

Firstly, treatment is indicated after two seizures, given the interval between them is not more than 1 or 2 years. Other element to be considered is the individual wish of the patient (7).

Secondly, because about 50% of individuals with a first unprovoked seizure who are not treated will never have a second seizure, and because AED treatment carries a substantial risk of adverse effects, it is reasonable to defer treatment until after seizure recurrence in most cases. However, early treatment might be justified in patients with a high recurrence risk, such as for example in patients with a first unprovoked seizure after a stroke or a seizure caused by other identifiable lesions (7,8,9,13). After having decided whether or not to treat the patient, the next question is off course which anti- epileptic drug should be administered in first line?

For focal seizures—by far the most prevalent seizure type in adults—the American Academy of Neurology guidelines (10) lists many old and new AEDs without expressing any preferences among them; the UK National Institute for Health and Clinical Excellence (11) recommends preferential use of older agents unless there are specific reasons for doing otherwise; the Scottish Intercollegiate Guideline Network (SIGN) identifies specifically carbamazepine, valproate, lamotrigine, and oxcarbazepine as first-line agents (12); and the ILAE rates phenytoin and carbamazepine as the AEDs with the highest quality of evidence for efficacy and effectiveness (13). In Belgium, the first choice treatment as initial monotherapy in focal seizures is carbamazepine, followed by levetiracetam and lamotrigine (14).

There are several properties in addition to efficacy that might affect the choice of AEDs. These include adverse effects such as rare idiosyncratic reactions, teratogenic effects and chronic side-effects. Enzyme-inducing effects and potential for drug interactions are also important, as are the availability of parenteral formulations and the possibility of rapidly reaching an efficacious target dose in some cases. Selection of an AED needs an individual approach, in which elements such as childbearing potential, old age and co-morbidities play an important role. The dosage of the AEDs should be the lowest possible to achieve sustained seizure freedom and minimal side-effects. If a change in AED treatment is indicated, the conventional recommendation is to switch gradually to monotherapy with another drug (15). Other authors, however, feel that a combination therapy could be tried earlier, particularly in severe epilepsies when the first AED seems to have been partially effective and well tolerated, and the probability of seizure freedom with monotherapy is regarded as low (9).

Patients who do not achieve sustained seizure freedom after adequate trials of at least two appropriate AEDs, given alone or in combination, meet ILAE criteria for pharmaco-resistance (17). The rationale for this definition is that the probability of seizure freedom with another AED decreases in proportion to the number of drugs tried unsuccessfully in the past, and is probably no greater than 20% after failure of two such drugs (13). Aspects to be considered when trying an additional AED include its spectrum of efficacy, its adverse effects profile, its expected effect based on the patient's characteristics, and the possibility of pharmacokinetic and pharmacodynamic drug interactions, which can require dosage adjustments (7,8,13).

Finally, when seizure freedom cannot be achieved, the ultimate goal is the best possible quality of life, to be obtained through a compromise between reduction in seizure frequency or severity and the burden of side-effects.

Efficacy spectrum of the main AED in different subtypes in adults
Effective against focal and most generalised seizures
Valproate
Benzodiazepines
Phenobarbital
Primidone
Lamotrigine
Levetiracetam
Topiramate
Zonisamide
Rufinamide
Felbamate
Primarily effective against focal seizures, with or without secondary generalisation
Carbamazepine
Phenytoin
Gabapentin
Lacosamide
Oxcarbazepine
Esclicarbazepine
Pregabaline
Tiagabine
Vigabatrin
Effective against absences seizures
Ethoxsuximide

Table 2 : Efficacy spectrum of the main AEDs in different seizure types in adults (13,16)

2.3 Pre-surgical evaluation and treatment options for refractory epilepsy

Nearly all patients with recurrent seizures treated with anti-epileptic drugs respond well. Nevertheless, one third of the patients are not fully controlled or experience intolerable side effects (8). Epilepsy is regarded as refractory when seizures are difficult to control despite adequate mono- or poly-therapy with anti-epileptic drugs that do not cause significant adverse events (8,17). Consequently, these patients suffer from long term morbidity, disability and underemployment. For these patients, referral to a Reference Centre for Refractory Epilepsy may offer new opportunities.

The first step in identifying patients for possible surgical therapy is to confirm the diagnosis of epilepsy and to attempt to localize the onset of seizures. Importantly, non-epileptic attacks, particularly psychiatric disorders are diagnosed in 10-20% of patients admitted to monitoring units. Moreover, cardiovascular and sleep disturbances may also be misdiagnosed as epilepsy as well (18).

The second step is to determine whether a patient can benefit from epilepsy surgery or other alternative treatment, a multidisciplinary team of epileptologists, neuroradiologists, neurosurgeons and neuropsychologists is required. The pre-surgical evaluation has as a main goal to identify the epileptogenic onset zone and to determine whether this zone can be surgically removed without injury to brain regions that mediate key neurological functions. For this purpose, a series of complementary technical investigations are necessary. The pre-surgical work up consists of a (1) precise and concise personal and familial history of epilepsy history, (2) a long term video-EEG monitoring to record habitual seizures, (3) optimal 3 Tesla MRI of the brain, (4) a positron emission tomography (PET), (5) a neuropsychological assessment to clarify cognitive deficits. Once the patient has been discussed in a multidisciplinary staff, additional examinations such as a single-photon emission computed tomography (SPECT), magneto-encephalography (MEG), a Wada test or fMRI can be performed to further characterize the epileptogenic zone or to determine laterality of memory function and language dominance and other eloquent area's (19-21).

Epilepsy surgery is an efficacious alternative therapy for selected patients with intractable epilepsy (22-27). Patients with medial temporal lobe epilepsy (and lesional epilepsy), may be suitable candidates for epilepsy surgery (28, 29). The hallmark pathology of medial temporal lobe epilepsy is medial temporal sclerosis (30, 31). Histological examination of surgically excised hippocampus in these patients shows focal cell loss and gliosis. These patients have a good postoperative outcome. Approximately 60-80% of patients with unilateral mesial temporal sclerosis, but also patients with a low-grade glial neoplasm or cavernous hemangioma, commonly become seizure-free after surgical treatment. In contrast, post-operative outcome is distinctly less favourable in individuals with focal cortical dysplasia and non-lesional epilepsy (32, 33). Potential reasons for reduced efficacy of surgery include the extra-temporal onset of seizures and the non-favourable localization of epileptic brain tissue (34, 35).

The management of the patient with medically refractory epilepsy who is not a candidate for a 'curative' surgical procedure can be very challenging. Treatment options for these individuals include continued AED treatment; 'palliative' surgical procedures, such as callosotomy or a subtotal resection of the epileptogenic zone, AED investigational studies, vagus nerve stimulation (VNS), deep brain stimulation and ketogenic diet.

2.4 Neurostimulation

2.4.1 Extra-cranial neurostimulation

2.4.1.1 VNS

VNS consists of stimulation of the tenth cranial nerve or vagus nerve and was developed in the eighties (VNS Therapy^{TM} device, Cyberonics, USA). Through an implanted programmable pulse generator and a bipolar helical electrode, electrical pulses are administered to the left vagus nerve in the neck. VNS is a widely recognized adjunctive treatment for patients with refractory epilepsy who cannot benefit from epilepsy surgery. Improvement resulting from treatment is not immediate but tends to increase over time (36, 39).

2.4.1.2 Repetitive Transcranial magnetic Stimulation (rTMS)

Transcranial magnetic stimulation (TMS) is a non-invasive, generally well-tolerated method for cortical stimulation that is based on principles of electromagnetic induction, where small intra-cranial electric currents are generated by a strong fluctuating extra-cranial magnetic field (40). The presumed mechanisms underlying these lasting changes in cortical excitability are similar to those of long-term depression (LTD) and long-term potentiation (LTP) of synaptic strength, which are seen with low- and high-frequency electrical brain stimulation respectively (41). Anti-seizure properties of rTMS were studied previously, but results of randomized, double-blind and sham controlled studies show diverting results (43, 44). For this reason rTMS has not yet been widely adopted as a treatment of refractory epilepsy.

2.4.1.3. Transcranial Dirrect Current Stimulation (tDCS)

tDCS involves the application of electrical currents to the scalp via anodal or cathodal electrodes and influence cortical excitability. In a double-blinded, sham-controlled, randomized trial tDCS significantly reduced epileptiform discharges.

Moreover, in the month after tDCS, the number of epileptic seizures decreased trend-wise while it was stable in the group treated with sham tDCS (42). Future clinical research will point out whether this treatment can be used as a new neurostimulation treatment for epilepsy.

2.4.2. Intra-cranial neurostimulation : Deep Brain Stimulation

2.4.2.1. Hippocampal stimulation in patients with hippocampal sclerosis

High frequency stimulation of the hippocampus as epileptic focus, showed to have seizure reducing effects in two studies (45,46). One randomized double-blind examination studied efficacy of bilateral hippocampal stimulation in 9 patients with temporal lobe epilepsy due to uni- or bilateral hippocampal sclerosis. From those patients 5/9 became seizure-free en 4/9 experienced a reduction of > 50 % (45). These positive results were reproduced by our group, in which an open prospective study with bilateral temporal lobe epilepsy showed 1/10 seizure-free patient, 6/10 experienced a seizure reduction of > 50%, while in 3/10 seizures diminished with less than 50% (46, 47). Currently, our centre is performing a double-blind randomized study of selective amygdalo hippocampectomy versus medial temporal lobe deep brain stimulation (CoRaStiR).

2.4.2.2. Responsive Neurostimulation System (ongoing clinical trial)

NeuroPace Inc., is sponsoring an investigational device study of the Responsive Neurostimulation System (RNS) for treating refractory epilepsy. The pivotal trial is a randomized, double-blind, sham-stimulation controlled investigation conducted in the United States. The RNS System is designed to detect abnormal electrical activity in the brain and to deliver small amounts of electrical stimulation to suppress seizures before any seizure symptoms occur. The RNS is placed within the skull and underneath the scalp and is connected to one or two wires containing electrodes that are placed within the brain or that rest on the brain surface over the area of seizure focus. This type of treatment is still under research and it is not yet known whether it will work for the treatment of epilepsy (48, 49)

2.4.2.3. Stimulation of the anterior nucleus of the thalamus

In epileptic patients, the effect of anterior nucleus high frequency stimulation has been reported in different recent open label trials (50-52), although reduction in seizure frequency varied from 14-76 % according to the study performed (50, 53, 54). In addition, one report showed that the insertion of the electrode itself reduced seizures (52) and one observed benefits that did not differ between stimulation-on and stimulation-off periods (50). The randomized, double-blind SANTE study (Stimulation of the Anterior Nucleus of the Thalamus for epilepsy) was published, in which bilateral anterior nucleus stimulation of the thalamus induced seizure reduction during 2 year follow-up. After 2 years of stimulation, seizures were reduced by a median 56%, a 50%-responder rate improvement occurred in 54% of patients, seizures were less severe and quality-of-life was improved. Additional

clinical experience may help establishing the best candidates and to further refine the risk-benefit ratio of this treatment (55).

2.5. VNS: overview of clinical efficacy

2.5.1. History

The historical basis of stimulating peripheral nerves for treatment of seizures dates back to the sixteenth and seventeenth century. Greek author Pelops described that ligature of the limb in which the seizure started, could terminate the progress of the seizure from that specific limb. Later on, in the nineteen century, Odier and Brown-Séquard showed that ligatures are as efficacious in preventing seizures caused by a organic brain disease (56). At the end of the century Gowers attributed this effect to a raised resistance in the sensory and motor nerve cells in the brain that correspond with the limb involved, which in turn would prevent spread of the discharge (57). Finally, in 1991, Zabara and Terry launched the first implantable neurocybernetic prosthesis system (58).

2.5.2. Clinical efficacy: short overview

Clinical efficacy trials started out with the randomized controlled trials phase-1 EO1 and EO2 in which a small amount of patients (N=14) were implanted with a VNS device. After 3 to 22 months of follow-up, a 50% seizure reduction was observed (59-61). Beneficial effect in the patient population was sustained after a longer follow-up of 14-35 months (62). Thereafter, two prospective double-blind randomized studies, EO3 and EO5 were started.

In these studies, larger patient groups were divided in a “high” (mean stimulation intensity 1.3 mA, 30 Hz, 500 µsec, 30 sec on, 5 min off) and “low” stimulation group (single increase to point of patient perception, with a mean stimulation intensity of 1.2mA, 1 Hz, 130 µsec, 30 sec on, 3 hours off). In both studies the “high” stimulation group produced better seizure reducing effects that ranged between 24 to 28% compared to maximal 15% in the low stimulation group (63-65).

These study results lead to a Food and Drug Administration (FDA) approval and were subsequently followed by open label extension trials, in which was shown that the effect of VNS clearly increased over time to values between 35 en 44% seizure reduction, but ultimately reaches a plateau phase after 2 years of stimulation (62,63,66-69). In the following years, a growing amount of clinical data confirmed efficacy of VNS. For example, a joint study of two epilepsy centres in Belgium and in the USA recruited 118 patients with a minimum follow up of 6 months and found a reduction of 55% in mean monthly seizure frequency (70).

In addition, in a retrospective Belgian multicenter study in which 138 patients with a minimal follow-up of one year were included, an overall seizure reduction of 51% was observed (71).

VNS also appeared to have beneficial effects in certain epilepsy syndromes, such as for example Lennox-Gastaut (72-74). In addition, generalized epilepsy syndromes responded equally well to VNS than focal epilepsy syndromes (75).

A few case reports that describe a beneficial effect of VNS in both convulsive and non-convulsive status epilepticus (76, 77).

2.5.3. Side effects and tolerability

2.5.3.1. Acute side effects

Postoperative infections occur in 3-6% of patients. Fluid accumulation at the generator site with or without infections occurs in 1-2% of patients. These side effects are treated with oral antibiotics, but in rare cases the generator or electrode must be removed (65, 78).

Ventricular asystole during testing of the device (1 mA, 20Hz, 500µsec, 17 sec) on implantation has been reported, in patients who did not have any history of cardiac dysfunction, nor showed any cardiac problems after surgery (79- 81). All those patients had been able to use VNS postoperatively. Adverse cardiac complications at start or during ramping up of stimulation intensity have not been observed (82), although one case report described a late brady- arrhythmia after 2 years of stimulation (83).

Until recently it was thought that the stimulation electrode should be implanted on the left vagus nerve, as it contains less sino-atrial fibres compared to the right side and consequently would provoke less cardiac side effects. In contrast, several other studies report implantation of the right vagus nerve in humans, mainly due to complications related to left vagus nerve surgery. Infection of the implant or wound opening with explantation as a consequence, intra-operative bleeding in the approach of the left vagal nerve, but also mechanical dysfunction due to fibrosis and high impedance of the electrode, have been reported as reasons to implant the right vagus nerve (84, 85, 86). Right vagus nerve stimulation induced at 0.75 mA in one case bradycardia and ventricular extra systoles (85) and respiratory symptoms and secondary tachycardia were presented in a second case (84).

Recent technology advancements, more specifically the introduction of the CardioFit, have proposed stimulation of the right vagus nerve as a therapy for chronic heart failure. The initial results indicate that stimulation of the right vagus nerve in patients with heart failure, improves left ventricular function and long term survival (87).

2.5.3.2. Adverse events related to long-term use & tolerability

The most common side effects are stimulus-related coughing, throat pain, hoarseness and hiccup, all of which tend to improve over time (88). Less often dyspnoea during exercise and vomiting were reported (63). No changes in autonomic functions were reported (i.e. blood pressure, heart rate, lung function or blood chemistry) (89). VNS had no effects on AED serum levels (62).

Psychiatric side effects have been described. In patients with pre existing psychiatric disorders decreased sedation and increased alertness may manifest itself as psychosis (90, 91).

The mortality and rates of sudden death in epilepsy (SUDEP) of patients receiving VNS therapy are comparable with those of other groups of patients with medically refractory seizures (92). Performing a body MRI could heat the electrode leads and thereby damage local tissue. When used according to the manufacturer's guidelines, a brain MRI conducted with a send-and-receive head coil is safe (93).

Relative contra-indications include progressive neurologic or systemic diseases, cardiac arrhythmia, asthma, chronic obstructive pulmonary disease, active peptic ulcer and insulin dependent diabetes mellitus (94).

2.6. VNS Research

Currently, the precise mechanism of action of VNS as a treatment for refractory epilepsy remains to be elucidated (89). VNS seems to exert an acute seizure aborting effect together with a sustained long-term seizure preventing effect (65, 95). Stimulation parameters were derived from animal experimental studies in which anti-seizure effects of VNS were proven (96-102). Later on, these pre-clinical VNS parameters were applied in early clinical randomized controlled trials (56, 68, 103, 104). However, in none of these studies, stimulation parameters were derived from individual electrophysiological properties of the human vagus nerve. Beside unravelling and understanding the effects of VNS in the brain, research on activation of the vagus nerve itself remains a key point, as adequate activation of the vagus nerve is ultimately necessary to achieve any positive effects. For this purpose, different techniques representing stimulation of the vagus nerve in the literature were reported. This was done by means of characterization of vagal evoked scalp potentials in humans (105), but also by analyzing the effect of VNS on clinically-commonly evoked potentials (106,107), by studying the effects of VNS in the brain with functional imaging studies (108) and finally by examining the neurochemical effects of VNS in CSF (109) or its relation to serum cytokine production (110). Only a small number of studies have investigated local activation of the vagus nerve by means of compound action potentials (CAP's) of the vagus nerve (111,112). There were some attempts to correlate different biological activation parameters to seizure outcome in VNS practice, but data

were insufficient to drawn any strong conclusions. Consequently VNS is still being used in a rather arbitrary way, without any support of physiological activation information of the vagus nerve itself. Introduction of CAP's into the clinical practice, could teach clinicians how to choose stimulation parameters more adequately, which in turn could have important clinical repercussions. CAP's may in the future be relevant for the choice of cost-effective stimulation parameters and help identifying VNS-responders and non-responders.

Reference List

1. Hauser WA, Annegers JF, Rocca WA. Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota. Mayo Clin Proc 1996;71:576-86.

2. Forsgren L, Beghi E, Oun A, Sillanpaa M. The epidemiology of epilepsy in Europe - a systematic review. Eur J Neurol 2005;12:245-53.

3. Fisher RS, van Emde BW, Blume W et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 2005;46:470-2.

4. Fisher RS, Leppik I. Debate: When does a seizure imply epilepsy? Epilepsia 2008;49 (Suppl 9):7-12.

5. Berg AT, Berkovic SF, Brodie MJ et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 2010;51:676-85.

6. Blume WT, Luders HO, Mizrahi E, Tassinari C, van Emde BW, Engel J, Jr. Glossary of descriptive terminology for ictal semiology: report of the ILAE task force on classification and terminology. Epilepsia 2001;42:1212-8.

7. Brodie MJ, French JA. Management of epilepsy in adolescents and adults. Lancet 2000;356:323-9.

8. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med 2000;342:314-9.

9. Kwan P, Brodie MJ. Epilepsy after the first drug fails: substitution or add-on? Seizure 2000;9:464-8.

10. French JA, Kanner AM, Bautista J, et al. Effi cacy and tolerability of the new anti-epileptic drugs, I: treatment of new-onset epilepsy: report of the TTA and QSS Subcommittees of the AmericanAcademy of Neurology and the American Epilepsy Society.Epilepsia 2004; 45: 401–09.

11. National Institute for Clinical Excellence. Clinical guideline 20the epilepsies: the diagnosis and management of the epilepsiesin adults and children in primary and secondary care. October 2004. http://www.nice.org.uk/nicemedia/live/10954/29532/29532.pdf

12. Scottisch Intercollegiate Guidelines Network. Diagnosis and management of epilpesy in adults. A nationale clinical guideline. April 2033, updated Oct 20, 2005 http://www.sign.ac.uk/pdf/sign70.pdf

13. Emilio Perucca and Torbjörn Tomson. The pharmacological treatment of epilepsy in adults; Lancet Neurology 2011;11:446-56.

14. Paul Boon, Sebestiaan Engelborghs, Henri Hauman, An jansen, Lieven lagae, Bejamin Legros, Michel Osseman, bernard Sadzot, Etienne Urbain and Kenou Van Rijckevorsel Acta Neurol. Belg 2008;108:118-130.

15. Cretin B, Hirsch E. Adjunctive anti-epileptic drugs in adult epilepsy: how the first add-on could be the last. Expert Opin Pharmacother 2010;11:1053-67.

16. Kwan P, Brodie MJ. Emerging drugs for epilepsy. Expert Opin Emerg Drugs 2007; 12:407-22.

17. Berg AT, Kelly MM. Defining intractability: comparisons among published definitions. Epilepsia 2006;47:431-6.

18. Wiliam H theodore, Roger J Porter. Epilepsy, 100 Elementary principles. Third edition. W.B. Saunders Company Ltd, 1995.

19. Unnwongse K, Wehner T, Foldvary-Schaefer N. Selecting patients for epilepsy surgery. Curr Neurol. Neurosci. Rep. 2010;10:299-307.

20. Kar BR, Rao SL, Chandramouli BA, Thennarasu K, Satishchandra P. Neuropsychological lateralization of brain dysfunction in children with mesial temporal sclerosis: a pre-surgical evaluation. J Child Neurol 2010;25:705-14.

21. Poon TL, Cheung FC, Lui CH. Magnetoencephalography and its role in evaluation for epilepsy surgery. Hong Kong Med J 2010;16:44-7.

22. Siegel AM. Pre-surgical evaluation and surgical treatment of medically refractory epilepsy. Neurosurg Rev 2004;27:1-18.

23. Siegel AM, Cascino GD, Meyer FB et al. Resective reoperation for failed epilepsy surgery: seizure outcome in 64 patients. Neurology 2004;63:2298-302.

24. Siegel AM, Cascino GD, Meyer FB, Marsh WR, Scheithauer BW, Sharbrough FW. Surgical outcome and predictive factors in adult patients with intractable epilepsy and focal cortical dysplasia. Acta Neurol Scand 2006;113:65-71.

25. Cohen-Gadol AA, Wilhelmi BG, Collignon F et al. Long-term outcome of epilepsy surgery among 399 patients with nonlesional seizure foci including mesial temporal lobe sclerosis. J Neurosurg 2006;104:513-24.

26. Spencer SS, Berg AT, Vickrey BG et al. Predicting long-term seizure outcome after resective epilepsy surgery: the multicenter study. Neurology 2005;65:912-8.

27. Spencer SS, Berg AT, Vickrey BG et al. Initial outcomes in the Multicenter Study of Epilepsy Surgery. Neurology 2003;61:1680-5.

28. Radhakrishnan K, So EL, Silbert PL et al. Prognostic implications of seizure recurrence in the first year after anterior temporal lobectomy. Epilepsia 2003;44:77-80.

29. Radhakrishnan VV, Rao MB, Radhakrishnan K et al. Pathology of temporal lobe epilepsy: An analysis of 100 consecutive surgical specimens from patients with medically refractory epilepsy. Neurol India 1999;47:196-201.

30. Luby M, Spencer DD, Kim JH, deLanerolle N, McCarthy G. Hippocampal MRI volumetrics and temporal lobe substrates in medial temporal lobe epilepsy. Magn Reson Imaging 1995;13:1065-71.

31. Jeong SW, Lee SK, Kim KK, Kim H, Kim JY, Chung CK. Prognostic factors in anterior temporal lobe resections for mesial temporal lobe epilepsy: multivariate analysis. Epilepsia 1999;40:1735-9.

32. Palmini A, Andermann F, Olivier A, Tampieri D, Robitaille Y. Focal neuronal migration disorders and intractable partial epilepsy: results of surgical treatment. Ann Neurol 1991;30:750-7.

33. Mosewich RK, So EL, O'Brien TJ et al. Factors predictive of the outcome of frontal lobe epilepsy surgery. Epilepsia 2000;41:843-9.

34. Roper SN. Surgical treatment of the extratemporal epilepsies. Epilepsia 2009;50 Suppl 8:69-74.

35. Seo JH, Noh BH, Lee JS et al. Outcome of surgical treatment in non-lesional intractable childhood epilepsy. Seizure 2009;18:625-9.

36. Boon P, Vonck K, Vandekerckhove T, De Reuck J, Calliauw L. Vagus nerve stimulation for refractory epilepsy. Neurosurgery Quarterly 2001;11:66-72.

37. De Herdt V, Boon P, Ceulemans B et al. Vagus nerve stimulation for refractory epilepsy: a Belgian multicenter study. Eur J Paediatr.Neurol 2007;11[5]:261-269.

38. Vonck K, Boon P, D'Have M, Vandekerckhove T, O'Connor S, De Reuck J. Long-term results of vagus nerve stimulation in refractory epilepsy. Seizure 1999;8[6]:328-334.

39. Vonck K, Boon P, Goossens L et al. Neurostimulation for refractory epilepsy. Acta Neurol Belg. 2003;103[4]:213-217.

40. Bae EH, Schrader LM, Machii K et al. Safety and tolerability of repetitive transcranial magnetic stimulation in patients with epilepsy: a review of the literature. Epilepsy Behav 2007;10:521-8.

41. Kandel ER. The molecular biology of memory storage: a dialog between genes and synapses. Biosci Rep 2001;21:565-611.

42. Fregni F, Otachi PT, Do VA et al. A randomized clinical trial of repetitive transcranial magnetic stimulation in patients with refractory epilepsy. Ann Neurol 2006;60:447-55.

43. Theodore WH, Hunter K, Chen R et al. Transcranial magnetic stimulation for the treatment of seizures: a controlled study. Neurology 2002;59:560-2.

44. Cantello R, Rossi S, Varrasi C et al. Slow repetitive TMS for drug-resistant epilepsy: clinical and EEG findings of a placebo-controlled trial. Epilepsia 2007;48:366-74.

45. Velasco AL, Velasco F, Velasco M, Trejo D, Castro G, Carrillo-Ruiz JD. Electrical stimulation of the hippocampal epileptic foci for seizure control: A double-blind, long-term follow-up study. Epilepsia 2007;48:1895-903.

46. Vonck K, Boon P, Achten E, De Reuck J, Caemaert J. Long-term amygdalohippocampal stimulation for refractory temporal lobe epilepsy. Annals of Neurology 2002;52:556-65.

47. Boon P, Vonck K, De Herdt V, Van Dycke A, Goethals M, Goossens L, Van Zandijcke M, De Smedt T, Dewaele I, Achten R, Wadman W, Dewaele F, Caemaert J, Van Roost D. Deep brain stimulation in patients with refractory temporal lobe epilepsy. Epilepsia 2007; 48(8):1551-60

48. Fountas KN, Smith JR. A novel closed-loop stimulation system in the control of focal, medically refractory epilepsy. Acta Neurochir Suppl 2007;97:357-62

49. Sun FT, Morrell MJ, Wharen RE Jr. Responsive cortical stimulation for the treatment of epilepsy. Neurotherapeutics. 2008 Jan;5(1):68-74.

50. Hodaie M, Wennberg RA, Dostrovsky JO, Lozano AM. Chronic anterior thalamus stimulation for intractable epilepsy. Epilepsia 2002;43[6]:603-608.

51. Kerrigan JF, Litt B, Fisher RS et al. Electrical stimulation of the anterior nucleus of the thalamus for the treatment of intractable epilepsy. Epilepsia 2004;45:346-54.

52. Lim SN, Lee ST, Tsai YT et al. Electrical stimulation of the anterior nucleus of the thalamus for intractable epilepsy: A long-term follow-up study. Epilepsia 2007;48:342-7.

53. Osorio I, Overman J, Giftakis J, Wilkinson SB. High frequency thalamic stimulation for inoperable mesial temporal epilepsy. Epilepsia 2007;48:1561-71.

54. Andrade DM, Zumsteg D, Hamani C et al. Long-term follow-up of patients with thalamic deep brain stimulation for epilepsy. Neurology 2006;66:1571-3.

55. Robert Fisher, Vicenta Salanova et al. Sante Study group. Epilepsia 2010;51:899-908

56. Odier Louis. Manuel de Médecine-Pratique. BiblioBazaar, 2010.

57. Gowers WR. Epilepsy and other convulsive diseases, their causes, symptoms and treatment . William Wood & Co, 1985.

58. Terry R, Tarver WB, Zabara J. An Implantable neurocybernetic prosthesis system. Epilepsia 1991;14:86-93.

59. Uthman BM, Wilder BJ, Hammond EJ, Reid SA. Efficacy and safety of vagus nerve stimulation in patients with complex partial seizures. Epilepsia 1990;31 Suppl 2:S44-S50.

60. Penry JK, Dean JC. Prevention of intractable partial seizures by intermittent vagal stimulation in humans: preliminary results. Epilepsia 1990;31 Suppl 2:S40-S43.

61. Wilder BJ, Uthman BM, Hammond EJ. Vagal-Stimulation for Control of Complex Partial Seizures in Medically Refractory Epileptic Patients. Pace-Pacing and Clinical Electrophysiology 1991;14:108-115.

62. Uthman BM, Wilder BJ, Penry JK et al. Treatment of epilepsy by stimulation of the vagus nerve. Neurology 1993;43[7]:1338-1345.

63. Handforth A, DeGiorgio CM, Schachter SC et al. Vagus nerve stimulation therapy for partial-onset seizures: a randomized active-control trial. Neurology 1998;51[1]:48-55.

64. A randomized controlled trial of chronic vagus nerve stimulation for treatment of medically intractable seizures. The Vagus Nerve Stimulation Study Group. Neurology 1995;45:224-30.

65. DeGiorgio CM, Schachter SC, Handforth A et al. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. Epilepsia 2000;41[9]:1195-1200

66. Holder LK, Wernicke JF, Tarver WB. Treatment of refractory partial seizures: preliminary results of a controlled study. Pacing Clin Electrophysiol 1992;15[10 Pt 2], 1557-71

67. George RE. Long-Term Efficacy of Vagal Nerve-Stimulation in the Treatment of Partial Seizures - Results of 93 Patients. Journal of Neurosurgery 1994;80:A401.

68. Morris GL, III, Mueller WM. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group E01-E05. Neurology 1999;53[8]:1731-1735.

69. Salinsky MC, Uthman BM, Ristanovic RK, Wernicke JF, Tarver WB. Vagus nerve stimulation for the treatment of medically intractable seizures. Results of a 1-year open-extension trial. Vagus Nerve Stimulation Study Group. Arch Neurol 1996;53[11]: 1176-1180.

70. Vonck K, Thadani V, Gilbert K et al. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience. Journal of Clinical Neurophysiology 2004;21[4]:283-289.

71. De Herdt V, Vonck K, Verschelde H et al. Vagus nerve stimulation for refractory epilepsy: Pooled results from epilepsy centres in Belgium. Epilepsia 2006;47:205-6.

72. Aldenkamp AP, Majoie HJ, Berfelo MW et al. Long-term effects of 24-month treatment with vagus nerve stimulation on behaviour in children with Lennox-Gastaut syndrome. Epilepsy Behav. 2002;3[5]:475-479.

73. Hosain S, Nikalov B, Harden C, Li M, Fraser R, Labar D. Vagus nerve stimulation treatment for Lennox-Gastaut syndrome. J Child Neurol 2000;15[8]:509-512.

74. Frost M, Gates J, Helmers SL et al. Vagus nerve stimulation in children with refractory seizures associated with Lennox-Gastaut syndrome. Epilepsia 2001;42[9]: 1148-1152.

75. Ng M, Devinsky O. Vagus nerve stimulation for refractory idiopathic generalised epilepsy. Seizure 2004;13:176-8.

76. De Herdt V, Waterschoot L, Vonck K et al. Vagus nerve stimulation for refractory status epilepticus. Eur J Paediatr Neurol 2009;13:286-9.

77. Patwardhan RV, Dellabadia J, Rashidi M, Grier L, Nanda A. Control of refractory status epilepticus precipitated by anticonvulsant withdrawal using left vagal nerve stimulation: a case report. Surgical Neurology 2005;64:170-3.

78. Ben Menachem E. Vagus nerve stimulation, side effects, and long-term safety. Journal of Clinical Neurophysiology 2001;18[5]:415-418.

79. Tatum WO. Ventricular asystole during vagus nerve stimulation for epilepsy in humans - Reply. Neurology 2000;54:776.

80. Ardesch JJ, Buschman HP, van der Burgh PH, Wagener-Schimmel LJ, Van Der Aa HE, Hageman G. Cardiac responses of vagus nerve stimulation: intraoperative bradycardia and subsequent chronic stimulation. Clin Neurol Neurosurg 2007;109[10]:849-852.

81. Ali II, Pirzada NA, Kanjwal Y et al. Complete heart block with ventricular asystole during left vagus nerve stimulation for epilepsy. Epilepsy Behav.2004; 5[5]:768-771.

82. Ramsay RE, Uthman BM, Augustinsson LE et al. Vagus nerve stimulation for treatment of partial seizures: 2. safety, side effects, and tolerability. first international vagus nerve stimulation study group. Epilepsia 1994;35[3]:627-636.

83. Amark P, Stodberg T, Wallstedt L. Late onset bradyarrhythmia during vagus nerve stimulation. Epilepsia 2007;48[5]:1023-1024.

84. McGregor A, Wheless J. Right-sided vagus nerve stimulation - Response.Epilepsia2005;46: 1152-3.

85. Spuck S, Nowak G, Renneberg A, Tronnier V, Sperner J. Right-sided vagus nerve stimulation in humans: An effective therapy? Epilepsy Res. 2008;82[2-3]:232-234.

86. Navas M, Navarrete EG, Pascual JM et al. Treatment of refractory epilepsy in adult patients with right-sided vagus nerve stimulation. Epilepsy Res 2010;90:1-7.

87 De Ferarri gaetano M., Crijns harry J.G.M., Borggrefe M. et al Chronic vagus nerve stimulation: an new and promising therapeutic approach for chronic heart failure. European Heart Journal 2011;32:847-855

88. Morris GL, Mueller WM, Vagus Nerve Stimulation Study Grp. Long-term treatment with vagus nerve stimulation in the refractory epilepsy patients. Epilepsia 1999;40:90.

89. Ben-Menachem E. Vagus-nerve stimulation for the treatment of epilepsy. Lancet Neurol 2002;1:477-82.

90. Blumer D, Davies K, Alexander A, Morgan S. Major Psychiatric Disorders Subsequent to Treating Epilepsy by Vagus Nerve Stimulation. Epilepsy Behav. 2001;2[5]:466-472.

91. De Herdt V, Boon P, Vonck K et al. Are psychotic symptoms related to vagus nerve stimulation in epilepsy patients? Acta Neurol.Belg. 2003:103[3]:170-175.

92. Annegers JF, Coan SP, Hauser WA, Leestma J. Epilepsy, vagal nerve stimulation by the NCP (R) system, all-cause mortality, and sudden, unexpected, unexplained death. Epilepsia 1999;40:165.

93. Gorny KR, Bernstein MA, Watson RE, Jr. 3 Tesla MRI of patients with a vagus nerve stimulator: initial experience using a T/R head coil under controlled conditions. J Magn Reson Imaging 2010;31:475-81.

94. Elliot S Krames, P.Hunter Peckham, Ali R.Rezai. Neuromodulation. First edition. London: Elsevier, 2009.

95. Zabara J. inhibition of experimenetal seizures in canines by repetitive vagal stimulation. Epilepsia 1992;33:1005-1012

96. McLachlan RS. Electrical-Stimulation of the Vagus Nerve for the Treatment of Epilepsy - Support from Experimental-Models. Annals of Neurology 1992;32(2):245.

97. Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. Epilepsia 1990;31 Suppl 2:S7-19.

98. Woodbury JW, Woodbury DM. Vagal stimulation reduces the severity of maximal electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording. Pacing Clin Electrophysiol 1991;14(1):94-107.

99. Zabara J. Inhibition of experimental seizures in canines by repetitive vagal stimulation. Epilepsia 1992;33(6):1005-1012.

100. Naritoku DK, Terry WJ, Helfert RH. Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. Epilepsy Res 1995;22(1):53-62.

101.Takaya M, Terry WJ, Naritoku DK. Vagus nerve stimulation induces a sustained anticonvulsant effect. Epilepsia 1996;37(11):1111-1116.

102.De Herdt, V, De WJ, Raedt R, Wyckhuys T, El TR, Vonck K, et al. Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures. Acta Neurologica scandinavica.2010 Apr; 121(4):271-276

103.Ramsay RE, Uthman BM, Augustinsson LE, Upton AR, Naritoku D, Willis J, et al. Vagus nerve stimulation for treatment of partial seizures: 2. safety, side effects, and tolerability. first international vagus nerve stimulation study group. Epilepsia 1994;35(3):627-636.

104.Handforth A, DeSalles AAF, Asatourian AA, Krahl SE, Us Vns SG. Seizure response to long-interval vagus nerve stimulation (VNS) is predictive of response to conventional VNS, suggesting a long-duration anti-epileptic mechanism. Neurology 1998;50(4):S05006.

105.Tougas G, Hudoba P, Fitzpatrick D, Hunt RH, Upton AR. Cerebral-evoked potential responses following direct vagal and esophageal electrical stimulation in humans. Am J Physiol 1993;264[3]:G486-G491

106.Naritoku DK, Morales A, Pencek TL, Winkler D. Chronic Vagus Nerve-Stimulation Increases the Latency of the Thalamocortical Somatosensory Evoked-Potential. Pace-Pacing and Clinical Electrophysiology 1992;15:1572-8.

107.Brazdil M, Chadim P, Daniel P et al. Effect of vagal nerve stimulation on auditory and visual event-related potentials. Eur J Neurol 2001;8[5]:457-461.

108. Chae JH, Nahas Z, Lomarev M et al. A review of functional neuroimaging studies of vagus nerve stimulation (VNS). J Psychiatr Res 2003;37:443-55.

109.BenMenachem E, Hamberger A, Hedner T et al. Effects of Vagus Nerve-Stimulation on Amino-Acids and Other Metabolites in the Csf of Patients with Partial Seizures. Epilepsy Research 1995;20:221-7.

110.De Herdt V, Bogaert S, Bracke KR et al. Effects of vagus nerve stimulation on pro- and anti-inflammatory cytokine induction in patients with refractory epilepsy. J Neuroimmunol 2009;214:104-8.

111.Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. Epilepsia 1990;31 Suppl 2:S7-19.

112.Woodbury JW, Woodbury DM. Vagal stimulation reduces the severity of maximal electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording. Pacing Clin Electrophysiol 1991;14(1):94-107.

CHAPTER 3

CHAPTER 3: THEORETICAL NEUROPHYSIOLOGICAL BACKGROUND

3.1 The vagus nerve

3.1.1 Anatomy

The vagus nerve carries somatic and visceral afferents and efferents. Afferents compose about 80% of the cervical portion of the vagus and are mainly narrow- calibre unmyelinated C fibres, which predominate over faster conducting myelinated A and B fibres. Viscero-motor efferents originate from the nucleus dorsalis, while efferents that innervate the striated muscles of the pharynx and larynx have their origin in the nucleus ambiguous. Afferent fibres have their origin in the nodose ganglion and conduct viscero-sensory information from organs to the brain via the nucleus tractus solitarius (NTS) in the brainstem. Each vagus nerve bifurcates on entering the medulla to synapse in the NTS bilaterally. In addition to viscera-sensible information, afferents are also represented by taste fibres from the dorsal part of the tongue and sensory information of the external auditory canal including the inner side of the tragus, although the latter fibres make synapse in nucleus spinalis trigeminus and not in the NTS. The NTS projects to inferior and medial cerebellar regions and to multiple pontine and mesencephalic nuclei (parabrachial nucleus, locus coeruleus (LC), raphe magne nucleus). From the NTS, there are direct and indirect projections to various structures in the brain, such as amygdale and thalamus which play a key role in the process of epileptogenesis. In addition, vagus nerve also has wide spread indirect cortical connections, such as the anterior insula, infralimbic cortex and prefrontal cortex (1, 2).

3.1.2 Physiology

The diffuse pathways of the vagus nerve mediate important visceral reflexes such as coughing, vomiting, swallowing, control of blood pressure and heart rate (2). Heart rate is mostly influenced by the right vagus nerve, which has dense projections to the atria of the heart (1).

3.2 General principles of stimulation

3.2.1 Physiology of excitation

Neural membranes at rest exhibit a transmembrane potential (TMP) of -60 to -100mV, which is the result of alignment of negative charges along the inner surface and of positive charges along the outer surface of the membrane. This dipole layer is maintained by diffusion and active ionic transport mechanisms across the lipid bilayer.

Passive changes in TMP occur when a constant current is applied to a membrane and resting level reaches a new steady state level. This process happens in an exponential way governed by the membrane time constant. Time constant equals the duration to achieve 63% of the steady state membrane potential and correlates with the product of membrane resistance and capacitance.

When the passive changes decrease TMP to a threshold of -40 to -60mV (Fig. 1a), ion channels briefly open and allow inward current of sodium ions, reversing the TMP to +20mV. An outflow of potassium ions occurs next, together with an inhibition of sodium inflow, which restores the TMP to a resting level. The resulting sequence of rapid TMP fluctuation as described above is called an action potential (Fig. 1b, c, d) (3).

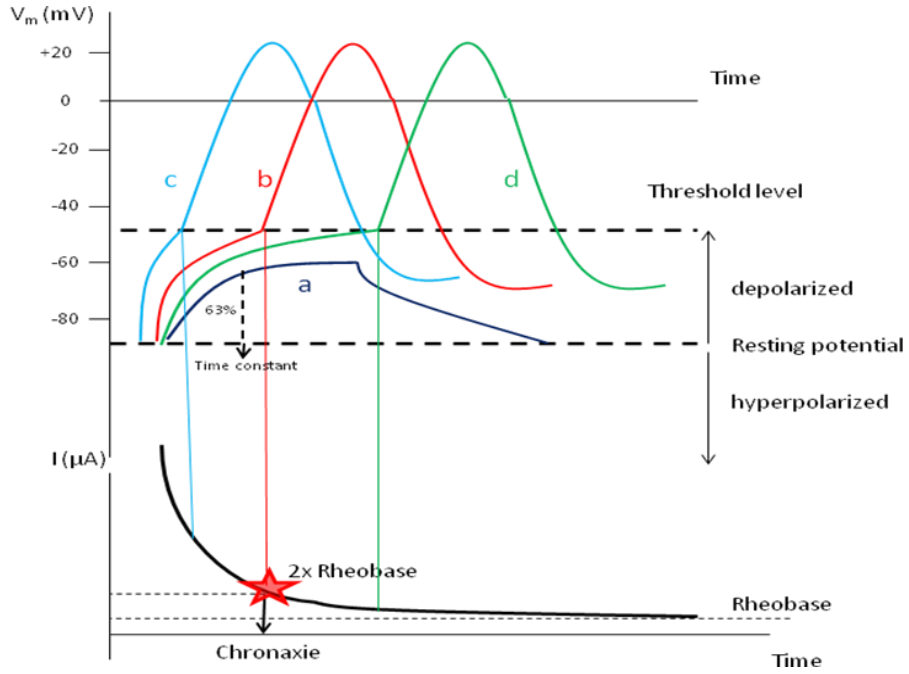


Fig. 1 (a,b,c,d) Changes in transmembrane potential by different constant current pulses. Fig. inspired from Jayakar P, Adv Neurol 1993;63:17-27.

The relation between stimulus parameters and tissue excitability are illustrated in Fig. 1. With very brief pulse durations despite use of high intensity no excitation will take place. Likewise, excitation will not occur below a certain minimum level called the rheobase, which is the minimum intensity needed to obtain an excitation with a stimulus of infinite duration. The chronaxie of the strength duration curve is the pulse duration required to elicit a response with an intensity that equals twice the rheobase.

This point is considered to be most efficient stimulation and requires the least energy (Fig. 1 b). A shorter pulse width than the chronaxie, involves that cells will respond with less stimulus charge for a given current level (Fig. 1 c) Chronaxie and Rheobase principles were published for the first time by Louis Lapicque's in his famous paper on 'Définition experimentale de l'excitabilité' that was published 100 years ago (4). Later on, strength duration curves were further described by the Hill's Equitation (5).

$$Th(D) = \frac{Rh}{1 + e^{-D\frac{\ln(2)}{Chr}}}$$

In which Th(D) = magnitude of the stimulus, *Rh*= Rheobase current, D= pulse duration, *Chr*=chronaxie, ln(2)= natural logarithm of 2

In addition, the equitation was further simplified by Weiis which allows estimations of chronaxie on the basis of two threshold and pulse duration values.

$$Th(D) = Rh\left(\frac{Chr}{\ln(2).D} + \frac{1}{2}\right)$$

Magnitude of Rheobase is dependent on the separation between the electrode and target exicitable tissues, while chronaxie is primarly dependent on the tissues, with typical values of less than 1 msec for neural tissues and greater than 10 msec for muscle. Both parameters are influenced by changes in membrane potential, impedance and myelinisation (6).

3.2.2 Extracellular Field recordings

3.2.2.1 Action potential along a nerve

Electric signals that are measured in the extracellular space and are generated by the electric fields produced by the activity of a single neuron or groups of neurons are called 'Extracellular Field recordings'. Typically these are recorded between two points in the extracellular space rather than across the membrane of neurons. Examples of Extracellular field potentials include recordings of a passing action potential of a nerve, or more complex electroencephalographic recordings of a small part of the brain from the scalp.

If an action potential is elicited at A, the membrane will momentarily reverse polarity and local currents will flow into the nerve cells as is indicated by a ‘’sink’’ (Fig.2).

While the site where currents exist the cells is called “source” If we were recording extra-cellularly at point A (with respect to a distant ground), we would record a negative potential during the action potential, while in B we would record a positivity (Fig. 2) In the example demonstrated in Fig. 3, action potential is not stationary, but propagates over the nerve from the left to the right. Therefore local current sinks and sources changes over time. As the action potential passes over the different recordings sites, the shape of the extracellular field potential will evolve.

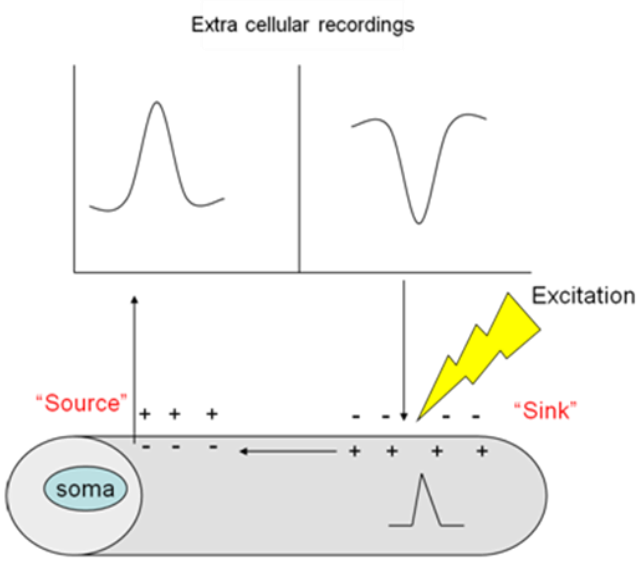


Fig. 2 Extracellular field recordings sink and source

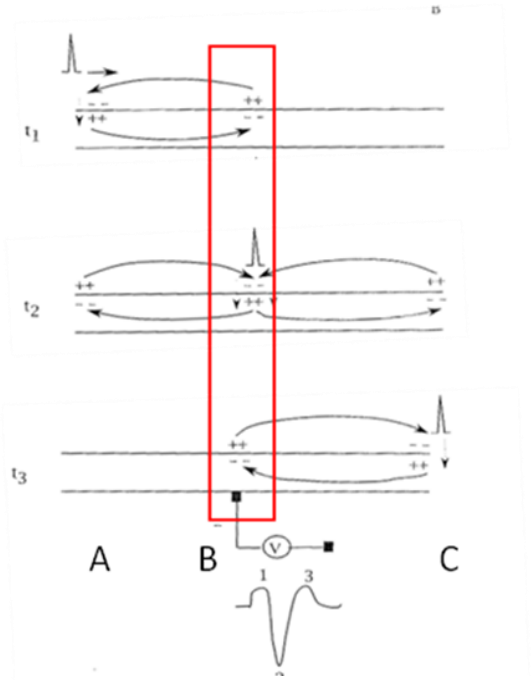
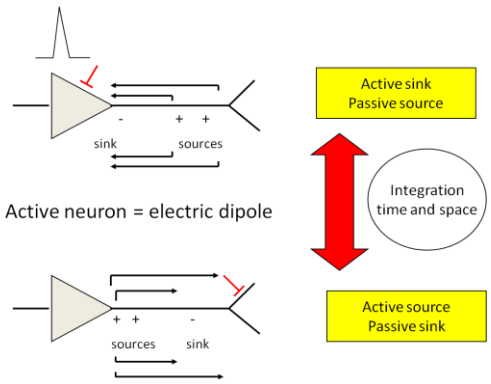


Fig.3 Propagation of an action potential

To summarize, when an action potential occurs at place A, but the recording site is located further on the nerve on place B, the extracellular field potential will first be positive, then show a large negative peak as the result of inward current of Na that has now reached point B. Once the action potential reached point C, extracellular field potential becomes positive again (Fig. 3)(7).

3.2.2.2. Active neuron is an electric dipole

In Fig. 4 for example an action potential is initiated in the soma and is called active sink, while changements in the dendrites are called a passive source. The neuron can be considered as an electric dipole, because during the action potential the dendrites are positive with respect to the soma. At the same time, excitatory inputs can reach dendrites creating new active sinks.



All these extracellular signals are not stationary in time and space, the resultant extracellular field recording of a group of neurons results from these local excitatory and inhibitory synapses.

Fig. 4 Neurons are active dipoles. Extracellular recordings results from of a complex integration in time and space of different sinks and sources.

3.2.2.3. Volume conductor theory

In addition to integration of sinks and sources, the measured extracellular field also depends on the solid angle made with the dipole. The solid angle depends on the physical size of the dipole and the distance between the observation point and the dipole. In Fig. 5.an example is shown, where a small part of the EEG represented as dipole illustrating some type of nervous tissue in which there is a separation of charge. The observation point at the left would measure a negative potential with respect to a distant ground while the one on the right side would measure a positive potential. For EEG, predictions are thus very difficult to make, as observation points are on the surface of the scalp, while dipoles from neurons come from the underlying cortex that show multiple folding and curving (7).

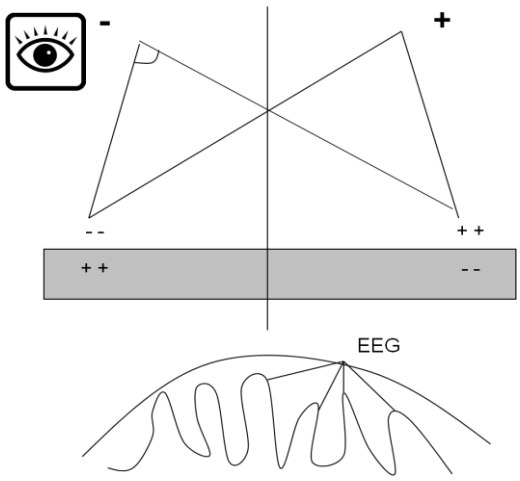


Fig. 5 Electrical field in a volume conductor

3.2.2.4 Classification of fields

Optimal recording of extracellular fields that result from the integration of sinks and sources in time and space depends also the internal arrangements of these specific neurons. There are three main types of geometrical arrangements of neurons: open field, closed field and open-closed field (Fig. 6).

The open field is encountered when neurons organize in a laminar array in which dendrites are facing in one direction and the somata in the other. This types of field is typically found in the cerebellum, neocortex and hippocampus (Fig. 7).These are highly laminated structures that allow adding up of dipoles which renders recordings of extra -cellular fields more effective. In contrast, when a spherical array of neurons in which or somata or dendrites are directed toward periphery, synchronous activation produces dipoles with spherical symmetry. Measurements outside the field record a zero potential as dipoles cancel each other within the sphere (7).

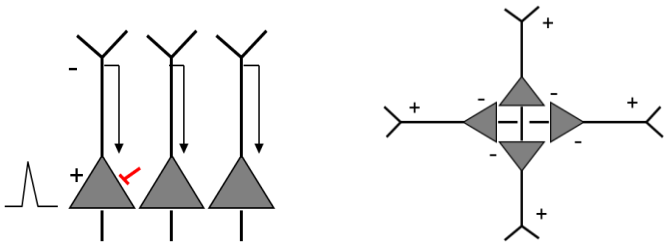


Fig. 6 Open and closed fields

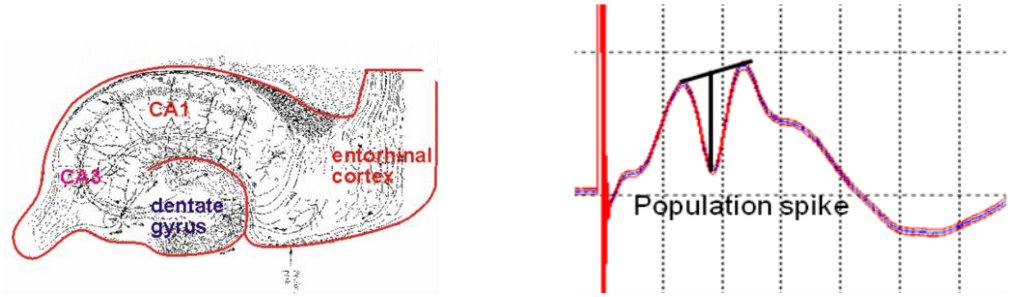


Fig. 7: In vivo stimulation of right perforant path of rat hippocampus and recording of a population spike of dendate gyrus. This example illustrates the importance of open linearly arranged neurons to be able to record extracellular field potentials

3.2.2.5 Compound action potential (CAP)

In mammals, large peripheral nerves, such for example the vagus nerve, are a result of bundles of thousands on individual axons enclosed in a loose connective tissue sheet, the epineurium. Within the epineurium, axons are further sub grouped into smaller fascicles that are embedded in perineum.

Finally, individual axons are covered by individual endonurium. The axons within a compound nerve include afferent (sensory) and efferent (motor and autonomic) nerves. Individual axons vary in diameter, myelinisation, excitability, threshold and conduction speed.

The compound action potential is the algebraic sum of many 'all or none'' action potentials arising more or less simultaneously in a large number of individual axons in a large compound nerve.

The CAP does not occur naturally, but results from an experimentally or clinically induced stimulus with extracellular stimulating electrodes and can be recorded with extracellular electrodes, that measure the summed electrical response of all the excited axons in the nerve. The properties of the CAP are threshold, amplitude, conduction velocity are determined by the type and number of individual axons recruited. The number and type of axons excited depends on the intensity of the applied stimulus. The CAP differs from ''all-or-none'' action potential response of a single neuron in several ways.

The CAP demonstrated by extracellular recording from many axons, is a graded response whose magnitude increases with intensity of stimulation. This is due to different thresholds of excitation of the different axons. In terms of excitability Aα>Aβ>Aɣ>Aδ>B>C, see table 1. At low stimulus intensities only the largest axons are activated, but as the stimulus gradually increases, more and more smaller axons are recruited (8,9).

Table 1: Mammalian Axon Properties at 37°C

Fibre types	**Diameter (µm)**	**Conduction velocity (msec)**	**Action potential duration (msec)**	**Absolute refractory period (msec)**	**Functions**
Aα	12-22	70-100	0.4-0.5	0.2-1	Efferent alpha Afferent muscle spindles, tendon organs
Aβ	5-13	30-70	0.4-0.5	0.2-1	Afferent, cutaneous, touch, pressure
Aɣ	3-8	15-40	0.4-0.7	0.2-1	Gamma motor neurons
Aδ	1-5	12-30	0.2-1	1.2	Afferent, fast pain, temperature
B	1-3	3-15	1.2	2	Efferent autonomic (only pre-ganglionic)
C	0.2-1.2	0.2-2	2		Afferent, ''slow'' Pain, efferent autonomic postganglionic

3.2.3 Effects of stimulation on neural tissue

As mentioned above CAP is induced by an experimentally induced extracellular stimulus. This subchapter will describe which kind of electrical stimuli can be applied and what the effects are of electrical stimulation on neural tissue.

3.2.3.1 Electrochemical processes

Electrical stimulation with metal electrodes requires a flow of ionic charge in biological tissues. When a metal electrode is placed into a physiological medium such as extracellular fluid, an interface is formed between the two phases. In the metal electrode phase and in attached electrical circuits, charge is carried out by electrons.

In the physiological medium, charge is carried out by ions, including sodium, potassium and chloride in the extracellular space. When a voltage source is applied across two electrodes, so that one electrode is driven to relatively negative potential and the other to a relatively positive potential, the interface that is driven negative (cathode), will have an excess of negative charge. This will attract positive cations in solution towards the electrode and repel anions. In the interfacial region, there will reach net electro neutrality, because the negative charge excess on the electrode will equal the positive charge solution near the interface. At the second electrode, the opposite occurs, i.e. repulsion of anions by the negative electrode is countered by attraction of anions at the positive electrode (anode). If the total amount of charge delivered is sufficiently small, no transfer of electrons across the interface will take place and the interface can be modelled by a simple capacitor. If polarity of stimulus is reversed, directions of currents reverse and subsequently charge redistributions reverse and the charge that was injected from the electrode into the electrolyte and stored by the capacitor may be recovered (10). This capacitive mechanism is safe, as no chemical changes are induced in the neural tissue. However, the amount of charge that can be injected this way is limited and is not able to produce an action potential. Further charge injection will lead to reduction and oxidation reactions in the solution, which can lead to storage of these electrochemical products near the surface. In general, reduction requires addition of an electron and occurs at the cathode, while oxidation demands a removal of an electron, which finds place at the anode. Unlike, capacitive mechanisms, Faradaic charge injection forms toxic products in solution that cannot be recovered upon reversing the direction of the current. Next question is how we can induce action potentials by stimulating nerves extracellularly without immediately inducing toxic effects?

Faradaic reactions are divided into reversible and irreversible reactions (11). The degree of reversibility depends on relative rates of kinetics (electron transfer at the electrode) and mass

transport. A Faradaic reaction with very fast kinetics relative to the rate of mass transport, will allow electrochemical products not to move far away from the electrode surface and when direction of currents is reversed, some product that has recently been formed can be reversed back to its initial form. In contrast, when slow kinetics are involved, chemical reactions are able to diffuse away from the electrode interface (see Fig.8) and reversion of current will not reverse the chemical reactions, as products have diffused away from the electrode contacts.

In this case, chemical reactants or formation of gas bubbles lead to irreversible Faradaic reactions, which leads to corrosion of the electrodes and production of toxic molecules (11).

One of the parameters that determine the limit of injected charge and reversible Faradaic reaction are parameters of stimulation waveform. Other factors that play a role are the kind of material used, shape and size of the electrodes and electrical composition of the electrolyte in which the stimulus is given (10).

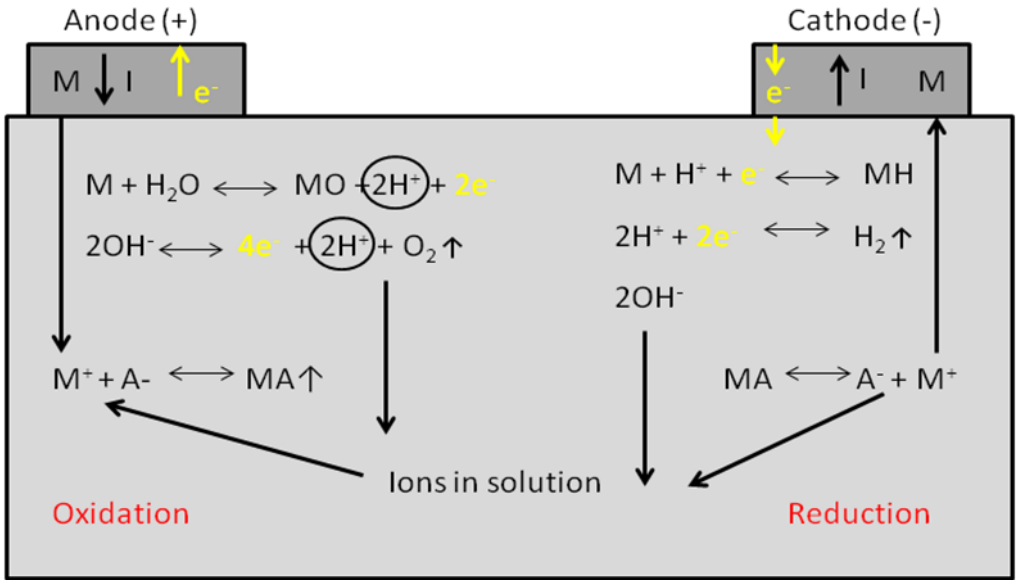


Fig.8. Electrochemical processes induced by electrical stimulation (12)

3.2.4 Stimulus waveforms and stimulation parameters

Pulse train stimulus is described by the pulse waveform, amplitude, duration, polarity and frequency

Fig. 9 summarizes key features of various stimulation waveform types. The cathodic monophasic waveform illustrated in Fig. 9a, consists of pulses of current passed in one direction and is the most efficacious for stimulation. However, monophasic pulses are not used in long term where tissue damage must be avoided. Greater negative over potentials are reached during monophasic pulsing then with biphasic pulsing. Furthermore, electrode potential remains longer negative and the

charged electrode capacitance slowly discharges through Faradaic reactions, allowing deleterious reduction reactions.

Biphasic waveforms are illustrated in Fig. 9b. The first stimulating phase elicits the desired physiological effect such as initiation of an action potential and the second (reversal) phase is used to reverse direction of electrochemical processes. Reversal of both phases is possible too (see later ADNS-300 pulse) The charge balanced biphasic waveform is widely used to prevent tissue damage, although we must bear in mind that biphasic pulses do not completely protect tissue from injury. The charge *imbalanced* waveform (Fig. 9c) may be used to reduce the most positive potentials during the anodic phase of a balanced waveform and prevent electrode corrosion. In addition to electrode corrosion, a second concern with biphasic pulses is that the reversal phase may reverse some of the desired physiological effect of the stimulation phase. This effect causes an increased threshold for biphasic pulses compared to monophasic pulses. An interphase delay between stimulating and reversing phases may lower the threshold of biphasic pulses (Fig.9d) A delay of 100µsec is typically sufficient to prevent the suppressing effect of the reversal phase and be short enough to prevent accumulation of toxic Faradaic reactions.

Finally, the ADNS-300 for therapeutic vagus nerve stimulation used a particular stimulation waveform (Fig.9e). An initial sub treshold long recovery phase was followed by a sharp cathodic pulse. This initial low amplitude recovery phase permitted necessary local recovery from electrochemical process without activating the heart. As the recovery phase was chosen to be low in amplitude, duration had to be longer to achieve a charge balanced pulse. Initiation of this long low amplitude phase before cathodic stimulation also prevents artifact related problems when recording CAPs.

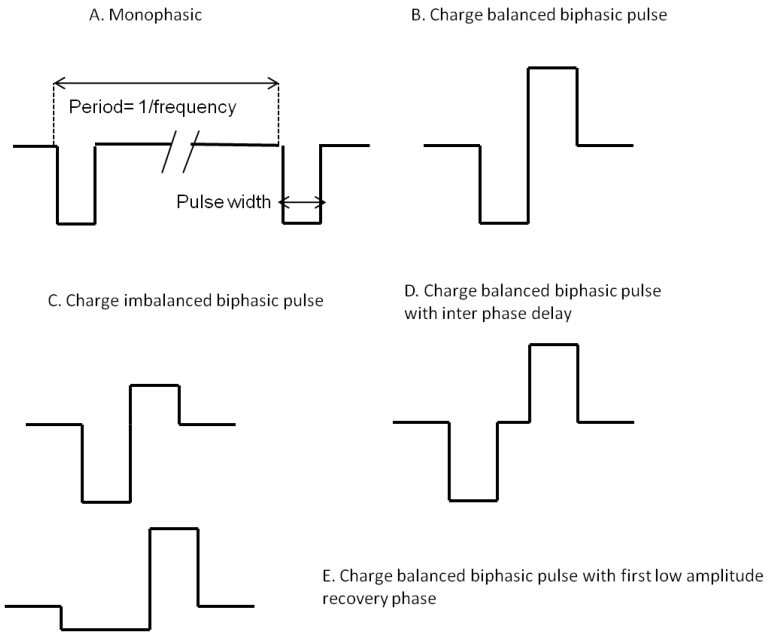


Fig.9 (a,b,c,d,e) Different stimulation pulses

3.2.5 Electrode implant biocompatibility and general methodology

Cuff electrodes are composed of an insulating tubular sheath that completely encircles the nerve and contains two or more electrode contacts exposed at the inner surface that are connected to insulated lead wires. Cuff electrodes have the particular advantage of confining the stimulating current to the inner space of the electrode, thus avoiding stimulation of other neighbouring tissues (13) and diminishing power consumption of the stimulator system. Moreover, cuff electrodes allow correct positioning of electrode leads to minimize mechanical distortion of electrode leads and probability of lead failure (14). Implanted electrodes must fulfil to a certain amount of biocompatibility issues, which are represented by three main pillars (i) degree of inflammation responses (ii) mechanical and (iii) electrical aspects of nerve damage.

Implant biocompatibility should ideally provoke a restricted immune reaction, although no reaction at all is not feasible. After implantation an acute inflammatory response with development of oedema and invasion of inflammatory cells will take place, which is followed by a chronic inflammation (15) and finally fibrosis granulation tissue will encapsulate the implant and isolate it from local tissue environment (16).

With regard to mechanical properties, cuff electrodes should be flexible and self sizing in order to avoid stretching and compression of the nerve (17). On electrical level, biocompatibility implies prevention of tissue damage provoked by creation of toxic electro-chemical reaction products at the electrode surface by Faradaic processes. In addition, high duration, high frequency biphasic stimulation can lead to altered extra axonal environment and ionic homeostasis which might precipitate injury (17).

<u>General methodology: electrodes for experimental rats</u>

Fig. 10 Fabrication of spiral cuff electrodes

The self-sizing spiral cuff electrode is composed of two 80µm thick silicon rubber sheets (Statice Santé, France) glued together with an adhesive which polymerizes at room temperature (Part A and B MED 4-4210, Nusil). The internal sheet is stretched (stretch factor of 0.5) for curling of the spiral cuff. The cuff has an internal diameter of 1 mm and a total length of 9 mm. The sheets have three pieces of platinum (Alfa Aesar, 99.9% metal basis, 0.25 mm thick) inserted between them, which form stimulation and recording contacts. Anode and cathode (3x1 mm) are 1 mm separated from each other, while the recording contact (1x1mm) is situated 2 mm distal to the cathode. In order to create internal contacts, windows of 500µm size are cut out in the internal sheet at the level of the platinum contacts. Teflon coated stainless steel wires (FWM 1x7x0.02/316LVM/EFTE, Fort Wayne metals) of 20 cm were welded to each end of platinum contact (Fig. 10, 11).

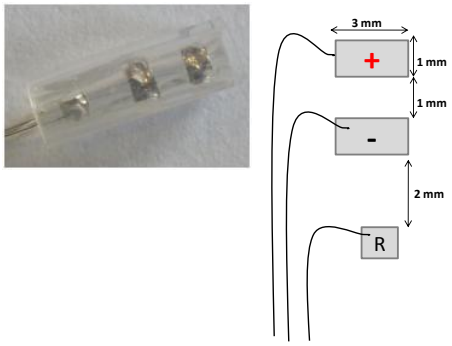


Fig.11: Custom made self sizing cuff electrode for combined stimulation and recording of the vagus nerve in rats.

3.3 Overview of biological markers reflecting electrical activation of the vagus nerve

3.3.1 Introduction

Epilepsy is one of the most common neurological disorders, yet one third of patients do not respond favourably to classical anti-epileptic drugs and a considerable amount of patients report major side effects. Epilepsy surgery or neurostimulation are treatment alternatives for some types of epilepsy. Although vagus nerve stimulation (VNS) has been introduced many years ago and more than 50,000 patients were implanted with a vagus nerve stimulator, there are no predictive factors for response. Duration of epilepsy (18-21), age at time of implantation (22), type of epilepsy or epilepsy syndrome (23-29), age at time of epilepsy onset, do not seem to be independent predictors of outcome(21). In

addition to analysis of large patients groups, identification of neurochemical and electrophysiological effects of VNS might lead to more efficient recognition of predictive factors for clinical response. This subchapter will review different parameters that reflect electrical stimulation and activation of the vagus nerve and highlight those that were correlated with seizure outcome after VNS.

3.3.2. Vagus nerve stimulation induced evoked potentials

3.3.2.1 Human experiments

Evoked potential (EP) recording is a special type of electro-encephalography (EEG) where an electrical potential is recorded from the nervous system following presentation of a stimulus. In clinical practice, auditory, visual and somatosensory evoked potentials are commonly used to evaluate integrity of neural functioning. In addition to appreciating functioning of CNS pathways, EP's can also provide us with a proof of adequate stimulation.

Therefore, VNS-induced EPs could be used as an indicator for effective stimulation and successful transmission to the brain. Only a few studies have measured VNS-induced EPs probably because somatotopic cortical representation of vagal information is not fully understood (30).

Tougas et al. compared cerebral EPs in response to direct vagal and esophageal stimulation in humans in order to examine the potential usefulness of studying esophageal EPs as a measure of vagal afferent neural function (31). Stimulation of the vagus nerve was provided by a Cyberonics model 100 generator who delivered 200 µsec square wave pulses of 1 mA at a rate of 2 Hz. Evoked potentials over midline at Cz (international 21 electrode system) were obtained 6 weeks after implantation. Vagal and esophageal EPs were similar and consisted of three negative peaks (N_1, N_2, N_3) with two intercalated peaks (P_1 and P_2). Mean latency to the first peak (N_1) was 71 +/- 12 msec while the latest component (N_3) appeared 328 +/- 46 msec after stimulation of the left vagus nerve. Authors found a significant delay in latencies of the evoked responses obtained in epileptic patients treated with VNS compared to healthy patients. The measured conduction velocity of the afferent response was 7.5 m/sec in epileptic subjects and 10 m/sec in healthy controls, suggesting that afferent conduction is the result of A-fibres rather than slower C afferent fibres activation. As control subjects did not have epilepsy nor VNS, it remains difficult to interpret these results, but authors hypothesized their findings could be due to treatment with anti-epileptic drug or to long standing poorly controlled epilepsy, although afferent neural damage after surgery may be another possible cause for the reported delayed responses. Unfortunately, authors did not check whether a correlation between VNS induced seizure reduction and vagal evoked EP's existed.

The previous study was put into question when Hammond et al. performed a study in which in similar way left vagus nerve was stimulated with a Cyberonics model 100 generator (250 µsec, 1 mA, 5 Hz), but a single negative potential of high amplitude with a peak at about 12 msec and a widespread field was recorded (32).

The generators were shown to be left anterior cervical skeletal muscles by abolishing the vagus evoked potential with a neuromuscular agent, and then showing the return of the vagus evoked potential after the effects of the blocker were reversed. This response was initiated at low stimulation intensity of 1 mA, while Tougas stimulated up to 14 mA and did not report any interfering muscle activity (31).

Interestingly, Hammond proposed activation of the recurrent laryngeal nerve and contraction of laryngeal muscles as possible explanation for the myogenic EP. Indeed, all patients reported effects on their voice, indicating activation of the recurrent laryngeal nerve.

Penry JK also reported a cortical vagal EP with a latency of 8.2 msec, though no further analysis was done to check whether the EP was myogenic in origin or not (33).

Besides left cervical vagus nerve stimulation with an implanted device, transcutaneous stimulation of the vagus nerve (t-VNS) was proposed by Ventureyra (34). Indeed, the vagal nerve has a cutaneous representation in the external auditory canal including the inner side of the tragus. Cutaneous stimuli of this region are transported via the auricular nerve to the jugular ganglion and from there with the vagus nerve into the medulla oblongata and the nucleus tractus solitarius (NTS). Falgatter et al described a vagal somatosensory evoked potential (VSEP) in 5 patients, which consisted of two positive peaks and one negative peak ($P_1N_1P_2$) and was most prominent at C4-P4 (35). Mean latency for P_1 was 2.4 msec, while N_1 and P_2 appeared at 3.58 and 4.88 msec respectively. VSEP was only observed when stimulating within the innervation area of the auricular branch of the vagus nerve, while no EP was elicited at other sites. Authors proposed VSEP to be a far field potential of postsynaptic brain activity from the vagus nerve nuclei, although it was not excluded that the VSEP was a result of the activation of vagus nerve in combination with other brainstem nerves, such as facial and trigeminal nerve. In addition, latencies were significantly longer in elderly healthy patients as compared to younger participants (36). In context of search of non invasive diagnostic tools, Polak et al. found significantly longer VSEP latencies in ten patients with Alzheimer disease and mild cognitive impairment (37, 38). This study was based on the knowledge that several vagal nuclei in the brainstem show pathological changes and autonomic functions are often impaired in the course of Alzheimer disease. In a similar way as it was applied for disease of Alzheimer, research on whether

VSEP could function as a predictive tool to identify VNS responders has still to be performed, as no literature addresses this question.

Considering t-VNS, we must bear in mind that transcutaneous approach of stimulating is far away of being a valid alternative for left cervical stimulation, although one study already has shown that t-VNS shares similar features in bold-fMRI brain activation patterns with invasive vagus nerve stimulation (39).

3.3.2.2 Animal studies

In rats, vagal evoked potentials have been recorded from different brain regions.

The NTS is a main gate of vagal afferents to the rest of the brain and evoked potentials in this structure were described by Nosaka for the first time in 1978 (40).

Results were reproduced by Ito and showed a negative deflection with 4 msec of latency and a second negative peak at 25 msec. Both Authors described the first peak as a myelinated (A) response and the second one as an unmyelinated (C) response. Interestingly, latency of C response decreased as stimulus intensity increased (41).

Insular cortex is widely accepted to incorporate visceral sensory information transmitted from the vagus nerve. Ito has identified two distinct insular cortical responses, an early and late positive negative (PN) potential in the granular layer and an equivalent early and late negative positive (NP) potential in the agranular layer of the insular cortex, which indicates a local generator of the evoked potential (42).

The early PN and NP potential resulted from activation of myelinated fibres of the vagus nerve and had a latency in the range of 20 to 40 msec, while the late response only appeared when c afferents were stimulated and occurred between 40 to 90 msec. In addition to activation in the insular cortex, Ito also examined whether visceral information projects to an area around S1 (primary somatosensory cortex), as natural esophageal stimulation induces activity changes in this region in different non-invasive imaging studies (fMRI, MEG and PET) (43-45). Moreover, older studies such as the study by O'Brien, have demonstrated evoked bilateral cortical responses in monkeys after stimulation of the cervical nerve in the region ventral to the precentral cortex with a latency of < 50 msec (46). Although regarded as 'motor cortex', the area ventral to the motor cortex is known to be occupied by the S1. Based on these elements, Ito further analyzed this subject in rats and described an S1 field potential, which was comparable in waveform, latency and amplitude to previous granular insular potential (positive negative, peak latency 62 +/- 14 ms) and located at the most rostral part of parietal granular cortex. Authors concluded that the rat S1 contains a region representing general

visceral information, topographically located as if the visceral organs protruded from the mouth in the Homunculus of Penfield (30). Furthermore, it was shown through pharmacological studies that the three vagal afferent projection sites, namely the S1, granular and agranular insular cortex, functioned independently from each other (30, 41, 42).

One important limitation in these vagal field potential studies is the fact that stimulation of the cervical vagal nerve must have activated laryngeal afferents, which are pure somatic in nature and consequently the cortical evoked potential recorded probably represents activation of both oropharyngeal as well as afferents from visceral tissues (31).

In addition to insular and lateral sensorimotor cortices, vagal activation was also found in the thalamus. Based upon anatomical information, Ito et al. found an evoked potential in the expected gustatory/visceral thalamic relay nucleus in monkeys (47). In addition, he reported an earlier and larger response in the adjacent parafascicular nucleus (Pf) of the thalamus (onset 18msec, peak 35 msec), which is regarded as an integral component of the striatal network controlling movement. Stimulation in the region of the Pf or its main projection target, the basal ganglia, was shown to have anti-epileptic effects (48, 49). In addition, functional imaging studies indicate that VNS produces strong activation of the basal ganglia (50).

Finally, vagal evoked responses were reported in the cerebellum of rabbits, more specifically in the medial region in lobule VIIa, which was shown to play a role in cardiovascular control (51). Electrical stimulation of this region produced an inhibition of the renal sympathic nerve activity and a fall in blood pressure in anesthetized rabbits (52).

In summary, vagal evoked potentials were observed in the NTS, thalamus, lateral portion of sensorimotor cortex, insula and cerebellum in different species (rats, rabbits and monkeys). In humans, no similar experiments were described, as introduction of depth electrodes remains invasive and clinical value of vagal evoked potentials remains to be proven. For this purpose, more extensive animal research in which different vagal evoked potentials are correlated with seizure outcome after treatment with VNS is needed.

3.3.3. Effect of VNS on clinically commonly used evoked potentials

In addition to vagal evoked scalp potentials, Hammond also studied whether VNS induces changes in other EP's, such as auditory brainstem evoked potentials, auditory 40 Hz potentials or cognitive evoked potentials, though no effects were found (32).

In contrast, Naritoku et al reported significant prolongation of the cervicomedullary to thalamocortical potential (N_{13}-N_{20}) on somatosensory evoked potential in three patients (53). Two of the three patients experienced an improved seizure control.

As patient population was small no conclusions were made whether N_{13}-N_{20} interval could be used as a predictive factor for beneficial response. Furthermore, anti epileptic drug regiments were not specified for the included patients although increases of interpeak latencies of SSEP were described during treatment with for example carabamazepine and phenytoin (54).

Therefore influence of anti-epileptic drug treatment on increased latencies of SSEP is not fully excluded. Contrary to the study of Hammond, Bradzil et al. described in nine patients treated with VNS higher N_2/P_3 amplitudes of visual EPs (which are cognitive evoked potentials) prior to implantation of the VNS system compared to 3-6 months after device activation (55). As alertness possibly played a role in P_3 parameters, alert state was monitored in patients by means of EEG and motor responses. Both remained comparable preceding and following VNS. In addition, a direct association between visual N_2/P_3 peak-to-peak amplitude increase and clinical effects of VNS treatment was found, although this was based on a small amount of patients.

3.3.4. Functional imaging studies

Different neuro-imaging techniques, like single photon computed tomography (SPECT), positron emission computed tomography (PET) and functional magnetic resonance imaging (fMRI), have been used to assess changes in brain activations patterns after VNS. There is growing literature about VNS combined with functional neuro-imaging, but divergent methodologies make it difficult to compare results. For a systematic review we refer to article of Chae et al. (56).

This subchapter of the thesis will highlight some particular findings especially those that were correlated with seizure reduction after VNS.

Henry et al. performed several acute and chronic PET studies. First, authors studied the acute effects in 10 patients who had a H_2O (15) IV injection before receiving VNS and again in less than 20h after VNS initiation (57). By use of the magnet function which triggers a 30 sec train of VNS, injection of H_2O (15) O was delivered at the beginning of stimulation when the magnet was placed over the VNS generator.

VNS was given at high and low levels in two groups of 5 patients. Bilateral thalamic increases in cerebral blood flow (CBF) occurred in both high- and low-stimulation groups during VNS. In addition, significant blood flow increases were observed in both groups in the hypothalami and in the left and

right anterior insular cortices, while significant decreases in amygdalar, hippocampal and posterior cingulated gyral blood flow were found.

The authors suggested that the VNS induced decreases might have been a reflection of anti-convulsant effects of the device, with lower sustaining repetitive ictal firing in these regions, although no intracellular recordings were performed to confirm this hypothesis.

In addition, the high stimulation group had significant blood flow increases in the left and right orbitofrontal gyri, right entorhinal cortex and the right temporal pole, which did not occur in the low stimulation group, indicating a dose dependent VNS effect. Most importantly, Henry et al. were able to demonstrate that acute bilateral thalamic cerebral increases in CBF were correlated with decreased seizure frequency, although no definite dose-response correlation was described. Chronic VNS after three months of treatment showed similar PET changes, although initial described decreases in bilateral hippocampus, amygdala and cingulate gyrus and increases in the bilateral insula were not detected in the chronic study. During the chronic study, no CBF changes were observed in any region that did not have CBF changes during immediate-effect studies (58,59). Interestingly, acute thalamic activations persisted during chronic VNS. In general, authors concluded that subcortical regions which showed CBF changes in the short term study persisted in showing the same activation in the long-term VNS study, while cortical changes did not persist. Differences might have been a reflection of the brain's adaptation to chronic VNS, as identical PET protocols were applied and no changes in VNS parameters or anti epileptic drug treatment were made. Ko et al. performed H_2O (15) PET scans in patients who were treated with VNS during 5 to 16 months and compared scans before and after switching on and off the stimulator (2 mA, 30 Hz) (60) The difference between PET with VNS and without revealed that left VNS activated right thalamus, right posterior temporal cortex, left putamen and left inferior cerebellum.

In contrast with the PET studies of Henry, our group showed decreased thalamic activity in a SPECT study with ^{99m}TC-ethyl cysteinate dimer (ECD:Neurolite) (61, 62).

A baseline scan was taken 10 min after injection of the bolus of Neurolite while VNS was off and a second scan later the same day with VNS switched on from 0.25 mA to 0.5 mA, depending on individual tolerance. The second dose was injected at the end of a 30 sec train of VNS. Decreased thalamic CBF in acute VNS suggested reduction of seizure onset or propagation through inhibition of the thalamic relay centre.

The difference between the study of Vonck et al. and Henry may be partially explained by the fact that Henry took PET images during the VNS on cycle, while the SPECT study images were taken just after VNS was stopped. Moreover, PET and SPECT studies are quite different imaging techniques, which also may account for some of the observed differences.

Nevertheless, it can be speculated that during VNS thalamus has an increased blood flow and that immediately after there is a decreased CBF compared to baseline, which could be considered as a kind of rebound phenomena. With respect to seizure reduction, Vonck et al found a correlation between increased uptake in the left medial inferior temporal lobe and higher seizure reduction. Moreover, decreased left thalamic uptake was positively correlated with clinical efficacy. Interestingly, thalamic decrease in CBF persisted in chronic SPECT and moreover, no other alterations were observed (63). In addition to PET and SPECT, BOLD fMRI studies were performed in patients with refractory epilepsy and VNS. This technique doesn't use any radioactive tracer, but is based on detecting changes in cerebral blood oxygenation. Importantly, in a study of Liu, two patients with thalamic activation were shown to have a more than 50% seizure reduction, while the other three participating patients were non responders and showed no thalamic activation (64). Additionally, the patient with greater seizure control had a more robust thalamic activation pattern. Although sampling size was small, important role of the thalamus is highlighted once again.

3.3.5. Cerebrospinal fluid (CSF)

Ben-Menachem analysed CSF in patients with refractory epilepsy treated with VNS, before initiation and after three months of therapy and correlated these findings with seizure reduction up to 9 months of VNS (65). A trend for decrease of excitatory amino acid asparagine was correlated with decreased seizure frequency at maximal follow-up, while GABA, an inhibitory amino acid, was elevated in both responders and non- responders. Surprisingly, GABA seemed to be elevated even more in non-responders. Similarly, elevated ethanolamine (EA) and phospho-ethanolamine (PEA) at three and 9 months respectively also correlated with increased seizure reduction. These substrates are brain phospholipids and mark turnover of neural membranes and were shown to be increased in extracellular spaces in experimentally induced seizures in animals (66). Possibly, responders were patients with initially highest seizure frequency, which could be an explanation for higher levels of EA and PEA, but no baseline levels before implantation were available.

On the other hand, VNS might have induced more neuroplastic changes in the brain of responders, in this case EA and PEA possibly could function as a potential VNS predictor, although this a very speculative idea.

3.3.6. Transcranial Direct Current Stimulation (tDCS)

tDCS is non-invasive stimulation technique which was shown to suppress epileptic activity and epileptic seizures in animal experiments (67,68) In patients, tDCS was demonstrated to decrease frequency of epileptic discharges on EEG and to produce a trend to seizure reduction 1 month after application of stimulation (69,70). Currently, a study is being carried out at University of Luebeck (Germany), to examine whether tDCS can be used as a predictive tool in VNS responder identification. A prior positive effect after a single tDCS on short time seizure reduction and epileptiform discharges on EEG will be correlated be VNS outcome in patients with refractory epilepsy

3.3. 7. Effects of VNS on IL-8

The role of VNS in controlling and modulating inflammatory responses was investigated by our group by measuring cytokines produced by peripheral blood mononuclear cells before and after VNS (71). IL-8 was significantly decreased after 6 months of VNS. No correlation could be made between decrease of IL-8 and seizure outcome, but patient population was small (n=10). Il-8 is a chemokine produced by macrophages and other immune cells and it has a major role in the inflammatory response. Future studies on larger amounts of patients might clarify whether responders and non responders have a different cytokine profile after VNS.

Reference List

1. Henry TR. Therapeutic mechanisms of vagus nerve stimulation. Neurology 2002;59:S3-14.

2. Vonck K, Van LK, Dedeurwaerdere S, Caemaert J, De RJ, Boon P. The mechanism of actio of vagus nerve stimulation for refractory epilepsy: the current status. J Clin Neurophysiol 2001;18:394-401

3. Jayakar P. Physiological principles of electrical stimulation. Adv Neurol 1993;63:17-27.

4. Irnich W. The terms "chronaxie" and "rheobase" are 100 years old. Pacing Clin Electrophysiol 2010;33:491-6.

5. Bostock H. The strength-duration relationship for excitation of myelinated nerve: computed dependence on membrane parameters. J Physiol 1983;341:59-74.

6. J.Thomas Mortimer, Narendra Bhadra. In: Elliot S Krames, P Hunter Peckham, Ali R.Rezai, eds. Neuromodulation. Elsevier, 2010:109-21.

7. Johnston Daniel, Miao-sin Wu Samuel. Foundations of Cellular Neurophysiology. A Bradford Book, 1995.

8. GASSER HS. The Control of Excitation in the Nervous System. Bull N Y Acad Med 1937;13:324-48.

9. Choh-Luh Li, George Mathews, Anthony Frank Bak. Action potential of somatic and autonomic nerves . Experimental Neurology 1977;56[3]:527-537.

10. Merrill DR, Bikson M, Jefferys JG. Electrical stimulation of excitable tissue: design of efficacious and safe protocols. J Neurosci Methods 2005;141:171-98.

11. Allen J.Bard, Larry R.Faulkner. Electrochemical methods: fundamentals and applications. Wiley India Pvt. Ltd, 2006.

12. Jean Delbeke. Presentation « Implantable stimulation electrodes » UCL. 14-6-2006.

13. Navarro X, Krueger TB, Lago N, Micera S, Stieglitz T, Dario P. A critical review of interfaces with the peripheral nervous system for the control of neuroprostheses and hybrid bionic systems. J Peripher. Nerv.Syst 2005;10[3]:229-258.

14. Rodriguez FJ, Ceballos D, Schuttler M et al. Polyimide cuff electrodes for peripheral nerve stimulation. J Neurosci.Methods 2000;98[2]:105-118.

15. William DF, Roaf R. Implants in surgery. London: W.B. Saunders compagny Ltd., 1973.

16. Thil MA, Tran Duy D, Colin IM, Delbeke J. Time course of tissue remodelling and electrophysiology in the rat sciatic nerve after spiral cuff electrode implantation. J Neuroimmunol. 2006;185:103-114.

17. Agnew WF, McCreery DB. Considerations for safety with chronically implanted nerve electrodes. Epilepsia 1990;31 (Suppl 2):S27-S32.

18. Helmers SL, Wheless JW, Frost M et al. Vagus nerve stimulation therapy in pediatric patients with refractory epilepsy: retrospective study. J Child Neurol 2001;16[11]:843-848.

19. Labar D. Vagus nerve stimulation for 1 year in 269 patients on unchanged anti-epileptic drugs. Seizure. 2004;13[6]:392-398.

20. Ben Renfroe J, Wheless JW. Earlier use of adjunctive vagus nerve stimulation therapy for refractory epilepsy. Neurology 2002;59:S26-S30.

21. Janszky J, Hoppe M, Behne F, Tuxhorn I, Pannek HW, Ebner A. Vagus nerve stimulation: predictors of seizure freedom. Journal of Neurology, Neurosurgery and Psychiatry 2005;76[3]:384-389.

22. Sirven JI, Sperling M, Naritoku D et al. Vagus nerve stimulation therapy for epilepsy in older adults. Neurology 2000;54[5]:1179-1182.

23. Labar D, Murphy J, Tecoma E. Vagus nerve stimulation for medication-resistant generalized epilepsy. E04 VNS Study Group. Neurology 1999;52[7]:1510-1512.

24. Frost M, Gates J, Helmers SL et al. Vagus nerve stimulation in children with refractory seizures associated with Lennox-Gastaut syndrome. Epilepsia 2001;42[9]:1148-1152.

25. Karceski S. Vagus nerve stimulation and Lennox-Gastaut syndrome: a review of the literature and data from the VNS patient registry. CNS.Spectr. 2001;6[9]:766-770.

26. Majoie HJM, Berfelo MW, Aldenkamp AP, Evers SMAA, Kessels AGH, Renier WO. Vagus nerve stimulation in children with therapy-resistant epilepsy diagnosed as Lennox-Gastaut syndrome - Clinical results, neuropsychological effects, and cost-effectiveness. J Clin Neurophysiol 2001;18:419-28.

27. Valencia I, Holder DL, Helmers SL, Madsen JR, Riviello JJ, Jr. Vagus nerve stimulation in pediatric epilepsy: a review. Pediatr.Neurol 2001;25[5]:368-376.

28. Ng M, Devinsky O. Vagus nerve stimulation for refractory idiopathic generalised epilepsy. Seizure. 2004;13[3]:176-178.

29. Holmes MD, Silbergeld DL, Drouhard D, Wilensky AJ, Ojemann LM. Effect of vagus nerve stimulation on adults with pharmacoresistant generalized epilepsy syndromes. Seizure. 2004;13[5]:340-345.

30. Ito S. Visceral region in the rat primary somatosensory cortex identified by vagal evoked potential. J Comp Neurol 2002;444:10-24.

31. Tougas G, Hudoba P, Fitzpatrick D, Hunt RH, Upton AR. Cerebral-evoked potential responses following direct vagal and esophageal electrical stimulation in humans. Am J Physiol 1993;264[3]:G486-G491.

32. Hammond EJ, Uthman BM, Reid SA, Wilder BJ. Electrophysiologic studies of cervical vagus nerve stimulation in humans: ii. evoked potentials. Epilepsia 1992;33[6]:1021-1028.

33. Penry JK, Dean JC. Prevention of intractable partial seizures by intermittent vagal stimulation in humans: preliminary results. Epilepsia 1990;31 (Suppl 2):S40-S43.

34. Ventureyra ECG. Transcutaneous vagus nerve stimulation for partial onset seizure therapy - A new concept. Childs Nervous System 2000;16:101-102.

35. Fallgatter AJ, Neuhauser B, Herrmann MJ et al. Far field potentials from the brain stem after transcutaneous vagus nerve stimulation. J Neural Transm. 2003;110[12]:1437-1443.

36. Fallgatter AJ, Ehlis AC, Ringel TM, Herrmann MJ. Age effect on far field potentials from the brain stem after transcutaneous vagus nerve stimulation. Int.J Psychophysiol. 2005;56[1]:37-43.

37. Polak T, Ehlis AC, Langer JBM et al. Non-invasive measurement of vagus activity in the brainstem - a methodological progress towards earlier diagnosis of dementias? Journal of Neural Transmission 2007;114:613-9.

38. Polak T, Markulin F, Ehlis AC et al. Auricular vagus somatosensory evoked potentials in vascular dementia. J Neural Transm 2009;116:473-7.

39. Kraus T, Hosl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. Journal of Neural Transmission 2007;114:1485-93.

40. Nosaka S, Kamaike T, Yasunaga K. Central vagal organization in rats: an electrophysiological study. Exp Neurol 1978;60[3]:405-419.

41. Ito S. Multiple projection of vagal non-myelinated afferents to the anterior insular cortex in rats. Neurosci Lett 1992;148:151-4.

42. Ito S. Electrophysiological evidence for projections of myelinated and non-myelinated primary vagal afferents to the rat insular cortex. Neurosci Lett 1994;179:29-32.

43. Binkofski F, Schnitzler A, Enck P et al. Somatic and limbic cortex activation in esophageal distention: a functional magnetic resonance imaging study. Ann Neurol 1998;44:811-5.

44. Aziz Q, Thompson DG, Ng VW et al. Cortical processing of human somatic and visceral sensation. J Neurosci 2000;20:2657-63.

45. Hobson AR, Furlong PL, Worthen SF et al. Real-time imaging of human cortical activity evoked by painful esophageal stimulation. Gastroenterology 2005;128:610-9.

46. O'Brien JH, Pimpaneau A, Albe-Fessard D. Evoked cortical responses to vagal, laryngeal and facial afferents in monkeys under chloralose anaesthesia. Electroencephalogr Clin Neurophysiol 1971;31:7-20.

47. Ito S, Craig AD. Vagal-evoked activity in the parafascicular nucleus of the primate thalamus. Journal of neurophysiology 2005;94[4]:2976-2982.

48. Velasco F, Velasco AL, Velasco M, Jimenez F, Carrillo-Ruiz JD, Castro G. Deep brain stimulation for treatment of the epilepsies: the centromedian thalamic target. Acta Neurochir.Suppl 2007;97[Pt 2]:337-342.

49. Velasco M, Velasco F, Velasco AL, Jimenez F, Brito F, Marquez I. Acute and chronic electrical stimulation of the centromedian thalamic nucleus: modulation of reticulo-cortical systems and predictor factors for generalized seizure control. Arch Med Res 2000;31[3]:304-315.

50. Narayanan JT, Watts R, Haddad N, Labar DR, Li PM, Filippi CG. Cerebral activation during vagus nerve stimulation: a functional MR study. Epilepsia 2002;43[12]:1509-1514.

51. Kondo M, Sears TA, Sadakane K, Nisimaru N. Vagal afferent projections to lobule VIIa of the rabbit cerebellar vermis related to cardiovascular control. Neurosci Res 1998;30:111-7.

52. Nisimaru N, Okahara K, Yanai S. Cerebellar control of the cardiovascular responses during postural changes in conscious rabbits. Neurosci Res 1998;32:267-71.

53. Naritoku DK, Morales A, Pencek TL, Winkler D. Chronic Vagus Nerve-Stimulation Increases the Latency of the Thalamocortical Somatosensory Evoked-Potential. Pace-Pacing and Clinical Electrophysiology 1992;15:1572-8.

54. Mervaala E, Keranen T, Tiihonen P, Riekkinen P. The effects of carbamazepine and sodium valproate on SEPs and BAEPs. Electroencephalogr Clin Neurophysiol 1987;68:475-8.

55. Brazdil M, Chadim P, Daniel P et al. Effect of vagal nerve stimulation on auditory and visual event-related potentials. Eur J Neurol 2001;8[5]:457-461.

56. Chae JH, Nahas Z, Lomarev M, Denslow S, Lorberbaum JP, Bohning DE, et al. A review of functional neuroimaging studies of vagus nerve stimulation (VNS). J Psychiatr Res 2003;37(6): 443-455

57. Henry TR, Bakay RA, Votaw JR et al. Brain blood flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: I. Acute effects at high and low levels of stimulation. Epilepsia 1998;39[9]:983-990.

58. Henry TR, Votaw JR, Pennell PB et al. Acute blood flow changes and efficacy of vagus nerve stimulation in partial epilepsy. Neurology 1999;52[6]:1166-1173.

59. Henry TR, Bakay RA, Pennell PB, Epstein CM, Votaw JR. Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. prolonged effects at high and low levels of stimulation. Epilepsia 2004;45[9]:1064-1070.

60. Ko D, Heck C, Grafton S et al. Vagus nerve stimulation activates central nervous system structures in epileptic patients during PET H_2O (15) blood flow imaging. Neurosurgery 1996;39[2]:426-430.

61. Van Laere K, Vonck K, Boon P, Brans B, Vandekerckhove T, Dierckx R. Vagus nerve stimulation in refractory epilepsy: SPECT activation study. J Nucl.Med 2000;41[7]: 1145-1154.

62. Vonck K, Boon P, Van Laere K et al. Acute single photon emission computed tomographic study of vagus nerve stimulation in refractory epilepsy. Epilepsia 2000;41[5]: 601-609.

63. Vonck K, De Herdt V, Bosman T, Dedeurwaerdere S, Van Laere K, Boon P. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. Seizure-European Journal of Epilepsy 2008;17:699-706.

64. Liu WC, Mosier K, Kalnin AJ, Marks D. BOLD fMRI activation induced by vagus nerve stimulation in seizure patients. Journal of Neurology, Neurosurgery and Psychiatry 2003;74[6]:811-813.

65. BenMenachem E, Hamberger A, Hedner T et al. Effects of Vagus Nerve-Stimulation on Amino-Acids and Other Metabolites in the Csf of Patients with Partial Seizures. Epilepsy Research 1995;20:221-7.

66. Porcellati GaAG. Handbook of Neurochemistry. Plenum Press, new York, 1983.

67. Liebetanz D, Fregni F, Monte-Silva KK et al. After-effects of transcranial direct current stimulation (tDCS) on cortical spreading depression. Neurosci Lett 2006;398:85-90.

68. Liebetanz D, Klinker F, Hering D et al. Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. Epilepsia 2006;47:1216-24.

69. Kuba R, Guzaninova M, Brazdil M, Novak Z, Chrastina J, Rektor I. Effect of vagal nerve stimulation on interictal epileptiform discharges: a scalp EEG study. Epilepsia 2002;43[10]:1181-1188

70. Santiago-Rodriguez E, Cardenas-Morales L, Alonso-Vanegas M et al. Vagus Nerve Stimulation decrease the EEG paroxysmal activity in patients with epilepsy. Journal of the Neurological Sciences 2005;238:S143

71. De Herdt V, Bogaert S, Bracke KR et al. Effects of vagus nerve stimulation on pro- and anti-inflammatory cytokine induction in patients with refractory epilepsy. J Neuroimmunol 2009;214:104-8.

CHAPTER 4

Chapter 4

Regarding optimization of VNS technology, this study describes in a retrospective way implementation of the latest Cyberonics VNS device, i.e. the Demipulse generator. Results showed that similar positive effects on seizure reduction were obtained compared to older generators. More importantly, main technical advancements were decreased size and improved software for the follow-up of impedance measurements and battery life.

Evolution in VNS therapy for refractory epilepsy,

experience with Demipulse devices at Ghent University Hospital

Riëm El Tahry, Veerle De Herdt, Robrecht Raedt, Annelies Van Dycke, Alfred Meurs, Frank Dewaele, Paul Boon, Dirk Van Roost, Kristl Vonck.

Seizure. 2010 Nov;19(9):531-5

Abstract

Rationale: Vagus nerve stimulation (VNS) is a frequently used treatment for patients with refractory epilepsy who are unsuitable candidates for epilepsy surgery. There has been a steady evolution in VNS technology, as generators´ volumes have become smaller and battery life expectancy longer. This pilot study is an open label retrospective study that describes our experience with the latest commercially available generator ie. the VNS Therapy™ Demipulse Model 103. Treatment efficacy and side-effects, as well as technical and practical enhancements useful for the patient and for the medical staff are discussed in this study.

Methods: Twenty patients (11F/9M) with a mean age of 40 years (range 8-61), who were considered unsuitable candidates for resective surgery, were implanted with a VNS Therapy™ Demipulse Model 103. Mean monthly seizure frequency reduction and side effects were evaluated 1 year after implantation.

Results: Mean monthly seizure frequency decreased significantly from 54 seizures/month (SEM 30; range 1-555) before treatment to 33 (SEM 24, range 0-445) following 12 months of treatment. ($p<0.05$). Seven patients (39%) were considered responders with a reduction in seizure frequency of more than 50%. One of those seven patients became seizure free. Side effects were stimulation-related tingling sensation in the throat and/or hoarseness, a painful sensation in the left neck or ear region and a lead breakage In addition; one case of “sudden unexpected death in epilepsy” (SUDEP) was reported.

Conclusion: Patients treated with VNS Therapy™ Demipulse generators proved to have a significant decrease in seizure frequency. In this patient group, VNS was well tolerated. The main technical advances are the decrease in size and improved options for battery life follow-up.

Keywords: vagus nerve stimulation, generator, Demipulse, refractory epilepsy

4.1. Introduction

Vagus nerve stimulation (VNS) is indicated in patients with medically or surgically refractory epilepsy. More than 50.000 patients worldwide have been implanted with VNS therapy™ devices since 1989. Initially, efficacy of VNS for refractory epilepsy was studied in the randomised double-blind placebo-controlled E03 and E05 studies, which included 114 and 198 patients with a follow-up of three months (1, 2). In these studies, seizure frequency reduction was compared between a high, so-called effective stimulation group and a low, so-called placebo stimulation group. The E03 study found a decrease in seizures of 24 % in the high stimulation group versus 6 % in the low stimulation group, while the E05 study found a 28 % decrease in seizure frequency in the high stimulation group versus 15 % in the low stimulation group (1, 2). Prospective and retrospective long-term open-label studies confirmed VNS efficacy and safety in adults and children with refractory epilepsy (3-11). In parallel with the increasing amount of clinical data, there has been a steady evolution in VNS technology, as the size of the generator has become smaller (Fig.1) and battery life expectancy has increased at each new generator release.

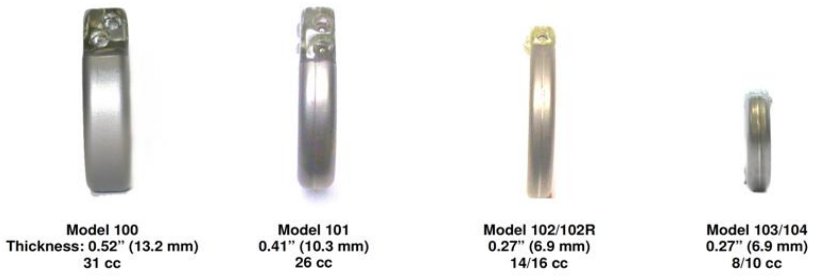


Fig. 1- Volume reduction of VNS generators over time

This pilot study is an open-label retrospective study that describes our experience with the latest commercially available generator ie. the VNS Therapy™ Demipulse Model 103. Treatment efficacy and side-effects, as well as technical and practical enhancements useful for neurologists, neurosurgeons and for the patient, are discussed in this study. This study is the first to date to report clinical experience with Demipulse generators.

4.2. Patients and Methods

4.2.1 Patient population and pre-surgical evaluation

Between 1/6/2007 and 1/12/2009, 40 patients were implanted with a VNS device (Cyberonics®, Houston, USA) at the Ghent University Hospital. All patients were diagnosed with refractory epilepsy and underwent long-term video-EEG monitoring for seizure detection, 3 Tesla brain MRI and PET scan as part of the pre-surgical evaluation. All patients were considered unsuitable candidates for resective surgery, either because the epileptic focus remained unidentified, or because it was located in functional cortex. Subsequently they were offered treatment with VNS. For the purpose of this study, medical records of patients implanted with a VNS therapy™ Demipulse Model 103 were evaluated. Patients with a post-implantation follow-up of at least 1 year were included in this study. Additionally, a documentation of seizure frequency before implantation and at maximal follow-up was required.

4.2.2 Implantation procedure

The system was implanted under general anaesthesia via two short incisions. The first incision was placed in a skin fold at the left base of the neck, about 3 cm above the clavicle and across the medial border of the sternocleidomastoid muscle. The left vagus nerve was searched for between the common carotid artery and the internal jugular vein, and exposed over a distance of 3 cm. Using optical magnification, the helical tether and the two helical electrodes were wrapped around the nerve. The lead was carried to the surface in smooth loops and anchored twice, both to the deep and to the superficial cervical fascia. Through a second, also transverse, incision below the left clavicle on the mamillary line, a small epifascial pouch was shaped in which the pulse generator (Cyberonics Demipulse, model 103 or 104) was placed. The lead was subcutaneously tunneled from the first to the second incision and plugged into the pulse generator. The system was telemetrically verified before the pulse generator was anchored to the pectoral fascia and the wounds were closed.

4.2.3 Ramping-up procedure and stimulation parameters

Stimulation was initiated 2 to 4 weeks after surgery at the epilepsy clinic. Stimulation intensity was gradually increased over the next months with steps of 0.25 mA until seizure control was reached or side effects appeared. The other stimulation parameters were programmed as follows: pulse width 250-500 µsec; frequency 20-30 Hz; on/off cycle 30 sec on/10 min off or 30 sec on /5min off. As part of normal clinical practice in patients treated with VNS at the Ghent University Hospital, anti-epileptic drug (AED) treatment was preferably left unchanged during the first 12 months of follow-up.

4.2.4 Outcome measures

The clinical data collected for this study included: gender and age at time of implantation; type of epilepsy; mean monthly seizure frequency before implantation and at maximal follow-up; number of anti-epileptic drugs taken before implantation and at maximal follow-up; stimulation intensity at maximum follow-up. In addition, properties of the surgical implantation procedure as well as user friendly characteristics for neurologist and patients were assessed.

Monthly seizure frequency pre-VNS was based on seizure frequency reported one month before date of surgery. Mean monthly seizure frequency post-VNS resulted from an average of two to three consecutive months at maximum follow-up.

Type of epilepsy was based on clinical semiology of the seizures and ictal and interictal electroencephalographic recordings.

Primary outcome measures included reduction in mean monthly seizure frequency and the percentage of patients with a seizure frequency reduction of 50% or more (responder rate). Secondary outcome measures were the changes in number of concomitant AEDs taken at maximum follow-up compared to before stimulation and stimulation output at maximum follow-up.

Outcome measures were first calculated for the entire study group. Subsequently, the study population was divided into two groups: responders (seizure frequency reduction of 50% or more) and non-responders (seizure frequency reduction between of less than 50%). Outcome parameters were assessed for the two groups separately.

4.2.5 Ethical approval

This retrospective study was approved by the Ethical Committee of Ghent University Hospital (EC 2005/238 and EC 2009/604). Informed consent was obtained from all patients.

4.2.6 Statistical analysis

Group mean differences in percentage of reductions in seizure frequency and differences in mean monthly seizure frequencies were tested non-parametrically. Statistical significance was set on $p < 0.05$. All calculations were performed using SPSS 15.0.

4.3. Results

4.3.1 Patient population

Twenty patients (11 females, 9 males) with a mean age of 40 years (range 8-61) were included in the study. Sixteen patients had localised epilepsy with complex partial seizures with or without secondary generalisation. Four patients had generalised epilepsy with tonic clonic seizures, absences or myoclonic seizures. Two patients had a follow-up of 6 and 8 months due to early discontinuation of VNS therapy (1 sudden unexplained death in epilepsy (SUDEP) and 1 lead breakage respectively). They were excluded from further statistical analysis.

4.3.2 Seizure frequency reduction and responder rate (Table 1)

Mean monthly seizure frequency before implantation was 54 seizures/month (SEM 30, range 1-555), mean monthly seizure frequency at maximum follow-up was 33 seizures/month (SEM 24, range 0-445) *(*Wilcoxon Signed Ranks test, *<0.05)* Seven patients (39%) were considered responders with a reduction in seizure frequency of more than 50%. One of those seven patients became seizure free. Eleven patients (61%) were non responders (reduction in seizure frequency of less than 50%). In the non responder group, three patients (16%)

responded with a seizure frequency reduction between 30 and 50%, two patients (11%) responded with a seizure frequency reduction of less than 30%, five patients (28%) experienced no change in seizure frequency and one patient reported a small increase in seizure frequency.

Table 1. Overview of primary and secondary outcome parameters in patient population

		Seizure reduction ≥ *50%* *N=7*	Seizure reduction ≤ *50%* *N=11*
Mean age at implantation (years)		34 (range 21-56)	32 (8-49)
Mean follow-up (months)		12	12
Mean Seizure frequency/month pre VNS (n)		42 (range 7-150)	62 (1-555)
Mean Seizure frequency/month post VNS (n)		4 (range 0-12,5)	51 (1-445)
Mean seizure reduction (%)		84	13
Number of AED before (n)		3 (2-5)	3 (3-4)
Number of AED after(n)		3 (2-5)	3 (2-5)
Mean Stimulation output (mA)		1,64 (0,75-2,25)	1,88 (1,5-2,5)
Epilepsy type	Focal	5	10
	Generalised	2	1

Table1: Overview of primary and secondary outcome parameters in patient population.

Responders started to respond to their VNS treatment at month 7 after date of implantation, while no effect was seen over time in the non responder group (see Fig 2) (Wilcoxon Signed Ranks test, $p<0.05$).

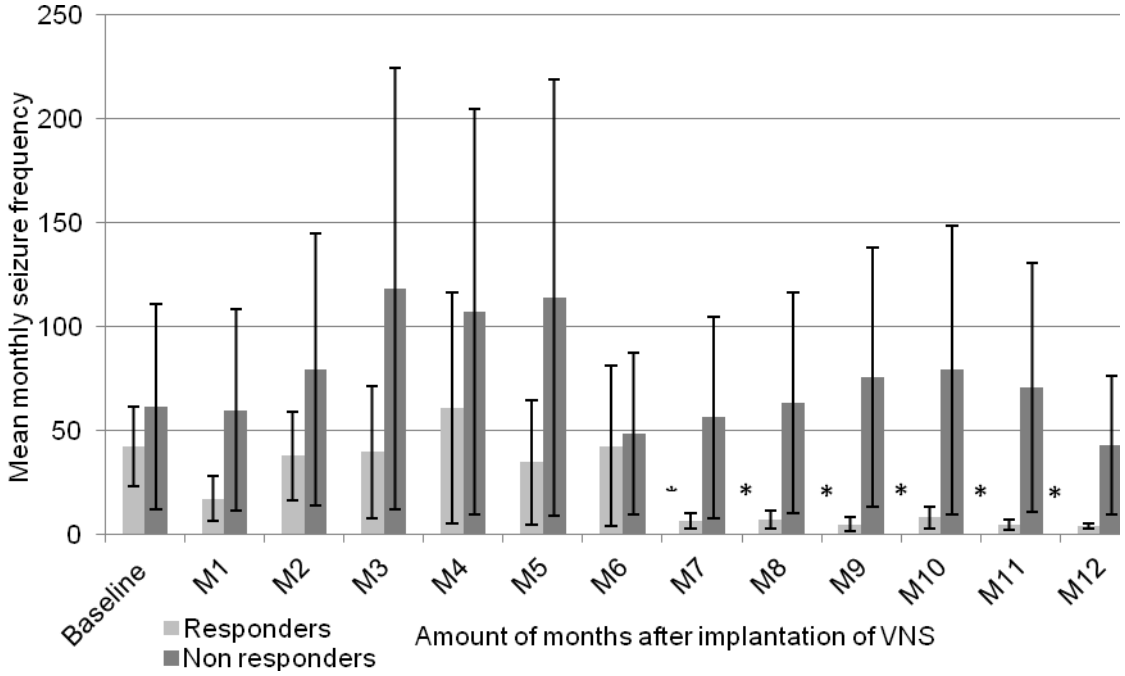


Fig. 2 Evolution of mean monthly seizure frequency in responders and partial responders compared to non responders over time. Responders started to respond to their VNS treatment at month 7 after date of implantation

4.3.3 Comparison between responders, partial responders and non-responders

There were no significant differences regarding seizure frequency before VNS, mean age at time of implantation and number of AEDs before implantation, between the responder and non-responder group (Table 1).

The mean stimulation output current at maximal follow-up was 1.79 mA (range 0.75 -2.5 mA) (Fig.3). Responders had a lower mean stimulation output at maximal follow-up (1.64 mA, range 0.75 - 2.25mA) in comparison to non-responders (1.88 mA, range 1.5 - 2.5 mA), although these differences were not statistically significant. Mean number of AEDs before and after implantation in the responder and non-responder was 3 (range of 2-5 for the responders and 2-4 for the non-responders). These results, although based on small amount of patients, are in accordance with data in the literature in which these variables did not appear to be independent predictors of outcome (7, 14-19).

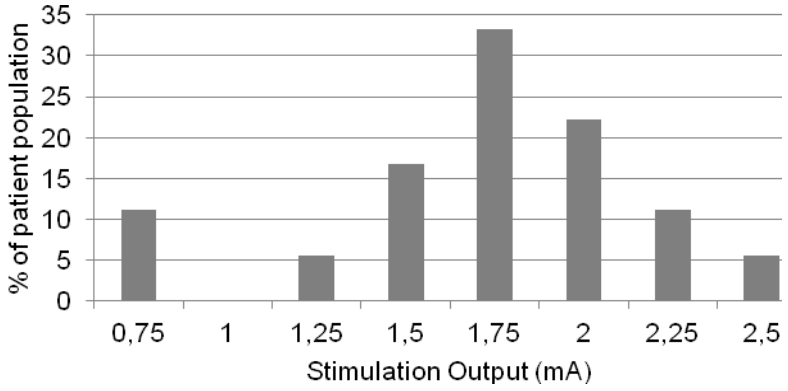


Fig. 3 Percentage of patients programmed at different stimulation outputs at maximal follow-up.

4.3.4 Reported side effects

All patients reported a stimulation-related tingling sensation in the throat and/or hoarseness, especially during the ramping-up period. Two patients reported a painful sensation in the left neck or ear during stimulation at 2 and 2.25 mA, respectively. Other stimulation parameters for both patients were: 20 Hz, 500 µsec, 30 sec on/5 min off. One of those patients also complained of a light dyspnoea when lying on her left side. The dyspnoea was not continuously present over time, even though stimulation parameters were not adjusted.

One mentally retarded patient, which was considered as a partial responder at 6 months of follow-up, had a habit to frequently rotate his generator subcutaneously, which resulted in a corkscrew shaped lead and finally to a lead breakage. Consequently, at 8 months of follow up, interrogation of his VNS device showed a very low lead impedance (< 200 ohm), which indicated a short-circuit of his VNS system. Subsequently, the output current was programmed at 0 mA. As seizure frequency did not increase in the following months, it was decided not to replace the VNS device.

4.4. SUDEP

One patient died suddenly at the age of 62 after 6 months of follow-up. She was found at home in asystole with bilateral light-rigid pupils. The patient was transported to an intensive care unit after receiving emergency care at home, but she died shortly after. No specific cause of death was identified on autopsy. This case was considered as a ‘sudden unexpected death in epilepsy (SUDEP).

4.5 Discussion

In this retrospective study we evaluated the efficacy, safety and practical enhancements of the latest commercially available VNS device, the VNS Therapy Demipulse 103 Model (Cyberonics®, Houston, USA), in the treatment of patients with refractory epilepsy.

Demipulse generators seem to have similar efficacy as previous generators in the treatment of refractory epilepsy. We evaluated VNS therapy with Demipulse generators after 12 months of stimulation. Our study yielded a responder rate of 39%. This result is comparable to the results obtained with previous models of VNS generators, such as the study by Morris et al, who described responder rates of 23% at 3 months and 37% at 1 year (12). Other retrospective and prospective studies reported similar or slightly higher responder rates after at least one year of stimulation, such as 26% (10), 48% (7), 50% (11) or 54%. (3). In our study, mean stimulation output in the responder group was 1.79 mA range (0.75 -2.5), which is similar to results reported by DeGiorgio (13).

Besides equivalent efficacy, Demipulse generators appeared to have similar tolerability as previous generators, as no new side effects were described in our study. (1-3, 12, 13). We report 1 case of SUDEP and one case in which VNS therapy was stopped due to a lead breakage. The relationship between VNS and SUDEP has been subject of research, although no correlations between VNS treatment and SUDEP were found (8, 20-22). Lead breakages were also reported in previous studies, especially in children en mentally retarded patients (23, 24).

Due to reduced generator volume, Demipulse generators are more easily implanted, which reduces considerably possible surgery related complications. Earlier versions of the VNS pulse generator required larger incisions and larger pouches for implantation; they also brought about more distinct skin eminences in the pectoral region and some tension on the wound edges. In order to avoid unaesthetic scars the insertion of the pulse generator was often performed via an incision on the anterior axillary line (and in some cases underneath the pectoralis muscle), which implicated a longer and broader range of dissection and, hence, more frequent problems of pouch hematoma and pain after surgery. The pulse generators n° 103 and 104, in virtue of their small size and weight, do not require such coping strategies. The short skin incision can be placed directly over the site of insertion in the pectoral region, and can be aesthetically closed with a running intradermal suture or with glue. Problems of pouch hematoma or pain have become virtually eliminated. Moreover, in our study no single infection of generator or lead implantation site was reported, although this is the most common observed surgical complication in published trials with older devices with an incidence varying around 3% (23, 25). Even though cosmetic advantages are not a priority in health care in general, it is worthwhile noting that epilepsy patients often deal with a lot of prejudice, which indirectly affects their social and economic integration in our society.

Discrete scars enhance their well being and ameliorate their daily social life. For this reason cosmetic advantage of Demipulse must be emphasized.

Besides the surgical and esthetical advantages of smaller generator volume, Demipulse generators also enhance treatment and clinical follow-up of patients. First of all, the generator life projection system displays the end of life (EOL) in exact amount of years and months in function of programmed parameters and warns the clinician 6 months ahead to foresee generator replacement. This is of particular value, as postponing generator replacement may result into permanent loss of seizure control (26). Battery life depends on many factors including the generator model, stimulation parameters, lead impedance and magnet use. The first model developed for human use, Model 100 (2002), had an expected battery life of 4-8 years. For the second generation of generators i.e. Model 101 (2003) battery life increased to 8-12 years. Model 102 and models 103/104 (2007, Demipulse) have similar life expectancies as model 101. Ideally, future technology development will allow transcutaneous battery recharge and render generator replacements unnecessary. This will further ameliorate quality of life of patients and reduce health care costs.

Another important feature useful for clinicians is the fact that Demipulse generators are capable of measuring lead impedances in Ohms, while older models only delivered DC-DC converter codes. This gives the clinician more accurate information about the good functioning of the VNS device. Lead impedance should vary between 200 Ohm (low impedance) and 7kOhm (high impedance). If high impedance is discovered upon interrogation of the device, a discontinuity of the lead or fibrosis between the nerve and the lead may be the reason. To check whether a lead breakage is present, a radiography or CT scan of the neck can be performed. In other cases a surgical revision of the device may be needed. Demipulse generators are able to measure lead impedance every 24 hours. If the impedance has reached “high” or “low” values between interrogations at office visits, a warning message will be displayed when interrogating the device. For the moment, the device does not inform the clinician about the exact date/hour on which lead impedance changed. This element could be useful to correlate with certain potentially harmful events, such as an important head or neck trauma which could explain breakage of the lead. In our study, one mentally retarded patient, showed a very low impedance (<200 ohm), which indicated a short circuit of his VNS device.

Despite the fact that VNS therapy as a treatment for epilepsy has proven to reduce significantly health care utilisation and its related costs (27, 28), Demipulse generators are not reimbursed in all countries. In addition to equal efficacy and tolerability in comparison with older devices,

Demipulse generators have better surgical characteristics and improve clinical follow-up of patients treated with VNS. Future studies with larger amount of patients are needed to confirm improved

capacities of Demipulse VNS devices, which hopefully will lead to reimbursements of the new model 103 in all concerned countries.

Reference List

1. Ben Menachem E, Manon-Espaillat R, Ristanovic R et al. Vagus nerve stimulation for treatment of partial seizures: 1. A controlled study of effect on seizures. First International Vagus Nerve Stimulation Study Group. Epilepsia,1994;35(3):616-626.

2. Handforth A, DeGiorgio CM, Schachter SC et al. Vagus nerve stimulation therapy for partial-onset seizures: a randomized active-control trial. Neurology,1998;51(1):48-55.

3. Chavel SM, Westerveld M, Spencer S. Long-term outcome of vagus nerve stimulation for refractory partial epilepsy. Epilepsy Behav.,2003; 4(3):302-309.

4. DeGiorgio C, Heck C, Bunch S et al. Vagus nerve stimulation for epilepsy: randomized comparison of three stimulation paradigms. Neurology,2005; 65(2):317-319.

5. Helmers SL, Wheless JW, Frost M et al. Vagus nerve stimulation therapy in pediatric patients with refractory epilepsy: retrospective study. J Child Neurol, 2001;16:843-8.

6. Holmes MD, Silbergeld DL, Drouhard D, Wilensky AJ, Ojemann LM. Effect of vagus nerve stimulation on adults with pharmacoresistant generalized epilepsy syndromes. Seizure, 2004;13(5):340-345.

7. Labar D. Vagus nerve stimulation for 1 year in 269 patients on unchanged anti-epileptic drugs. Seizure, 2004;13(6):392-398.

8. Morris GL, III, Mueller WM. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group E01-E05. Neurology,1999;53: 1731-5.

9. Scherrmann J, Hoppe C, Kral T, Schramm J, Elger CE. Vagus nerve stimulation: clinical experience in a large patient series. J Clin Neurophysiol, 2001;18:408-14.

10. Uthman BM, Reichl AM, Dean JC et al. Effectiveness of vagus nerve stimulation in epilepsy patients: a 12-year observation. Neurology,2004; 63(6):1124-1126.

11. Vonck K, Thadani V, Gilbert K et al. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience. Journal of Clinical Neurophysiology,2004; 21(4):283-289.

12. Morris GL, III, Mueller WM. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group E01-E05. Neurology,1999;53(8):1731-1735

13. DeGiorgio CM, Schachter SC, Handforth A et al. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. Epilepsia,2000; 41(9):1195-1200

14. Helmers SL, Wheless JW, Frost M et al. Vagus nerve stimulation therapy in pediatric patients with refractory epilepsy: retrospective study. J Child Neurol,2001;16(11):843-848.

15. Ben Renfroe J, Wheless JW. Earlier use of adjunctive vagus nerve stimulation therapy for refractory epilepsy. Neurology, 2002;59:S26-S30.

16. Janszky J, Hoppe M, Behne F, Tuxhorn I, Pannek HW, Ebner A. Vagus nerve stimulation: predictors of seizure freedom. Journal of Neurology, Neurosurgery and Psychiatry,2005;76(3):384-389.

17. Sirven JI, Sperling M, Naritoku D et al. Vagus nerve stimulation therapy for epilepsy in older adults. Neurology,2000;54(5):1179-1182.

18. Ng M, Devinsky O. Vagus nerve stimulation for refractory idiopathic generalised epilepsy. Seizure,2004;13(3):176-178.

19. Valencia I, Holder DL, Helmers SL, Madsen JR, Riviello JJ, Jr. Vagus nerve stimulation in pediatric epilepsy: a review. Pediatr.Neurol,2001; 25(5):368-376.

20. Annegers JF, Coan SP, Hauser WA, Leestma J, Duffell W, Tarver B. Epilepsy, vagal nerve stimulation by the NCP system, mortality, and sudden, unexpected, unexplained death. Epilepsia,1998;39(2):206-212.

21. Schachter SC. Therapeutic effects of vagus nerve stimulation in epilepsy and implications for sudden unexpected death in epilepsy. Clin Auton.Res,2006;16(1):29-32.

22. Annegers JF, Coan SP, Hauser WA, Leestma J. Epilepsy, vagal nerve stimulation by the NCP system, all-cause mortality, and sudden, unexpected, unexplained death. Epilepsia,2000;41(5):549-553.

23. Rychlicki F, Zamponi N, Cesaroni E et al. Complications of vagal nerve stimulation for epilepsy in children. Neurosurg.Rev 2006;29(2):103-107.

24. Rychlicki F, Zamponi N, Trignani R, Ricciuti RA, Iacoangeli M, Scerrati M. Vagus nerve stimulation: Clinical experience in drug-resistant pediatric epileptic patients. Seizure,2006;15(7):483-490.

25. Smyth MD, Tubbs RS, Bebin EM, Grabb PA, Blount JP. Complications of chronic vagus nerve stimulation for epilepsy in children. J Neurosurg 2003;99:500-3.

26. Vonck K, Dedeurwaerdere S, De Groote L et al. Generator replacement in epilepsy patients treated with vagus nerve stimulation. Seizure,2005; 14(2):89-99.

27. Bernstein AL, Barkan H, Hess T. Vagus nerve stimulation therapy for pharmacoresistant epilepsy: Effect on health care utilization. Epilepsy & Behavior, 2007;10:134-7.

28. Boon P, Vonck K, Vandekerckhove T et al. Vagus nerve stimulation for medically refractory epilepsy; efficacy and cost-benefit analysis. Acta Neurochir.(Wien),1999;141(5): 447-452.

CHAPTER 5

Chapter 5

Improvement of VNS technology is a growing field of interest. This chapter describes a pilot trial with a new VNS device, the ADNS-300. Main keyfeatures include a transcutaneous rechargeable generator and a combined stimulation and recording electrode. We report for the first time human vagus nerve CAP's in vivo with an implantable device. This study demonstrates the use of the ADNS-300 system for combined therapeutic stimulation and recording of CAPs in response to VNS.

A novel implantable vagus nerve stimulation system (ADNS-300)

for combined stimulation and recording of the vagus nerve:

pilot trial at Ghent University Hospital

Riëm El Tahry, Robrecht Raedt, Lies Mollet, Veerle De Herdt, Tine Wyckuys, Annelies Van Dycke, Alfred Meurs, Frank Dewaele, Dirk Van Roost, Pascal Doguet, Jean Delbeke, Wytse Wadman, Kristl Vonck, Paul Boon

Epilepsy Res. 2010 Dec; (2-3):231-9

Abstract

Purpose: Vagus nerve stimulation (VNS) is an established treatment for refractory epilepsy. The ADNS-300 is a new system for VNS that includes a rechargeable stimulus generator and an electrode for combined stimulation and recording. In this feasibility study, three patients were implanted with ADNS-300 for therapeutic VNS. In addition, Compound Action Potentials (CAPs) were recorded to evaluate activation of the vagus nerve in response to VNS.

Methods: Three patients were implanted with a cuff-electrode around the left vagus nerve that was connected to a rechargeable pulse generator under the left clavicula. Two weeks after surgery, therapeutic VNS (0.25 mA to 1.25 mA, 500µsec, 30 sec On, 10 min Off and 30 Hz) was initiated and stimulus-induced CAPs were recorded.

Results:

The ADNS-300 system was successfully implanted in all three patients and patients were appropriately stimulated during six months of follow-up.

A reduction in seizure frequency was demonstrated in two patients (43% and 40% in patient 1 and 3 respectively), while in patient 2 seizure frequency remained unchanged. CAPs could be recorded in patient 1 and 2, proving stimulation-induced activation of the vagus nerve.

Conclusion:

This feasibility study demonstrates that the ADNS-300 system can be used for combined therapeutic stimulation (in 3/3 patients) and recording of CAPs in response to VNS (in 2/3 patients) up to three weeks after surgery. Implantation in a larger number of patients will lead to a better understanding of the electrophysiology of the vagus nerve, which in turn could result in more adequate and individualized VNS parameter choice.

Keywords: vagus nerve stimulation, Compound action potential, epilepsy

5.1 Introduction:

Vagus nerve stimulation (VNS) is an established treatment for refractory epilepsy. More than 50,000 patients worldwide have been implanted with VNS therapy ™ (Cyberonics®) devices since 1989. Despite the fact that VNS is a recognised adjunctive treatment for epilepsy, the mechanism by which therapeutic VNS reduces seizure activity is not yet fully understood. Moreover, reduction in seizure frequency in patients treated with VNS varies considerably (1-9). Unfortunately, no predictive factors to identify responders have been found to date (5,6,10-18). Unravelling the mechanism of action of VNS could lead to improved treatment and follow-up of patients treated with VNS. In this context, a new VNS device, the Advanced Nerve Stimulator version 300 (ADNS-300, Neurotech SA, Louvain-La-Neuve, Belgium), allows recording of compound action potentials (CAPs) of the vagus nerve in addition to stimulation of the vagus nerve.

Recordings may result into better understanding of the electrophysiological properties of the vagus nerve. Optimal VNS parameters were derived from animal experimental studies in which anti-seizure effects of VNS were proven (19-25). Later on, these pre-clinical VNS parameters were applied in early clinical randomized controlled trials (1-4,8). However, in none of these studies, stimulation parameters were derived from individual electrophysiological properties of the human vagus nerve. The incorporated recording option of the ADNS-300 may remedy this shortcoming and may offer the opportunity to select stimulation parameters on an individual basis. Apart from this new feature, the ADNS-300 has a transcutaneous battery recharging system, that may reduce the need for generator replacements. The ADNS-300 is further designed to be implanted in approximately the same way as the Cyberonics device

This feasibility study aimed at evaluating the use of the ADNS-300 system for therapeutic VNS and reporting, for the first time, in vivo recording of CAPs of the vagus nerve with an implantable device.

5.2 Patients, materials and methods

5.2.1 Patients

Three patients with refractory epilepsy underwent a full pre-surgical evaluation that included a video-EEG monitoring, 3 Tesla MRI and FDG-PET of the brain. A multidisciplinary epilepsy surgery team considered these patients unsuitable candidates for resective surgery, either because no clear epileptic focus was found or because the epileptogenic focus was located in eloquent brain tissue.

Patients were then considered candidates for VNS treatment. Seizure frequency was evaluated prospectively three months before implantation and monthly after implantation up to a 6 months follow-up period.

5.2.2 ADNS 3.0 device (Fig 1)

5.2.2.1 Generator, electrode and lead

The ADNS-300 generator has a volume of 7 cc is surgically implanted in a subcutaneous pouch under the left clavicle and is connected with a subcutaneous lead to the stimulation/recording electrode around the left vagus nerve. The generator is a bipolar current source with a compliance of +/- 8V_{DC}.

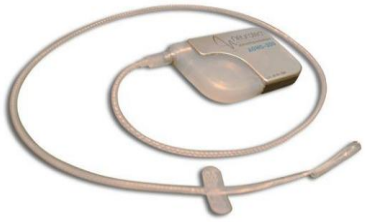


Fig. 1 T he ADNS-300 vagus nerve stimulator.

The electrode is a self-sizing spiral cuff consisting of two separate rings of 3 platinum contacts with a total surface of 0.6 mm² which are used as stimulation cathode and anode. A longitudinally arranged tripolar set of 0.2 mm² contacts provides the compound nerve action potentials recording derivation. The stimulation anode and cathode are spaced 4 mm from each other, while the first recording contact is situated 5 mm rostral from the stimulation cathode (Fig 2). The three recording contacts are separated by 5 mm intervals. The middle one is considered as the active recording contact referred to the outer two which are connected to each other. The total length of the cuff electrode is 22 mm. The stimulation anode is oriented caudally, while the cathode and recording contacts are directed rostrally, allowing recording of orthodromic signals. The inner cuff diameter is 1,9 mm and is designed to fit the nerve snugly.

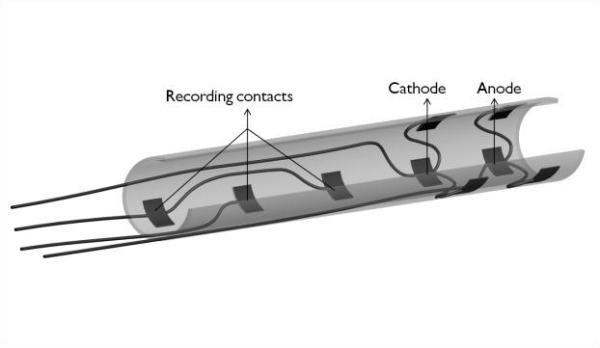


Fig. 2: Schematic representation of the ADNS-300 electrode

The lead is 43 cm long and is made of four helically wounded Teflon insulated multi-strand wires embedded in silicone rubber. From a surgical point of view the implantation of the ADNS-300 pulse generator does not differ from the implantation of the Cyberonics device. The electrode of the ADNS-300 system, however, resembles a miniature parchment roll whereas the Cyberonics electrode

consists of three separate springlike spirals. The latter are at implantation consecutively stretched via bits of strings at their ends in order to allow the vagus nerve to enter the spirals´ cores, where after the spirals regain their original shapes and thus contain the nerve. Some additional manipulation may, however, be necessary to wrap the spirals properly around the nerve. The ADNS-300 electrode is being unwrapped to a sheet that is placed underneath the vagus nerve. At releasing the sheet margins, the electrode folds back to its original roll-like shape to contain the nerve. In either system the lead is secured to the deep and the superficial tissue layers to protect the electrode and the nerve against traction.

5.2.2.2 Patient's and physician's external controller

The patient's external controller is a handheld battery-operated device that enables the patient to visualize the status of the implanted generator's battery, to carry out a pause or an additional acute stimulation at the onset of a seizure.

In both cases parameters of temporary interruption or additional stimulation are programmed beforehand by the neurologist. The external pebble does not allow the patient to adapt the stimulation parameters own handed.

The physician's external controller allows interrogating the implanted device, checking battery voltage as well as lead impedance, programming of new parameter settings and recording CAPs. These functions use a Medical Implant Communication Service (MICS) communication between the implant and the main computer system.

5.2.3 Ramping-up procedure and stimulation parameters

The stimulation paradigm was comparable to the one used in previous studies done by our group (26-30). Stimulation was initiated two weeks after surgery at the epilepsy clinic using the following parameters: pulse width 500µsec; frequency 30 Hz; duty cycle 30 sec on/10 min off. The stimulation intensity was gradually increased with steps of 0.20 mA or 0.25 mA at intervals of at least two weeks until either seizure control was reached or side effects appeared. Anti-epileptic drug treatment was kept unchanged during the entire follow-up. This regimen was applied in all three patients. Other possible parameter settings, such as pulse frequency, pulse duration and signal time on or off were identical to previous reported studies using the Cyberonics VNS device (26-30). Only the parameters described above were adjusted here.

5.2.4 Clinical outcome measures

The clinical data collected for this study included the following: gender, age, type of seizures and epilepsy, mean monthly seizure frequency before implantation and at the end of the follow-up period. The stimulation intensity reached at the end of the follow-up was also recorded. Pre-VNS monthly seizure frequencies were prospectively assessed during three consecutive months before the date of surgery. Mean post-VNS monthly seizure frequency was based on the reported seizure frequency at the sixth month after implantation. At each time point, patients carefully noted the number of seizures in a commonly used seizure diary.

5.2.5 CAPs

The vagus nerve was stimulated at a frequency of 1 or 2 Hz with a biphasic charge-balanced pulse of 500 μA and 250 μsec pulse width. The initial anodic 'recovery' or charge balancing phase had a lower (1/5 ratio) amplitude and longer (5/1) duration than the second cathodic stimulation phase. Amplitude and duration values refer to the current intensity and duration of this second phase. This 'recovery-before-stimulating-phase' scheme avoids the charge recovery to interfere with the CAP recording. To further reduce stimulation related artifacts, the amplifier was shorted from the start of the stimulation until 200 μs thereafter.

Signals were recorded starting 200 μs after the end of the second phase of the stimulation pulse. To obtain clean traces, 4 to 32 identical sweeps were recorded and averaged. In addition, battery level and impedance of the stimulation contacts were measured on consecutive visits. The contact impedance of the stimulation electrodes is expressed in kOhms and defined as the voltage to current ratio measured at the end of the second phase of a stimulation pulse with 500 μA amplitude and 150 μs duration.

5.2.6 Data analysis

Data analysis of the CAPs was carried out offline using Mathcad 13. Because of the proximity between the stimulating and recording electrodes, a large stimulus artifact hides the neural responses. Hence, artifact reducing algorithms were applied using Mathcad 13. First, the raw traces were compensated for the 66 msec time constant of the single pole high-pass filter of the implanted amplifier. The result is characterized by a DC offset and a trend that is estimated by linear regression on the last 2.5 msec of 12.8 msec traces which subsequently was subtracted from the signals. Finally, if an exponential could be fitted simultaneously on the first maximum value and on the trace area, it was subtracted from the trace as well. This subtraction is justified on the basis of the known

exponential post-stimulus potentials resulting from local ionic shifts induced by the applied current pulses (31).

Latency was measured from the beginning of the stimulus artifact to various peaks of the compound action potential waveforms.

5.2.7 Ethical approval

The ADNS-300 system is complying with applicable European and Belgian regulatory requirements and in particular the European Council Directive 90/385/EEC relevant to Active Implantable Medical Devices.

This study was approved by the Ethics Committee of Ghent University hospital (EC 2008/274). Informed consent was given by all patients.

5.3 Results

5.3.1 Feasibility

All three patients were successfully implanted with the ADNS-300 system. No postoperative complications occurred. Impedance values over consecutive visits remained below 8 kOhm (Fig. 3). Transcutaneous recharging of the generator was performed once to twice a week for about two hours, which was sufficient to allow appropriate therapeutic stimulation. Patients did not feel any discomfort while charging the battery.

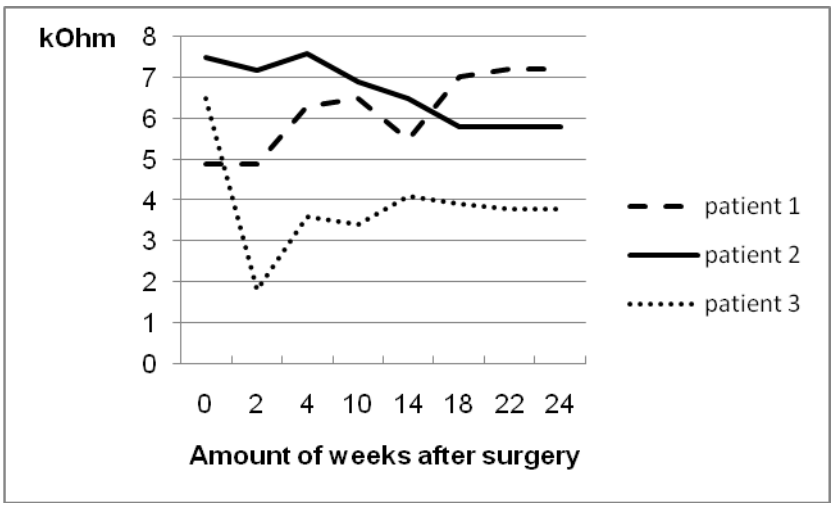


Fig.3: Stimulation electrode impedance (kOhms) history for all three patients.

Electrode contact impedance values are largely non-linear, i.e. dependent on the applied current or voltage. For this reason, it remains difficult to compare electrode impedances between two different VNS systems, as the method to estimate the impedance differ.

On the other hand, the total stimulation contact surface area is smaller for the Neurotech electrode compared to the Cyberonics device, also explaining some of the difference. More importantly, Fig 3 shows that impedance values remain quite stable over time.

5.3.2 Patient outcome (Table 1).

The first patient was a 59-year-old female with focal symptomatic epilepsy and seizure onset from the right parietal or frontal region. Seizures were characterized by an aura of a tingling sensation in the left hand, followed by an impairment of consciousness with important hyperventilation and motor automatisms of both shoulders and arms, most pronounced on the right side. The patient showed a seizure frequency reduction of 43% (28 seizures/month before VNS compared to 16 seizures/month after 6 months of VNS) At that time point, VNS had been ramped up to 1.25 mA.

The second patient was a 47-year-old male who developed post-lesional epilepsy 6 months after experiencing a stroke of the left medial cerebral artery. This resulted in a mixed aphasia and a residual minor hemiparesis of the right arm and leg.

The patient suffered from simple partial motor seizures with clonic movements of the right arm and leg, complex partial seizures with mainly staring and occasionally secondary generalization.

After six months follow-up, there was no reduction in the seizure frequency. VNS was ramped up until 0.75 mA and stopped thereafter due to the appearance of painful sensation in the left neck area.

The third patient was a 35-year-old female who experienced salmonella encephalitis at the age of 9 months. She developed a right hemispastic syndrome and postlesional epilepsy. Her seizures were characterized by an aura of a bad taste in the mouth, followed by a complex partial phase with behavioral arrest, orobuccal and manual automatisms which were occasionally accompanied by clonic movements of the right arm. This patient was rather cautious for her VNS treatment and had a very anxious character. As the ADNS-300 allowed up titrating in smaller steps of 0.2 mA, we have chosen to apply this option in her case. Seizure frequency decreased by 40% after 6 months of VNS (seizure frequency decreased from 15 to 9 seizures/ month). Output intensity at 6 months of follow-up was 1.2 mA.

In all cases, the 'temporary interruption' or 'additional stimulation at seizure onset' functions were not used by our patients, as they felt no need for it, either because hoarseness did not affect their daily life, or because seizures started too quickly, leaving them no time to switch on the external controller. There was no difference in tolerability whether the stimulus intensity was increased by 0.2 or 0.25 mA steps.

Table 1. Clinical features of patients and outcome after 6 months of follow-up

Patient (no.)	Age(year)/ gender	Type of seizures	Type of epilepsy	Focus EEG	MRI brain	Therapy	Mean seizure frequency/month before VNS	Mean seizure frequency/month after VNS	Stimulation output VNS (mA)
1	59/V	CPS	Focal symptomatic	Right frontal or parietal	Discrete loss of volume and structure R hippocampus	LEV,CBZ	28	16	1.25
2	47/M	SPS,CPS,SG	Focal symptomatic	Left frontotemporal	Encephalomalacia, hypoxia	PHT,CLZ	1	1	0,75
3	35/V	CPS	Focal symptomatic	Left frontotemporal	Atrophy L hemisphere, gliosis L thalamus	VPA,LTG	15	9	1.2

5.3.3 Reported side effects (Table 2).

All patients reported stimulation-related hoarseness, especially during the ramping-up period. In patient 3, this side effect completely disappeared after a few months of stimulation. In the other two patients, hoarseness persisted but did not lead to any inconvenience in daily life (Table 2). Patient 1 and 2 reported at 1 mA of output intensity and frequency of 30 Hz, a stimulus-related painful sensation in left side of the neck which radiated towards the left ear. Consequently, stimulation intensity had to be reduced to 0.75 mA in patient 2, while in patient 1 this side effect attenuated over time. Moreover, patient 2 experienced a beneficial effect of VNS and consequently intensity was further increased to 1.25 mA.

Table 2: Overview of reported side effects

Patient	Side effects	Intensity (mA)
1	Hoarseness	0.25
	Light discomfort in left neck	1
2	Hoarseness	0.25
	Pain left side of neck	1
3	Transient hoarseness	0.25

5.3.4 CAPs of the vagus nerve

CAPs were recorded at week 2 or 3 in patient 1 and 2 respectively. No reproducible CAP recording was possible in patient 3. X-ray could not show loss of continuity in this patient. In this patient, we checked whether stimulation pulses were delivered appropriately with an amplifier and oscilloscope setup. In fig. 4a and 4b two examples of vagal CAPs are presented from patient 1 and 2 respectively. In both cases the vagus nerve was stimulated with a pulse of 500 µA intensity, 250 µsec pulse duration. The first peak of the CAP appeared at 0.4 msec after the end of the stimulus and consisted of a sharp negative deflection (N_1), that was followed by a positive (P_1) (0.65 msec) and second negative deflection (N_2) (1.35-1.45 msec). Despite optimized filtering, N_1 was masked by a large initial artifact in patient 2. In contrast, the later waves P1 and N2 were more consistently recognised. Therefore, in patient 2, we chose to concentrate on the P_1-N_2 difference for amplitude measurements. In addition to P_1-N_2, more common and logical N_1-P_1 was calculated in patient 1. The maximal amplitude of N_1-P_1 in patient 1 was 58.26 µV, while P_1-N_2 was 19.44 µV. In patient 2, P1-N2 amplitude was 61.4 µV. The threshold for these responses was situated between 100 and 150 µA in both patients. When pulse duration was shortened to 100 µsec, the threshold stimulus intensity increased to a value between 200 and 250 µA in patient 1 and 250 and 500 µA in patient 2.

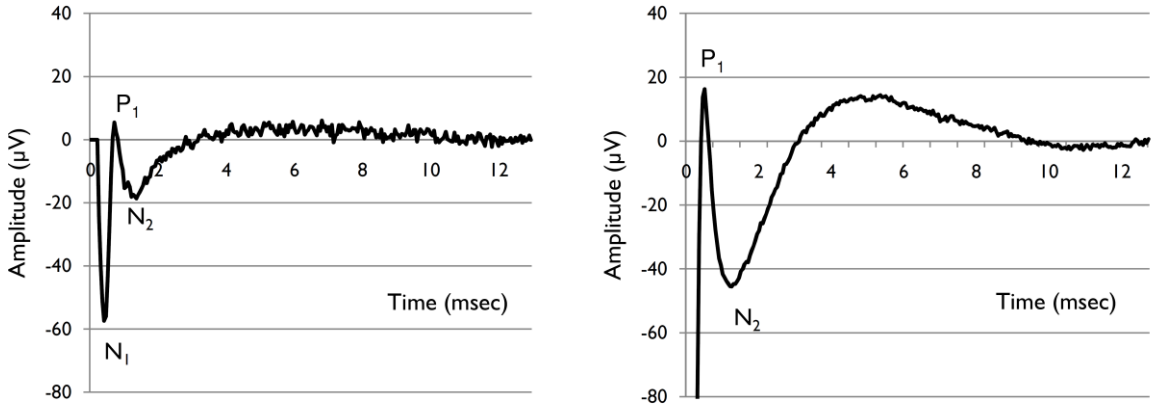


Fig 4 (a,b): CAPs obtained from the vagus nerve after applying a 500 µA stimulus of 250 µsec duration at week 2 to 3 after date of implantation. a: in patient 1 a $N_1P_1N_2$ CAP was measured. b: in patient 2 N_1 was masked by a large initial stimulation artefact and only P_1-N_2 was identifiable.

5.4 Discussion

Previous human studies on VNS mainly focused on clinical response in terms of seizure reduction (2-8,10-18,32-33) and a wide range of studies attempted to unravel the mechanism of action of VNS by

means of electrophysiological recordings, mainly EEG (34-40),evoked potentials (41-45) and functional radiological imaging (26,28,46-50). As the effect of VNS on the brain is ultimately dependent on the activation of the vagus nerve itself (20-21), a study of the electrophysiological nerve response is essential for a better understanding of VNS. To date, no biological markers of effective vagal stimulation are available. CAPs of the vagus nerve may serve as a new tool to indicate effective stimulation and choose more adequate stimulation parameters. The ADNS-300 offers such an opportunity to combine VNS with CAP recording.

Three patients with refractory epilepsy were successfully implanted with the new stimulator. Two of the three patients responded to the treatment and reported a 43% and 40% reduction of seizure frequency (patient 1 and 3).

Patient 2 experienced no improvement after 6 months of treatment but side effects limited further increase of the stimulation intensity baseline seizure frequency was already relatively low.

Moreover, evaluation of the VNS treatment was performed at month 6, while the literature recommends treating patients up to 12 or 18 months before drawing any conclusions about seizure outcome (4,7-9). In terms of side effects, patients reported stimulation-related hoarseness, which was transient in patient 3, but persisted in patient 1 and 2. In addition, patient 1 and 2 experienced pain in the left neck region at 1 mA and 0,75 mA respectively. Both side effects have already been described in earlier studies with Cyberonics® vagus nerve stimulator (1,2,4,7,32), although in the present cases these complaints as well as the therapeutic effect appeared at relatively low intensities compared to previous studies. This could be partially explained by the fact that the implanted electrode of the ADNS-300 has a cuff structure in which the shielding effect of the insulating silicone rubber sheet minimizes leakage of current to the surrounding tissues and provides more efficient stimulation of the vagus nerve.

Regarding user friendliness, the ADNS 300 generator can be recharged transcutaneously, resulting in a postponed surgical replacement of the generator and a prolonged battery lifespan of approximately 12 years. Interrogation of the device informs the physician about impedance values of the stimulation contacts and delivers a history chart of these measurements. In the event of a lead breakage, for example, the impedance history could be compared with the history reporting a trauma or other incidents that might explain the failure. In addition to the impedance history, the ADNS 300 also provides the physician with a battery recharge history. This information allows the physician to evaluate the patient's recharging habits.

A second part of this study was performed to evaluate whether CAPs of the vagus nerve could be measured with the ADNS-300.

In preclinical data, only one author has implemented a cuff electrode for combined stimulation and recording of the vagus nerve in a epilepsy model. Woodburry and Woodburry performed several experiments in the PTZ and MES model in 1990 and 1991 (20-21). They claimed to have recorded CAPs of the vagus nerve and derived useful stimulation parameters from these recordings.

In their study, the vagus nerve with was stimulated with a pulse of 200 µsec and 30 µA, which allowed to record a first component of the CAP which appeared at 1 msec. This volley of the CAP was considered to represent an activation of A type and B type fibres.

The distance between the stimulation and recording contacts was about 1 mm, so that the conduction velocity would have been about 1m/s, which is far too low to represent activation of myelinated fibres.

Moreover, the authors tried to record those signals chronically, but A and B fibres tended to lose their excitability after a few days. Therefore, to our knowledge, no animal study has prospectively analyzed the vagus nerve electrophysiology over time after surgery or correlated such recordings with seizure outcome. So far, only two studies have reported intra-operative CAPs recorded from the human vagus nerve (51,52), and no postoperative CAP data are available.

Evans described three different fibre populations (A, Aδ and C), on the basis of the latency of each waveform from the stimulus artifact onset (51). CAPs were recorded using a recording hook electrode placed 2 cm distal to the stimulation contacts. Mean conduction velocities of 18.8, 9.5 and 2.1 m/sec corresponding respectively to the different fibre types of the vagus nerve were described. The initial component, consistent with the activation of myelinated fibres, occurred after 1 msec, which might seem early compared to our findings at a fourfold shorter distance. The ADNS-300 uses one set of recording contacts and the stimulus to the nearest contact distance is very short, namely 5 mm. Hence a stimulus artifact must be dealt with, which could obscure early CAP components. In addition, it is also possible that in our 2 to 3 week postoperative recordings gliosis develops and the production of inflammatory cytokines slows down the nerve response at least temporarily (53). Amplitude range of recorded CAPs falls within values given by the group of Evans. The threshold to elicit a CAP in our study was low (between 100 and 150 µA for a stimulus of 250 µsec), which indicates that the obtained responses most likely correspond to the activation of large myelinated fibres.

Koo et al. also reported intra-operative myelinated fibre CAPs with completely different electrodes (52). These authors described a threshold intensity of 1 mA at a pulse width of 100 µsec to activate the CAP, while in our study intensities between half and a fourth of that level were sufficient to initiate a response.

Interestingly, important latency and threshold changes with age from childhood to adulthood were described. This indicates that an individual adjustment of the stimulus strength based on the observed nerve activation is absolutely necessary.

CAPs of the vagus nerve might help clinicians to select stimulation parameters more adequately. One of the important issues is whether higher stimulation currents can provide greater efficacy (32). Currently, there is no dose-response curve for VNS available. In clinical practice, due to the lack of electrophysiological information of the vagus nerve itself, VNS is gradually up titrated according to the reported seizure reduction or side effects.

This method requires months of adjustments and might lead to unnecessary strong stimuli. Moreover, long durations and high current intensities may carry a greater risk for nerve injury, use more power and activate nearby somatosensory nerves resulting in unnecessary pain or discomfort.

CAP measurements may one day replace this empiric and arbitrary upward titration with an objective calculation of the optimal parameters. In this context, stimulus response curves may help clinicians to determine the individual maximal intensity required to fully activate myelinated fibres of the vagus nerve, which in turn could be used as a patient specific VNS maximal output value.

Further clinical trials with the ADNS-300 are needed to fully characterize the vagus nerve CAP in humans. Implantation in a larger number of patients will lead to a better understanding of the electrophysiology of the vagus nerve, which hopefully will lead to more adequate and individual optimized choice of the VNS parameters. Hypothetically, non-responders could be those patients in whom the vagus nerve does not recover from surgery sufficiently and consequently less or no beneficial anti seizure effects may be achieved with VNS. In addition, the vagus nerve contains a number of functionally different fibres that contribute with different latencies and amplitudes to the CAP. It is unlikely that they all serve the anti-epileptic effect. By recording CAPs postoperatively over time and performing longitudinal prospective correlations between CAP recordings and seizure outcomes, more insight would be available in the subpopulations are to be targeted and with what stimulus parameters they are best activated.

5.5 Acknowledgements

Riëm El Tahry is supported by a grant from the Ghent University Research Fund (BOF). Prof. Dr. P. Boon is supported by grants from FWO; grants from BOF and by the Clinical Epilepsy Grant from Ghent University Hospital. Prof. Dr. K Vonck is supported by a grant of the BOF. This study was partially funded by Neurotech Belgium.

5.6 Disclosure of conflicts of interest

Pascal Doguet and Jean Delbeke are respectively employee and advisor at Neurotech. None of the other authors has any conflict of interest to disclose. All authors confirm they have read the Journal's position on issues involved in ethical publication and affirm this report is consistent with those guidelines.

Reference List

1. Ramsay RE, Uthman BM, Augustinsson LE, Upton AR, Naritoku D, Willis J, et al. Vagus nerve stimulation for treatment of partial seizures: 2. safety, side effects, and tolerability. first international vagus nerve stimulation study group. Epilepsia 1994;35(3):627-636.

2. BenMenachem E, Manonespaillat R, Ristanovic R, Wilder BJ, Stefan H, Mirza W, et al. Vagus Nerve-Stimulation for Treatment of Partial Seizures .1. A Controlled-Study of Effect on Seizures. Epilepsia 1994;35(3):616-26.

3. Handforth A, DeSalles AAF, Asatourian AA, Krahl SE, Us Vns SG. Seizure response to long-interval vagus nerve stimulation (VNS) is predictive of response to conventional VNS, suggesting a long-duration anti-epileptic mechanism. Neurology 1998;50(4):S05006.

4. Morris GL, III, Mueller WM. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group E01-E05. Neurology 1990;53(8):1731-1735.

5. Ben Renfroe J, Wheless JW. Earlier use of adjunctive vagus nerve stimulation therapy for refractory epilepsy. Neurology 2002;59(6):S26-S30.

6. Labar D. Vagus nerve stimulation for 1 year in 269 patients on unchanged anti-epileptic drugs. Seizure 2004;13(6):392-398.

7. Uthman BM, Reichl AM, Dean JC, Eisenschenk S, Gilmore R, Reid S, et al. Effectiveness of vagus nerve stimulation in epilepsy patients: a 12-year observation. Neurology 2004;63(6):1124-1126.

8. Holder LK, Wernicke JF, Tarver WB. Long-Term Follow-Up of 37 Patients with Refractory Partial Seizures Treated with Vagus Nerve-Stimulation. Journal of Epilepsy 1993;6(4): 206-14.

9. Hui AC, Lam JM, Wong KS, Kay R, Poon WS. Vagus nerve stimulation for refractory epilepsy: long term efficacy and side-effects. Chin Med J (Engl) 2004 Jan;117(1):58-6

10. Helmers S. Comparison of early and later initiation of vagus nerve stimulation for refractory epilepsy. Neurology 2001;56(8):A267-A268.

11. Janszky J, Hoppe M, Behne F, Tuxhorn I, Pannek HW, Ebner A. Vagus nerve stimulation: predictors of seizure freedom. Journal of Neurology, Neurosurgery and Psychiatry 2005;76(3): 384-389.

12. Sirven JI, Sperling M, Naritoku D, Schachter S, Labar D, Holmes M, et al. Vagus nerve stimulation therapy for epilepsy in older adults. Neurology 2000 14;54(5):1179-82.

13. Frost M, Gates J, Helmers SL, Wheless JW, Levisohn P, Tardo C, et al. Vagus nerve stimulation in children with refractory seizures associated with Lennox-Gastaut syndrome. Epilepsia 2001;42(9):1148-1152.

14. Karceski S. Vagus nerve stimulation and Lennox-Gastaut syndrome: a review of the literature and data from the VNS patient registry. CNS.Spectr. 2001;6(9):766-770.

15. Majoie HJM, Berfelo MW, Aldenkamp AP, Evers SMAA, Kessels AGH, Renier WO. Vagus nerve stimulation in children with therapy-resistant epilepsy diagnosed as Lennox-Gastaut syndrome - Clinical results, neuropsychological effects, and cost-effectiveness. J Clin Neurophysiol 2001;18(5):419-28.

16. Valencia I, Holder DL, Helmers SL, Madsen JR, Riviello JJ, Jr. Vagus nerve stimulation in pediatric epilepsy: a review. Pediatr.Neurol 2001;25(5):368-376.

17. Ng M, Devinsky O. Vagus nerve stimulation for refractory idiopathic generalised epilepsy. Seizure. 2004;13(3):176-178.

18. Holmes MD, Silbergeld DL, Drouhard D, Wilensky AJ, Ojemann LM. Effect of vagus nerve stimulation on adults with pharmacoresistant generalized epilepsy syndromes. Seizure. 2004;13(5):340-345.

19. McLachlan RS. Electrical-Stimulation of the Vagus Nerve for the Treatment of Epilepsy - Support from Experimental-Models. Annals of Neurology 1992;32(2):245.

20. Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. Epilepsia 1990;31 Suppl 2:S7-19.

21. Woodbury JW, Woodbury DM. Vagal stimulation reduces the severity of maximal electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording. Pacing Clin Electrophysiol 1991;14(1):94-107.

22. Zabara J. Inhibition of experimental seizures in canines by repetitive vagal stimulation. Epilepsia 1992;33(6):1005-1012.

23. Naritoku DK, Terry WJ, Helfert RH. Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. Epilepsy Res 1995;22(1):53-62.

24. Takaya M, Terry WJ, Naritoku DK. Vagus nerve stimulation induces a sustained anticonvulsant effect. Epilepsia 1996;37(11):1111-1116.

25. De Herdt, V, De WJ, Raedt R, Wyckhuys T, El TR, Vonck K, et al. Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures. Acta Neurologica scandinavica.2010 Apr; 121(4):271-276.

26. Vonck K, De Herdt, V, Bosman T, Dedeurwaerdere S, Van LK, Boon P. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. Seizure 2008 Dec;17(8):699-706.

27. De Herdt V, Boon P, Ceulemans B, Hauman H, Lagae L, Legros B, et al. Vagus nerve stimulation for refractory epilepsy: a Belgian multicenter study. Eur J Paediatr.Neurol 2007;11(5):261-269.

28. Vonck K, Boon P, Van Laere K, D'Have M, Vandekerckhove T, O'Connor S, et al. Acute single photon emission computed tomographic study of vagus nerve stimulation in refractory epilepsy. Epilepsia 2000;41(5):601-609.

29. Boon P, Vonck K, Van Walleghem P, D'Have M, Caemaert J, De Reuck J. Vagus nerve stimulation for epilepsy, clinical efficacy of programmed and magnet stimulation. Acta Neurochir.Suppl 2002;79:93-98.

30. Vonck K, Thadani V, Gilbert K, Dedeurwaerdere S, De Groote L, De Herdt V, et al. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience. Journal of Clinical Neurophysiology 2004;21(4):283-289.

31. Marin J, De Lannoy G, Delbeke J (2009) When can we recover charges with a biphasic charge balanced stimulation pulse? Proceedings of the IFESS conference: 55-57.

32. DeGiorgio CM, Thompson J, Lewis P, Arrambide S, Naritoku D, Handforth A, et al. Vagus nerve stimulation: analysis of device parameters in 154 patients during the long-term XE5 study. Epilepsia 2001;42(8):1017-1020.

33. DeGiorgio CM, Schachter SC, Handforth A, Salinsky M, Thompson J, Uthman B, et al. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. Epilepsia 2000;41(9):1195-1200.

34. Jaseja H (2009) EEG-desynchronization as the major mechanism of anti-epileptic action of vagal nerve stimulation in patients with intractable seizures: Clinical neurophysiological evidence. Med Hypotheses 74[5], 855-856.

35. Salinsky MC, Burchiel KJ. Vagus nerve stimulation has no effect on awake EEG rhythms in humans. Epilepsia 1993;34(2):299-304.

36. Wang H, Chen X, Lin Z, Shao Z, Sun B, Shen H, et al. Long-term effect of vagus nerve stimulation on interictal epileptiform discharges in refractory epilepsy. J Neurol Sci 2009;284(1-2):96-102.

37. Santiago-Rodriguez E, Alonso-Vanegas M, Cardenas-Morales L, Harmony T, Bernardino M, Fernandez-Bouzas A. Effects of two different cycles of vagus nerve stimulation on interictal epileptiform discharges. Seizure 2006;15(8):615-20.

38. Rizzo P, Beelke M, De CF, Canovaro P, Nobili L, Robert A, et al. Modifications of sleep EEG induced by chronic vagus nerve stimulation in patients affected by refractory epilepsy. Clin Neurophysiol 2004;115(3):658-64.

39. Kuba R, Guzaninova M, Brazdil M, Novak Z, Chrastina J, Rektor I. Effect of vagal nerve stimulation on interictal epileptiform discharges: a scalp EEG study. Epilepsia 2002;43(10):1181-8.

40. Koo B. EEG changes with vagus nerve stimulation. J Clin Neurophysiol 2001;18(5):434-41.

41. Upton AR, Tougas G, Talalla A, White A, Hudoba P, Fitzpatrick D, et al. Neurophysiological effects of left vagal stimulation in man. Pacing Clin Electrophysiol 1991;14(1):70-6.

42. Tougas G, Hudoba P, Fitzpatrick D, Hunt RH, Upton AR (1993) Cerebral-evoked potential responses following direct vagal and esophageal electrical stimulation in humans. Am J Physiol 264[3], 486-491.

43. Hammond EJ, Uthman BM, Reid SA, Wilder BJ. Electrophysiologic studies of cervical vagus nerve stimulation in humans: ii. evoked potentials. Epilepsia 1992;33(6):1021-1028.

44. Naritoku DK, Morales A, Pencek TL, Winkler D. Chronic Vagus Nerve-Stimulation Increases the Latency of the Thalamocortical Somatosensory Evoked-Potential. Pace-Pacing and Clinical Electrophysiology 1992;15(10):1572-8.

45. Brazdil M, Chadim P, Daniel P, Kuba R, Rektor I, Novak Z, et al. Effect of vagal nerve stimulation on auditory and visual event-related potentials. Eur J Neurol 2001;8(5): 457-461.

46. Chae JH, Nahas Z, Lomarev M, Denslow S, Lorberbaum JP, Bohning DE, et al. A review of functional neuroimaging studies of vagus nerve stimulation (VNS). J Psychiatr Res 2003;37(6): 443-455.

47. Henry TR, Votaw JR, Pennell PB, Epstein CM, Bakay RA, Faber TL, et al. Therapeutic response correlates with thalamic blood flow increases induced acutely by vagus nerve stimulation in partial epilepsy. Neurology 1998;50(4):S05008.

48. Henry TR, Votaw JR, Pennell PB, Epstein CM, Bakay RA, Faber TL, et al. Acute blood flow changes and efficacy of vagus nerve stimulation in partial epilepsy. Neurology 1999;52(6):1166-1173.

49. Henry TR, Bakay RA, Pennell PB, Epstein CM, Votaw JR. Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. prolonged effects at high and low levels of stimulation. Epilepsia 2004;45(9):1064-1070.

50. Van Laere K, Vonck K, Boon P, Brans B, Vandekerckhove T, Dierckx R. Vagus nerve stimulation in refractory epilepsy: SPECT activation study. J Nucl.Med 2000;41(7): 1145-1154.

51. Evans MS, Verma-Ahuja S, Naritoku DK, Espinosa JA. Intraoperative human vagus nerve compound action potentials. Acta Neurologica Scandinavica 2004;110(4):232-238.

52. Koo B, Ham SD, Sood S, Tarver B. Human vagus nerve electrophysiology: a guide to vagus nerve stimulation parameters. Journal of Clinical Neurophysiology 2001;18(5):429-433.

53. Thil MA, Tran Duy D, Colin IM, Delbeke J. Time course of tissue remodelling and electrophysiology in the rat sciatic nerve after spiral cuff electrode implantation. J Neuroimmunol. 2006;185:103-114.

CHAPTER 6

Chapter 6

This study describes further exeperience in one patient implanted with the ADNS-300 during one year of follow-up. CAP's were succesfully recoreded at different time points after surgery. In addition, different pulse widths were used, doses repons curves were recorded and finally a estimation of chronaxie and rheobase of myelinated fibers were calculated. These results give a first hint how individual electrophysiological information of the vagus nerve can be implemeted in clinical practice. This of particular value, as recordings of individual CAP's of the vagus nerve may one day replace the rather emperic way of uptitration of stimulation output. In addition, future exepriments may indicate wheter non-responders are patients with insuffient or delayed activation of the vagus nerve due to for example increased local inflammatory reactions.

Electrophysiological properties of the human vagus nerve recorded with the Advanced Nerve Stimulator version 3.00

Riëm El Tahry, Robrecht Raedt, Lies Mollet, Veerle De Herdt , Alfred Meurs, Frank Dewaele, Dirk Van Roost, Pascal Doguet, Jean Delbeke, Wytse Wadman, Paul Boon, Kristl Vonck

"In preparation"

Abstract

Rationale: VNS (vagus nerve stimulation) is a recognized adjunctive therapy for refractory epilepsy. Since its introduction in 1989, little efforts have been made to optimize the stimulation parameters. Individual recording of vagus nerve compound action potentials may represent one strategy to guide stimulation parameter adjustments.

Methods: In a patient with refractory epilepsy, the Advanced Nerve Stimulator version 300 (ADNS-3.00) was used for therapeutic stimulation of the vagus nerve. In addition, compound action potentials (CAPs) were recorded over a time span of 1 year. Recruitment curves of the CAPs were recorded at week 3, month 11 and 12. From these curves, maximal CAP amplitude (PN_{max}), intensity needed to reach 50% of the maximal amplitude response ($I_{50\%}$) and the slope factor (k) were determined. In addition, chronaxie and rheobase were calculated.

Results: Recruitment curves of the CAPs were successfully recorded at consecutive visits. Latency of P_1 kept stable between 0.8 0 msec +/-0.01 and 0.88 msec +/-0.01, while N_2 occurred between 1.15 msec +/-0.03 and 1.21 +/-0.02 msec. PN_{max} varied between 16 and 21.3 µV. Characteristics of the recorded dose response curves were similar at different visits. In addition, two different groups of fibres of myelinated fibers were recruited. Finally, the rheobase and chronaxie at week 3 values were found to be 125 µA and 121 µs respectively.

Conclusion: This study demonstrates for the first time that chronic vagal CAP recording in patients is feasible and may guide individual stimulation parameter settings.

Keywords: vagus nerve stimulation, epilepsy, compound action potentials, recruitment curve, chronaxie, rheobase

6.1 Introduction

VNS is a recognised adjunctive treatment for refractory epilepsy. The stimulation parameters applied in clinical practice were derived from a limited number of animal experiments (1-3). Since its introduction in 1989, little efforts have been made to optimize the stimulation parameters to improve efficacy. One strategy to guide stimulation parameter adjustments may be to explore individual vagus nerve responses to electrical stimulation. The Advanced Nerve Stimulator, version 3.00 delivers electrical pulses to the vagus nerve and is designed to record vagal nerve compound action potentials (CAPs). The CAP is the algebraic sum of many 'all or none'' action potentials arising more or less simultaneously in a large number of individual axons in a compound nerve, such as the vagus nerve. The CAP of the vagus nerve does not occur naturally, but results from an experimentally or clinical induced stimulus with extracellular stimulating electrodes and can be recorded with extracellular electrodes, which measure the summed electrical response of all the excited axons in the vagus nerve. This offers the opportunity to determine the electrical charge required to activate vagal nerve fibres in a given patient. Previously, our group has reported postoperative $N_1P_1N_2$ and P_1N_2 recorded CAPs from the vagus nerve in two implanted patients (4). Here, a 12 month follow-up study is presented during which we obtained successive recruitment curves of the vagus nerve in one patient. The patient received vagus nerve stimulation according to the standard paradigm used in our centre (5-7).

We report for the first time chronically obtained recruitment curves from a human vagus nerve and propose a novel approach to VNS therapy.

6.2 Methods

In one patient VNS therapy was delivered during the first year according to classical stimulation parameters (gradually increasing intensity of 0.25 mA every 2-3 weeks, 500 µs pulse duration, 30 Hz frequency, 30 sec On, 10 min Off). At different visits after implantation, namely week 3, month 11 and month 12, the vagus nerve was stimulated with a biphasic charge-balanced pulse and CAPs of the vagus nerve were recorded. The CAP is characterized by its latency (msec), which was calculated as the delay between the end of the cathodic phase of stimulation artifact and the occurrence of its first positive (P_1) and consecutive negative peak (N_2). In addition, the maximal amplitude of the CAP (µV) was calculated as the difference in voltage between P_1 and N_2 (= P_1-N_2).

Recruitment curves were obtained by increasing stimulus current intensity of the cathodic phase of the stimulus in steps of 50,100,250or 500µA starting at a minimum intensity of 50 µA until a maximum of 1mA. For this purpose, at week 3 pulse widths of 50 and 250 µsec were used, while at the following visits due to time constraints, only 50 µsec was used. In order to obtain clean traces, 2 to 32 identical sweeps were recorded and averaged. Subsequently, data were fitted to a Boltzman function:

$$PN(I) = \frac{PN_{\max}}{1 + e^{\frac{I50 - I}{k}}}$$

In which PN is the amplitude of the compound action potential and calculated as P_1-N_2. PN_{max} is the maximal value of PN and $I_{50\%}$ is the stimulation current whereby PN = PN_{max}/2. I is the stimulus intensity applied to the vagus nerve. The slope factor k describes the recruitment homogeneity of the vagus nerve fibres. The correlation coefficient R between the fitted model and the recruitment curve was > 0.95. Besides the latency of P_1 and N_2, PN_{max}, $I_{50\%}$, the slope factor k and the impedance of stimulation electrodes were measured at the consecutive visits. Finally, rheobase and chronaxie were calculated. The rheobase (Rh) is the minimum intensity needed to obtain an excitation with a stimulus of infinite duration. The chronaxie (Ch) is the pulse duration required to elicit a response with an intensity that equals twice the rheobase. The chronaxie is considered to represent the most efficient stimulation, which requires the least of energy to activate the vagus nerve adequately. These parameters were estimated on the basis of the thresholds Th (D) of two stimulation pulses with two different durations (D) recorded at week 3. For this purpose, the strength-duration model of Weiss was used (8).

$$Th(D) = Rh\left(\frac{Ch}{\ln(2).D} + \frac{1}{2}\right)$$

Further technical details about the stimulation as well as signal recording and filtering have been described earlier (4).

6.3 Ethical approval

This study complies with applicable European and Belgian regulatory requirements and in particular the European Council Directive 90/385/EEC relevant to Active Implantable Medical Devices.

This study was approved by the Ethics Committee of Ghent University hospital (EC 2008/274). Patient gave informed consent.

6.4 Results

6.4.1 Patient outcome

The patient is a 47-year-old male who developed post-lesional epilepsy 6 months after a stroke of the left medial cerebral artery. This resulted in a mixed aphasia and a residual minor hemi paresis of the right arm and leg. Before implantation, the patient suffered from 1 complex partial seizure with secondary generalized seizure a month. Several anti-epileptic drugs (AEDs) failed to obtain seizure freedom. Moreover the patient was very sensitive to various AED side effects which often limited the up-titration of his anti- epileptic drugs. Following VNS treatment, the patient became seizure free starting month 7. At that time, the stimulation parameters were 0.75 mA intensity, 500 µs duration, 30 Hz frequency with a 30 sec On, 10 min Off duty cycle. Treatment of the patient was modified 6 months after initiating the VNS because of complaints of extreme drowsiness and tiredness. Consequently, the Phenytoïne dosage was reduced from 300 mg to 200 mg/day and Pregabaline 300 mg/day was added to the patient's regimen. Therefore, it remains uncertain whether seizure freedom (from month 7 to 12) resulted from the addition of Pregabaline or VNS or the combination of both.

6.4.2 CAPs of the vagus nerve in vivo (Fig1.)

6.4.2.1 Latency of P_1 and N_2 of the CAP

Latency of the P_1-N_2 CAPs was reliably recorded over time. P_1 kept stable between 0.8 0 msec +/-0.01 and 0.88 msec +/-0.01, while N_2 occurred between 1.15 msec +/-0.03 and 1.21 +/-0.02 msec.

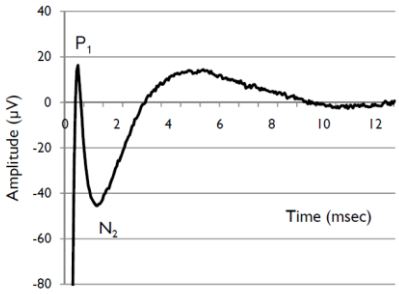


Fig 1: The vagus nerve was stimulated with pulses of 500µA intensity and 250 µs duration. A P_1N_2 compound action potential was simultaneously recorded with the ADNS-300 electrode

6.4.2.2 Amplitude of the CAP

Maximal amplitude PN_{max} of the CAPS were deduced from recruitment curve to 50 µsec pulses at each visit. At week 3, month 11 and 12, PN_{max} remained stable between 16 and 21.3 µV (Fig 2a,b,c). At week 3 the vagus nerve was additionally stimulated with a higher pulse width of 250 µsec. As shown in Fig 3, amplitude of the CAP increased up to 82.7 µV. From both curves, a first threshold is observed at a stimulation strength 500 µA x 50 µs (Fig 2a), corresponding to the activation of a first group of fibres, most likely the largest fibres in the nerve. Increasing the stimulation charge density, suggests the recruitment of an additional and different population of fibres, presumably of smaller diameter. These fibres had a threshold of approximately 150 µA x 250 µs (Fig 3). The low charge of the applied stimuli indicates that myelinated fibres are concerned.

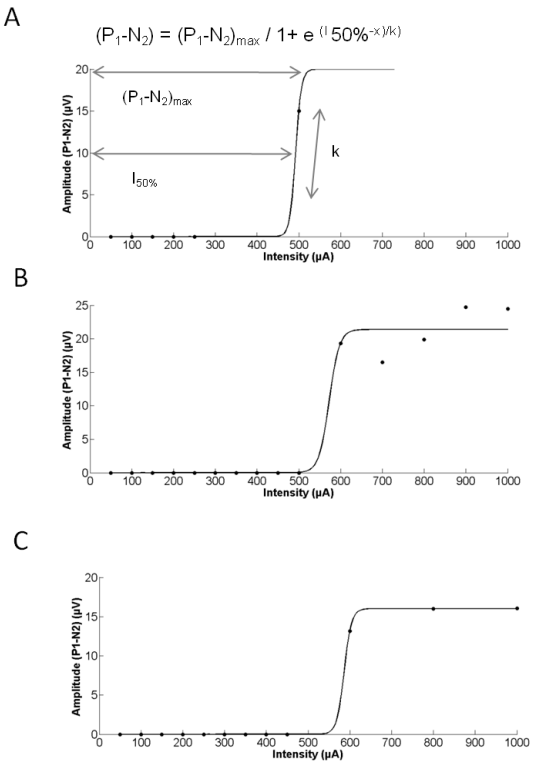


Fig 2 (a,b,c): The vagus nerve was stimulated with a biphasic balanced charged pulse of increasing intensity and pulse width of 50 µsec. Dose response curves were fitted to a Boltzman function $(P_1\text{-}N_2) = (P_1\text{-}N_2)_{max} / 1+ e^{(I\,50\%-x)/k)}$, from which $(P_1\text{-}N_2)_{max}$, $I_{50\%}$ and slope factor k were determined over time span of 1 year. A: week 3, B: month 11, C: month 12

6.4.2.3 Recruitment curves: $I_{50\%}$ and slope factor k

$I_{50\%}$ deduced from the fitted curves varied between 492 µA at week 3, 572 µA at month 11 and 594 µA at month 12. The slope factor k was 6.9 µA at week 3; 12,2 µA at month 11 and 3.7 µA at month 12. (Fig 2 a,b,c). When the vagus nerve is stimulated with larger charge density, recruitment curve showed a $I_{50\%}$ of 259 µA and a slope factor k of 53.2µA, corresponding to the activation of a second additional group of vagus nerve fibers (Fig 3). The equivalent amount of charge was 64.75mCoulomb, which is more than two fold of the applied charge for the 50 µs pulses above.

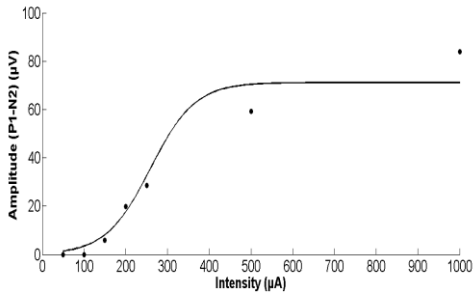


Fig 3: At week 3, the vagus nerve was additionally stimulated with a pulse with longer duration, i.e. 250 µsec. Data were fitted to a Boltzman function, $I_{50\%}$ and slope factor k were determined. This curve suggests that at least two groups of myelinated fibers are activated, as the second group is recruited only at higher charge levels, namely 150µA x 250 µs en further increases in amplitude as stimulus intensity increased.

6.4.2.4 Impedance

The impedance of stimulation contacts remained stable between 5.3 and 7.2 kOhm, which is in the range of impedance for which the constant current stimulator can function adequately. Consequently, it can be assumed that the stimulation electrode transferred electricity to the nerve in a satisfactory way.

6.4.2.5 Chronaxie and rheobase

Based on our experimental results of week 3, in which a threshold of 500 µA was necessary to activate the nerve using a pulse width of 50 µs and similarly 150 µA for a pulse width of 250 µs, using strength-duration model of Weiss we calculated a rheobase of 125 µA and a chronaxie of 121 µs.

6.5 Discussion

Although VNS is widely used as a treatment for refractory epilepsy, the actual choice of optimal stimulation parameters is arbitrary. Since its introduction in 1989, little efforts have been made to change the currently applied stimulation parameters to improve efficacy. One of the important shortcomings in the field of VNS is the lack of an objective evaluation of stimulation of the vagus nerve. Our study shows that CAPs of the human vagus nerve can be objectively recorded in response to an external electrical stimulus. Recruitment curves of the vagal nerve CAP were successfully recorded over a time span of 1 year after implantation.

6.5.1 Patient outcome

The patient implanted with the Advanced Nerve Stimulator version 300 vagus nerve stimulator was treated with VNS during the first year with a stimulation paradigm comparable to the one used in previous studies done by our group (4-7). Stimulation was initiated two weeks after surgery at the epilepsy clinic using the following parameters: pulse width 500µsec; frequency 30 Hz; duty cycle 30 sec on/10 min off. The stimulation intensity was gradually increased with steps 0.25 mA until 0.75 mA was reached. Beyond 0.75 mA the patient reported painful stimulation related sensation in the left neck region. Nevertheless, he became seizure free from month 7 on. This positive outcome may result from the adaptation of his AED regimen or combination of the latter with concomitant VNS treatment.

6.5.2 CAP characteristics

Latencies of P_1 and N_2 of the CAPs were short. P1 occurred between 0.8 0 msec +/-0.01 and 0.88 msec +/-0.01 and N2 between 1.15 msec +/-0.03 and 1.21 +/-0.02 msec. This suggests that large diameter myelinated fibres of the vagus were activated. Ideally, the determination of the fibre type is based on an estimation of the nerve conduction velocity (NCV), derived from latency value differences between two recording contacts at a distance from each other (9). However, in order to be implantable, the ADNS-3.00 electrode has a restricted length, which does not allow accurate conduction velocity measurements. More importantly, our study shows that recruitment curves of the vagus nerve CAP can be recorded repeatedly over time. The main drawback of our study is the fact that only a restricted number of data could be recorded due to time constraints. The limited amount of data does not permit statistical analysis. Therefore it remains difficult to claim that characteristics of the dose response curves remain stable over time, although initial results do tend to support this hypothesis. Future technology advancements will allow more efficient recording of the recruitment curves, which in turn will lead to more accurate determination of its characteristics.

In addition to the recruitment curves, we have calculated the chronaxie and rheobase of the identified vagus nerve fibres based on the model described by Weiss. Stimulation of the vagus nerve with pulse duration equal to the chronaxie is considered to be most efficient stimulation and thus requiring the least amount of energy. Ideally, the chronaxie could be calculated for each patient individually, enhancing stimulation efficiency and consequently spearing battery life. Taking into account the limited number of data and the fact that the rheobase calculation is also dependent on the electrodes used and distance to the nerve, our results (125 µA and 121 µs respectively) compare reasonably with findings in the literature. Chronaxie values of canine vagal A fibres in vivo were reported to be 75.4 ± 24.5 µs, with a rheobase of 630 µA ± 180 µA (9,10). Nevertheless, comparisons

should be interpreted with caution, as they are species dependent and very sensitive to temperature. The chronaxie value reported in our study suggests that the optimal stimulation pulse duration for VNS would be around 120µs, which is strikingly less than the pulse duration of 250 or 500 µs usually applied in clinical practice. This element emphasizes that clinicians might use much higher VNS electrical charges than really required.

6.5.3 Clinical implication of recruitment curves of human vagus nerve

In clinical practice, stimulation intensity is gradually increased over months, until a maximal tolerable level is reached or a reasonable seizure control is obtained. The procedure is a time consuming process and has no objective rational basis. Moreover, the common procedure of increasing the VNS intensity up to the maximum tolerable level, contrasts with the experimental evidence that only myelinated fibres need to be recruited in order to provide an anti seizure effect (11,12).

Individual recruitment curves could guide the clinician up to what level of current myelinated fibres can incrementally be recruited. The electrical charge required to fully activate these fibres, may then serve as an individual maximum stimulation intensity level. Consequently, the time needed to reach a maximal stimulation level can be reduced. In addition, unnecessary high output currents leading to painful sensations or discomfort may be prevented. Finally, reducing the stimulation strength will ultimately also reduce battery power consumption by the generator. In the context of individual adjustments of VNS parameters, until today only one author reported age dependent latency and threshold changes of the vagus nerve CAP derived from intra-operative recordings (13).

6.6 Conclusion

This study is the first to date to report chronic recruitment curves of human vagus nerve, most likely representing activation of large myelinated vagus nerve fibres. Implementation of CAPs in clinical VNS practice may help in the future to adapt the stimulation parameters on individual basis.

Finally, implantation of a large number of patients could clarify whether CAP recordings can be correlated with long-term seizure response and consequently function as possible predictive parameter.

6.7 Acknowledgements

Riëm El Tahry is supported by a grant from the Ghent University Research Fund (BOF). Prof. Dr. P. Boon is supported by grants from FWO; grants from BOF and by the Clinical Epilepsy Grant from Ghent University Hospital. Prof. Dr. K Vonck is supported by a grant of the BOF. This study was partially funded by Neurotech Belgium

6.8 Disclosure of conflicts of interest

Pascal Doguet and Jean Delbeke are respectively employee and advisor at Neurotech. None of the other authors has any conflict of interest to disclose.

All authors confirm they have read the Journal's position on issues involved in ethical publication and affirm this report is consistent with those guidelines

Reference list

1. Woodbury JW, Woodbury DM. Vagal stimulation reduces the severity of maximal electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording Pacing Clin Electrophysiol. 1991;14:94-107.

2. McLachlan RS Electrical-Stimulation of the Vagus Nerve for the Treatment of Epilepsy - Support from Experimental-Models. Annals of Neurology 1992;32:245.

3. Zabara J. Inhibition of experimental seizures in canines by repetitive vagal stimulation . Epilepsia 199;33:1005-10012.

4. El Tahry R., Raedt R, Mollet L, De Herdt, V, Wyckuys T, Van Dycke A, Meurs A, Dewaele F, Van Roost D, Doguet P, Delbeke J, Wadman W, Vonck K, Boon P.(2010) A novel implantable vagus nerve stimulation system (ADNS-300) for combined stimulation and recording of the vagus nerve: Pilot trial at Ghent University Hospital. Epilepsy Res.2010;92:231-239.

5. Vonck K, Thadani V, Gilbert K, Dedeurwaerdere S, De Groote L, De Herdt V, Goossens L, Gossiaux F, Achten E, Thiery E, Vingerhoets G, Van Roost D, Caemaert J, De Reuck J, Roberts D, Williamson P, Boon P. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience Journal of Clinical Neurophsiology. 2004;21:283-289.

6. De Herdt V, VonckV, Verschelde H, Legros B, Hauman H, Van Rijckevorsel K, Ceulemans B, Lagae L, Van Paesschen W, Sadzot B, Boon P. Vagus nerve stimulation for refractory epilepsy: Pooled results from epilepsy centres in Belgium Journal of Paediatric Neurology 2007;11:261-269.

7. El Tahry R., De Herdt, V, Raedt R, Van Dycke A, Meurs A, Dewaele F, Boon P, Van Roost D, Vonck K. Evolution in VNS therapy for refractory epilepsy, experience with Demipulse devices at Ghent University Hospital. Seizure 2010;19:531-535.

8. Bostock H, Baker M, Grafe P, Reid G. Changes in Excitability and Accommodation of Human Motor Axons Following Brief Periods of Ischemia Journal of Physiology-London 1991;441:513-35.

9. Krames Elliot S, Peckham P.Hunter,.Rezai Ali R (2009) Neuromodulation, First ed. Elsevier, London.

10. Smith C D, Geddes L A, Bourland J D, Foster K, Schoenlein W E. The Chronaxie and Propagation velocity of canine cervical Vagus Nerve fibres in Vivo Cardiovascular Engineering 2001;2:77-84.

11. Zagon A, Kemeny AA Slow hyperpolarization in cortical pyramidal neurones - A possible mechanism behind vagus nerve stimulation therapy for refractory epilepsy? Journal of Physiology-London 2000;526:59-60.

12. Krahl SE, Senanayake SS, Handforth A. Destruction of peripheral C-fibres does not alter subsequent vagus nerve stimulation-induced seizure suppression in rats. Epilepsia 2001;42:586-589.

13. Koo B, Ham SD, Sood S, Tarver B. Human vagus nerve electrophysiology: a guide to vagus nerve stimulation parameters. Journal of Clinical Neurophsiology. 2001;18:429-433.

CHAPTER 7

Chapter 7

The aim of this study was to identify a marker reflecting effective stimulation of the vagus nerve. In the first part of the study an electrophysiological response to VNS was measured using thin-point recording electrodes placed near the stimulation cuff electrode. By inducing lesions at various levels along the vagus and recurrent laryngeal nerves, performing simultaneous EMG recording and applying a muscle paralyzing agent, the electrophysiological response was identified to be a far field potential of a VNS-induced larynx compound muscle action potential (LCAMP).

During the second part of this study, a new self-sizing cuff electrode for combined stimulation and recording was designed and used to record VNS-induced LCAMP on a daily basis during two months. This study is the first to date to report chronic VNS induced LCAMP recordings. Importantly, nearly half the rats showed a delayed response and CAP's could only be recorded 2 to 7 weeks after surgery. This implicates that a local recovery period might be needed before the vagus nerve can be adequately stimulated. This is of particular clinical interest, as it points out that recording of CAP's may be important in decision when to start up-titration of VNS therapy. Moreover, it may be a partial explanation why some patients do not respond or respond only after a certain delay to their treatment.

Repeated assessment of larynx compound muscle action potentials using a self-sizing cuff electrode around the vagus nerve in experimental rats

Riëm El Tahry[*], Lies Mollet[*], Robrecht Raedt, Jean Delbeke, Veerle De Herdt, Tine Wyckhuys, Dimitri Hemelsoet, Alfred Meurs, Kristl Vonck, Wytse Wadman, Paul Boon.

[*]*Both authors equally contributed to this study*

Journal of Neuroscience Methods 2011 Jun 15;198(2):287-93

Abstract

Rationale: Vagus nerve stimulation (VNS) is an adjunctive treatment for patients with refractory epilepsy. In more than 30% of the patients VNS has no therapeutic effect. The goal of this study was to find an objective parameter that can be used as an indicator of effective stimulation of the vagus nerve.

Methods: The electrophysiological response to VNS was recorded from the vagus nerve, recurrent laryngeal nerve and larynx muscles. Nerve lesions and muscle relaxing agent were used to find the source of the electrophysiological response. A cuff-electrode for chronic stimulation and recording was implanted for chronic recording of the VNS-induced electrophysiological response after implantation. Dose-response curves were determined daily during a follow-up period of 2 months.

Results: VNS induced an electrophysiological response around 3 msec after start of the stimulation. This response was identified as a larynx compound action potential (LCMAP) LCMAP could be recorded immediately after surgery in 11/21 rats, while in the other 10/21 rats, a recovery period with an average of 25 days was required. Once the LCAMP could be recorded, the latency and overall characteristics of the doses response curves of the LCMAP remained stable during the entire follow-up period.

Conclusions: In this study, we provide an objective electrophysiological parameter for vagus nerve activation. LCAMP may indicate recovery of the vagus nerve after implantation, which may help to determine when uptitration of VNS therapy can be initiated. LCAMP could be of value in future experiments for objectification of VNS in animal models for epilepsy

Keywords: Vagus nerve stimulation, larynx muscle potential, neural electrode, epilepsy

7.1 Introduction

Epilepsy is a neurological disorder characterized by recurrent aberrant electrical activity in the central nervous system that typically manifests itself as seizures. It is estimated that 1-2% of the population is affected worldwide. About 30% of the patients with epilepsy do not respond to anti-epileptic drugs and are considered medically refractory (1,2). For these patients, alternative treatment modalities such as epilepsy surgery or neurostimulation, such as deep brain stimulation or vagus nerve stimulation, may be useful.

The vagus nerve is a mixed cranial nerve that consists of 80% afferent fibres innervating the heart, aorta, lungs and gastro intestinal tract and 20% efferent fibres that provide parasympathetic innervations of these structures and innervate the voluntary striated muscles of the larynx and pharynx through the recurrent laryngeal nerve, which is similar in rats (3,4) and humans (5,6). Moreover, the proportion of myelinated axons in the cervical left vagus nerve of rats is comparable to humans (7,8). At last, the left superior laryngeal nerve splits from the vagus nerve identically in rats and humans, as it runs back superiorly behind the aortic arch in a groove between oesophagus and trachea to finally enter into the larynx. (4,6)

Electrical stimulation of the left vagus nerve is used as an adjunctive treatment for patients with refractory seizures (9-14). The mechanism of action of vagus nerve stimulation (VNS) remains incompletely understood. There is little or no information available about the electrophysiology of the vagus nerve, although activation of the nerve is essential to its anti-epileptic effect (9-12). Many questions in VNS therapy remain unresolved, for example why some patients experience beneficial effects and others do not respond to the treatment. In current clinical epilepsy practice, no investigation is available to assess whether the vagus nerve is successfully activated by VNS or not. Defining a parameter reflecting stimulation-induced activation of the vagus nerve activation might provide a better understanding of the electrophysiological properties of the nerve. This in turn, could lead to further optimization of VNS treatment.

The aim of this study was to identify a marker reflecting effective stimulation of the vagus nerve. In the first part of the study an electrophysiological response to VNS was measured using thin-point recording electrodes placed near the stimulation cuff electrode. By inducing lesions at various levels along the vagus and recurrent laryngeal nerves, performing simultaneous EMG recording and applying a muscle paralyzing agent the electrophysiological response was identified to be a far field potential of a VNS-induced larynx compound muscle action potential (LCAMP). During the second part of this study a new self-sizing cuff electrode for combined stimulation and recording was designed and used to record VNS-induced LCAMP on a daily basis and for several weeks after

implantation of the stimulation electrode. In humans, intra-operative VNS-induced LCAMP were described (15), but no studies, animal nor human, report chronic LCAMP recordings

7.2 Materials and Methods

7.2.1 Design of the cuff-electrode

The self-sizing spiral cuff electrode is composed of two 80 µm thick silicone rubber sheets (Statice Santé, France) glued together with an adhesive which polymerizes at room temperature (Part A and B MED 4-4210, Nusil). The internal sheet is stretched during curling (stretch factor of 0.5) in order to obtain a self-curling spiral cuff. The cuff has an internal diameter of 1 mm and a total length of 9 mm.

For the acute experiments two pieces of platinum (Alfa Aesar, 99.9% metal basis, 0.25 mm thick) are inserted between the silicone sheets to form the stimulation contacts (Fig1b). The inter-electrode distance between stimulation the anode and cathode (each 3x1 mm) was 1 mm. Windows of 500µm diameter are cut out in the internal silicone sheet in order to give the platinum contacts access to the nerve.

For the chronic experiments a cuff electrode was manufactured with an extra contact for recording (Fig1a,c). Therefore a third piece of platinum (1x1 mm) was inserted between the silicone sheet at 2 mm from the cathode, near the cuff edge directed towards the head.

Teflon coated stainless steel wires (FWM 1x7x0.02/316LVM/EFTE, Fort Wayne metals) of 20 cm were welded to each platinum contact before their insertion between the electrode silicone sheets. Connector pins were soldered at the other extremity of the leads, allowing connection to an external stimulator or a recording device.

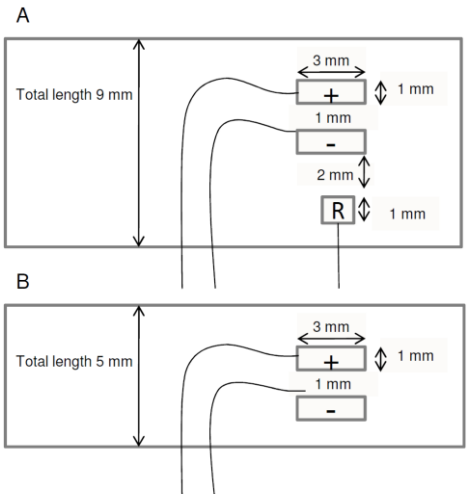


Fig 1 (A,B): Schematic representation of a combined stimulation and recording self sizing cuff electrode (A) and a simple stimulation electrode for acute use (B)

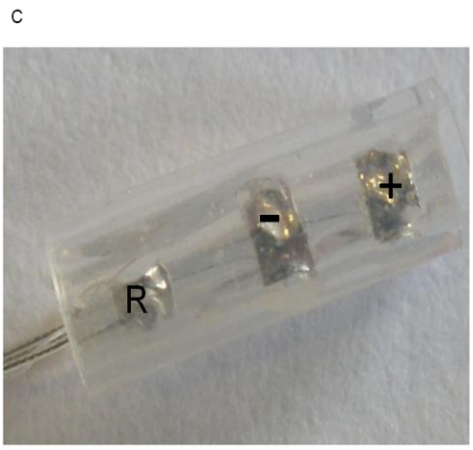


Fig 1 (C): photograph of a combined stimulation and recording self sizing cuff electrode

7.2.2 Animals

Adult male Wistar rats (Harlan, The Netherlands), were treated according to the guidelines approved by the European Ethics Committee (decree 86/609/EEC). The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University hospital (ECP 08/37). All animals were kept under environmentally controlled conditions (12h light/dark cycles, 20-23°C and 50% relative humidity) with food and water ad libitum.

7.2.3 Surgery

7.2.3.1 Acute experiments

Wistar rats (250- 350 g) (n=4) were implanted with a spiral cuff stimulation electrode around the left vagus nerve. Rats were anesthetized with gas isoflurane anesthesia (5% isoflurane for induction, 2% isoflurane for maintenance). An incision was made over the left anterior cervical region. The left vagus nerve was carefully dissected from the aortic sheet and the cuff electrode was wound around the nerve with the anode placed caudally. To record the electrophysiological response to VNS thin-point, stainless steel electrodes (125 µm diameter) were placed on different anatomical structures, including the vagus nerve, as well as the recurrent laryngeal nerve and the muscles surrounding the larynx. An epidural electrode, placed over posterior occipital cortex, was used as reference/ground electrode. In order to induce lesions along the nerves a nylon wire was strapped around the nerves.

Vecuronium (Norcuron, 1 ml of 2mg/ml solution), a muscle relaxing agent, was applied to paralyze the larynx muscles. At the end of each acute experiment, animals were sacrificed with an overdose of pentobarbital (180mg/kg i.p).

7.2.3.2 Chronic experiments

Wistar rats (250 - 350 g) (n=21) were implanted with a spiral cuff electrode for stimulation and recording of the left vagus nerve. The rats were anesthetized with a ketamine/xylazine (respectively 80 mg/kg and 7.5 mg/kg, i.p.) mixture. For chronic use, the electrode leads were tunnelled to an incision made in the skin above the skull. The connector pins were fixed to a skull head stage of acrylic cement. Four epidural stainless steel anchor screws were screwed bilaterally into the skull above parietal and occipital cortex. The posterior right screw, placed over occipital cortex, served as ground/reference for chronic electrophysiological recording.

7.2.4 Recording of the larynx compound action muscle potential

The vagus nerve was stimulated with biphasic square wave pulses of 100 µs duration in the acute experiments in order to keep stimulation artifact as low as possible and allow recording of possible early physiological signals in response to stimulation. For chronic experiments 500 µs block-pulses were used because this pulse width is mostly used in chronic studies on efficacy of VNS in humans and animals. Stimuli were delivered by a constant current stimulator. Dose-response curves were determined using a stimulus intensity ranging between 40 µA and 800 µA. Signals were recorded from stainless steel wire point electrodes placed on the vagus nerve, recurrent laryngeal nerve and larynx muscle or from the monopolar contact inside the cuff electrode . Signals were amplified 500 times before high pass filtering at 0.15 Hz in order to remove DC components. Thereafter, the data were digitized using a National Instruments acquisition board (NI DAQ PAD 6259) and finally stored on a personal computer. Recording and analysis of signals were done using Matlab (2007a, the MathWorks, Natick, Massachusetts).

7.2.5 Data analysis

Both in the acute and chronic experiments, the latency of the LCAMP was determined. The latency is defined as the delay between the onset of the stimulus artifact and the occurrence of the major negative peak. In addition, acute and chronic dose response curves of the LCAMP were determined. In the chronic experiments LCAMP dose response curves were determined on a daily basis for five days per week during a follow-up period of 8 weeks after surgery. At each session, the rats were anesthetized using isoflurane (induction 5%, maintenance 2%) in order to reduce movement artifacts. The time span, during which vagus nerve was stimulated but no response could be recorded, was considered to represent a recovery period after surgery.

A Boltzmann function: (M= Mmax/ 1+ e (I 50%-x)/k) was fitted to the measured dose-response curves. Mmax is defined as the maximal muscle potential amplitude. I50% is the intensity needed to obtain a response with half the maximal amplitude and slope factor k describes the recruitment homogeneity of the vagus nerve fibres (Fig 2). For each stimulus intensity, 20 sweeps were averaged to improve the signal to noise ratio. Besides the latency, I50% and k, the impedance of the stimulation contacts was recorded over time. The contact impedance of the stimulation electrodes was expressed in kOhms and defined as the voltage to current ratio measured at the end of a stimulation pulse of 100 µA amplitude and 500 µs duration. At the end of the 8 week follow-up period animals were sacrificed with an overdose of pentobarbital (180mg/kg i.p).

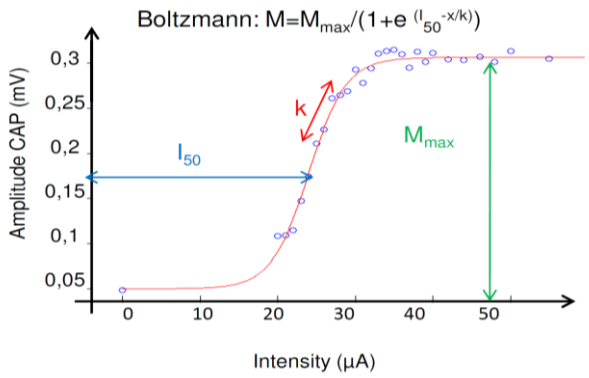


Fig 2: Example of a doses response curve of the larynx compound action muscle potential (LCAMP) in response to vagus nerve stimulation. A Boltzmann function (M= M_{max}/ 1+ e $^{(I_{50\%}-x)/k}$) was fitted to the doses response curve. I_{max} is defined as the intensity needed to achieve a response with maximal amplitude. $I_{50\%}$ is the intensity needed to obtain a response with a half the maximal amplitude and k is the slope factor.

7.2.6 Statistical analysis

From the chronic LCAMP recordings, parameters of the dose-response curves (latency, I50% and k) and electrode impedances were averaged for each rat and for each of the eight follow-up weeks. Sensitivity analysis showed that there was no bias effect of pooling the results of the animals with a recovery period to the results of the rats in which the LCAMP could be recorded from the start of the chronic recordings. Therefore, the means and standard errors of the mean (SEM), presented in the figures, were calculated from all rats in which an LCAMP could be recorded during a specific week. Statistical analysis was performed using mixed model linear regression analysis, including random intercepts in order to account for dependent observations. A Bonferonni correction was used to correct for type I error in multiple comparisons. Calculated residuals were normally distributed, which supports the validity of the used statistical model.

7.3 Results

7.3.1 Acute experiments: identification of the Larynx Compound Muscle Action Potential

VNS reproducibly induced a large negative peak at 2.6 msec +/- 0.2 msec after onset of the stimulation artifact (N1) (Fig. 3a). Based on its long latency and large amplitude we hypothesized that this response is a far field potential corresponding to larynx muscle activation induced by co-activating of the recurrent laryngeal nerve with VNS. The following observations support this hypothesis:

1)A lesion of the vagus nerve distal to the stimulation electrode but proximal to the aortic arch, abolished the recorded signals (Fig 3b), 2) a proximal lesion of the vagus nerve did not abolish the signal (Fig 3a). 3) an electromyography (EMG) recording of the laryngeal muscles shows a very large response at latency of 2.6 msec (Fig 3c). 4) Finally, all the signals recorded at the level of the vagus

nerve, recurrent laryngeal nerve and larynx muscle disappeared immediately when applying the muscle blocking agent Vecuronium (1 ml of Norcuron 4mg/2ml ampoule) to the larynx muscles.

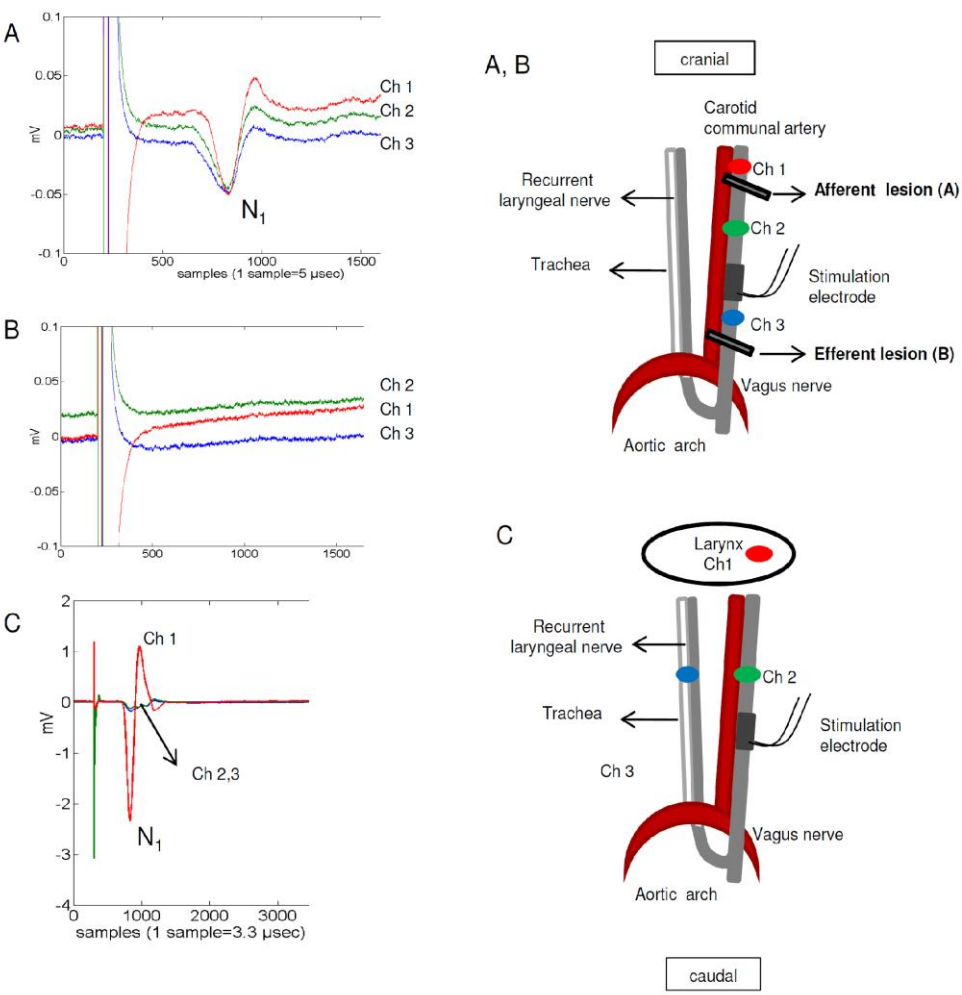


Fig 3 (A,B,C):

(A) Recorded Larynx Compound Muscle Action Potential (LCAMP) at different locations on the vagus nerve (Ch1,2,3), characterized by a major negative peak (N1). LCAMP remains preserved after proximal lesion of the vagus nerve cranial to stimulation electrode. This can be explained by the fact that the recorded signals on all channels result from a far field potential of the larynx contraction (see Fig3 C)

(B) Lesion of the vagus nerve distal to the stimulation electrode but proximal to the aortic arch, abolished all signals recorded at the level the vagus nerve (Ch1, 2, 3)

(C) Signals recorded from larynx muscles (Ch 1), the vagus nerve (Ch 2), the recurrent laryngeal nerve (Ch 3) showed no difference in latency. Moreover, the larynx EMG channel (Ch 1) exhibited much larger amplitude than signals recorded from other channels. All signals were abolished when applying Vecuronium into the larynx muscles

7.3.2 Acute and chronic recordings of the Larynx Compound Muscle Action Potential

In the acute experiments, latency of N1 of the LCAMP recorded at the level of the vagus nerve, was 2,6 msec +/- 0,2 msec after onset of the stimulation artifact. The I50 and slope factor of acute doses response curves were respectively 125,8 +/- 35,7 µA and 28 +/- 30µA.

In the chronic experiments, 21 rats were implanted with a cuff electrode for combined stimulation and recording of the vagus nerve.

LCMAP could be recorded immediately after surgery in 11/21 rats, while in the other 10/21 rats a recovery period ranging between 2 and 7 weeks (mean 25 days) was needed (Table 1).

Time after surgery (weeks)	Amount of rats with recordable LCAMP
0	11
1	11
2	13
3	13
4	13
5	16
6	20
7	21

Table 1 shows amount of rats per week after surgery in which LCAMP was recordable. In 11/21 rats, LCAMP could be measured immediately after implantation. In the remaining rats (10/21), a post-surgical recovery period was required before LCAMP could be recorded adequately.

The latency of N1 was 3.2 msec +/- 0.1 msec and did not change significantly over time during the 8 weeks of follow up (p=0.88) (Fig 4a). The I50% calculated from the doses response curves did not significantly change over time and varied between 56µA +/- 7µA and 74 µA +/- 18 µA (p=0.77) (p= 0.77) (Fig 4b). The slope factor of the doses response curves varied between 4.2µA +/- 0.7 µA and 6.7 µA +/-2.0 µA, indicating that implanted electrodes were able to activate vagus nerve fibres in a stable manner over the entire follow-up period (p=0.82) (Fig 4c).

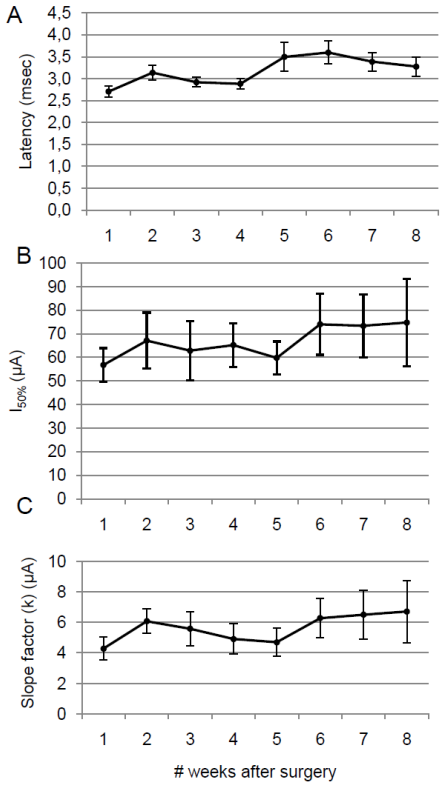


Fig 4 (A,B,C):

Means and standard errors of the mean (SEM) presented in the figures, were calculated from all rats in which an LCAMP could be recorded during a specific week.

(A) The latency of N_1 was 3.2 ms +/- 0.1 ms and did not change significantly over time during the 8 weeks of follow up

(B) I_{50} and (C) slope factor (k) deduced from the dose response curves remained stable during 8 weeks of follow-up.

The impedance of the stimulation contacts significantly increased over time ($p<0.001$), values of week 4 to week 8 being significantly higher in comparison to the first week (post-hoc analysis with bonferonni correction) (Fig 5).

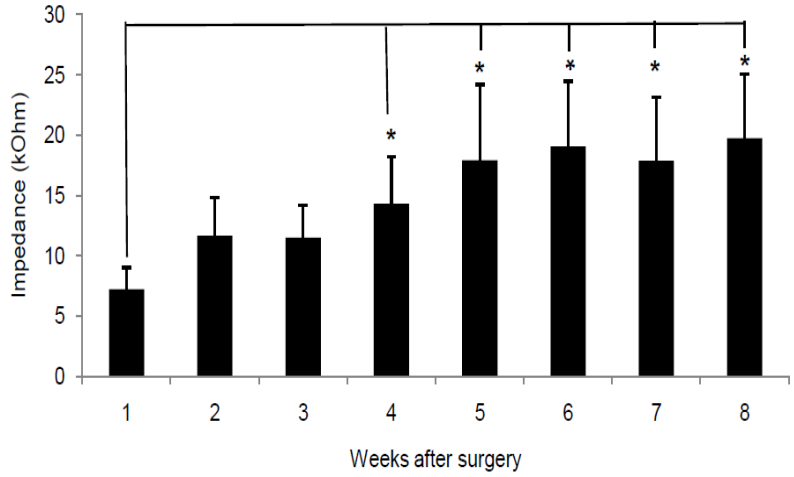


Fig. 5: The impedance of the stimulation contacts increased over time, but only values of week 4 to week 8 were statistically significantly higher in comparison to the first week

7.4 Discussion

The primary objective of this study was to identify an objective electrophysiological parameter to assess effective vagus nerve stimulation. In the first part of the study, the LCAMP was identified as a candidate marker for effective vagus nerve stimulation. In the second part of this study an implantable electrode system for chronic stimulation and recording of VNS-induced LCMAP in rodents was developed. In half of the rats implanted with this electrode system for chronic stimulation and recording a response to VNS could only be measured after delay of several weeks. From the moment the LCAMP could be measured up to eight weeks after electrode implantation input-output relationship between stimulus intensity and amplitude of the LCAMP remained stable over time although the impedance of the stimulation electrodes increased slightly during the eight weeks after implantation.

7.4.1 Characteristics of the LCAMP

By introducing lesions distal to the stimulation electrode and application of a muscle relaxing agent we showed that the measured electrophysiological response to VNS corresponded to muscle potentials of the larynx and not to any neural potential originating from the vagus nerve. Most likely the LCAMP is evoked by VNS-induced activation of the Aα efferent motor fibres of the vagus nerve. In rats but also in other mammals these vagal Aα fibres innervate the laryngeal muscles. The LCAMP was recordable in a reproducible manner during a follow-up period of two months, although a variable recovery period after surgery was found. The latency of the LCAMP, I50% and slope factor of dose response curves remained relatively stable during the follow-up period after electrode

implantation. However, as our experiments count a small group of animals (N=21), subtle changes over time cannot fully be excluded. Nevertheless, our results indicate that once the LCAMP could be recorded, the vagus nerve remained excitable, despite the development of a fibrous capsule around the electrode-nerve interface (16,17).

The increased impedance values of the stimulation electrodes between 4 and 8 weeks after implantation support the idea that a fibrous encapsulated electrode-nerve interface is formed. Importantly, values for the I50% and the slope factor of acutely dose response curves were larger than the same results obtained in chronic experiments, but a smaller pulse width was used. In addition, a shunting effect of physiological water that was added to the dissection pouch in order to moisturize the vagus nerve while performing surgery may also explain the obtained results.

7.4.2 LCMAP as a marker for vagus nerve stimulation

Laryngeal activation is the result of efferent stimulation, while VNS in epilepsy is focused on stimulating afferents in order to obtain beneficial effects in the brain (18-20). The use of LMCAP as a marker for adequate vagus nerve stimulation thus remains an indirect surrogate parameter, as it does not necessarily reflect activation of the specific fibre population with anti-seizure effect (20,21). Nevertheless, Aα fibres, provide motor activation of striated muscles of the larynx and represent a relatively low threshold fiber population. Importantly, these fibres are the most sensitive to anoxia and injury due to surgical manipulation (19,22). Consequently, alteration in Aα function may imply damage to other afferent vagus nerve fibres which are thought to provide anti-seizure effect of VNS. On the other hand, a histological study by Evans et al, in the rabbit vagus nerve showed that myelinated motor fibres of the vagus nerve seem to gather in the deep lateral part of the vagus nerve bundle, which implicates that damage to these fibres would not necessarily imply injury to the medial afferent myelinated fibres (23). Data about precise configuration of different fibres bundles in the cervical vagus nerve in humans is lacking, therefore possible hypothesises in this field remain purely speculative.

7.4.3 VNS induced vocal cord EMG in humans

A study performed in humans by Ardesh et al, in which intra-operative vocal cord EMG was recorded after VNS implantation showed a very similar VNS induced LCMAP. The shape was identical, but longer latencies and higher amplitudes were reported (15). Activation of the larynx in humans was obtained by applying a VNS pulse of 0.5 mA and 130 µsec, while in our experiments maximal muscle activation was already reached at approximately 65 µA and 100 µsec.

In humans, a temporary paresis of vocal cords after VNS surgery has been described (24-26), indicating that the surgical procedure and implantation of the electrode often causes a transient vagus nerve failure, hence requiring a recovery period before to become functional again.

7.4.4 Clinical relevance of the VNS induced LCAMP

In clinical practice, the idea that in some patients there is a delayed effect of VNS might in some cases be explained by a temporary failure of VNS-induced activation of the nerve due to nerve damage after electrode implantation. In our study, nearly half of the implanted rats required an average recovery period of 5 weeks (range 2-7) before VNS could efficiently induce a LCAMP. In clinical practice, ramping up of VNS output generally starts two weeks after surgery (27,28). Our results and the larynx studies cited above, suggest that an individualized approach might be more beneficial, although comparisons between humans and rats must be made with caution. Currently, there is no specific investigation indicating whether the vagus nerve recovered sufficiently to start uptitration of VNS therapy. In this context, recording a VNS- induced LCAMP by EMG of the larynx before and at different time points after surgery, could possibly serve as a new investigative tool in VNS therapy. More research on this topic is needed to confirm this idea.

Future experiments which investigate the relationship between LCAMP occurrence and therapeutic response to VNS treatment could be interesting. The lack of therapeutic effect of VNS may not only be the result of a lack of VNS effects on the brain, but also simply be the consequence of inadequate local recovery of the vagus nerve.

7.5 Conclusion

Twenty-one rats were successfully implanted with a custom-made self-sizing stimulation/recording electrode around the left vagus nerve, allowing repeated recording of the LCMAP over time. Our method provides an objective indication of effective vagus nerve activation, which could be of great value in all VNS experiments in animal models for epilepsy.

7.6 Acknowledgments

Riëm El Tahry is supported by a Bijzonder Onderzoeks Fonds (BOF) grant from University of Ghent. Prof. P. Boon is is supported by grants from the Fund for Scientific Research-Flanders (FWO); grants from the BOF and by the Clinical Epilepsy Grant from Ghent University Hospital.

Reference List

1. Hauser WA, Annegers JF, Rocca WA. Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester,Minnesota. Mayo Clin. Proc., 1996;71: 576-86.

2. Fisher RS, Van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J, Jr. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE),Epilepsia, 2005;46:470-2.

3. Dahlqvist A, Carlsöö B, Hellström S. Fibre components of the recurrent laryngeal nerve of the rat: a study by light and electron microscopy. Anat Rec. 1982; 204(4):365-70.

4. Paxinos G, The rat nervous system, Elsevier Academic Press: San Diego, 2004:77-84.

5. Jotz GP, De Campos D, Rodrigues MF, Xavier LL. Histological asymmetry of the human recurrent laryngeal nerve.J Voice, 2011; 25(1):8-14.

6. Berthoud HR, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. Auton Neurosci, 2000;20:1-17.

7. Hofmann HH, Schitzlein HN: The numbers of nerve fibers in the vagus nerve of man, Anat Rec., 1961;139:429-35.

8. Soltanpour N, Santer RM. Preservation of the cervical vagus nerve in rats: morphometric and enzyme histochemical evidence J Auton Nerv Syst, 1996;60:93-101.

9. Holder LK, Wernicke JF, Tarver WB. Treatment of refractory partial seizures: preliminary results of a controlled study.Pacing Clin Electrophysiol, 1992;15:1557-1571.

10. BenMenachem E, Manonespaillat R, Ristanovic R, Wilder BJ, Stefan H, Mirza W, Tarver WB, Wernicke JF, Augustinsson L, Barolat G, Bongartz U, Bergen D, Bucholz R, Burchiel K, Faught E, George R, Hufnagel A, Kuzniecky R, Landy H, Michael J, Naritoku D, Pencek T, Ramsay RE, Reid S, Richardson D, Rosenfeld W, Rosenwasser R, Salinsky M, Slater J, Sonnen A, Streletz L, Talalla A, Treig T, Upton ARM, Uthman B, Wegener K, Willis J. Vagus Nerve-Stimulation for Treatment of Partial Seizures .1. A Controlled-Study of Effect on Seizures.Epilepsia, 1994;35:616-26.

11. Handforth A, DeSalles AAF, Asatourian AA, Krahl SE, Us Vns SG. Seizure response to long-interval vagus nerve stimulation (VNS) is predictive of response to conventional VNS, suggesting a long-duration anti-epileptic mechanism.Neurology, 1998;50:S05006.

12. Handforth A, DeGiorgio CM, Schachter SC, Uthman BM, Naritoku DK, Tecoma ES, Henry TR, Collins SD, Vaughn BV, Gilmartin RC, Labar DR, Morris GL, III, Salinsky MC, Osorio I, Ristanovic RK, Labiner DM, Jones JC, Murphy JV, Ney GC, Wheless JW. Vagus nerve stimulation therapy for partial-onset seizures: a randomized active-control trial.Neurology, 1998;51:48-55.

13. DeGiorgio CM, Schachter SC, Handforth A, Salinsky M, Thompson J, Uthman B, Reed R, Collins S, Tecoma E, Morris GL, Vaughn B, Naritoku DK, Henry T, Labar D, Gilmartin R, Labiner D, Osorio I, Ristanovic R, Jones J, Murphy J, Ney G, Wheless J, Lewis P, Heck C. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures.Epilepsia, 2000;41:1195-2000.

14. Uthman B, Reichl A, Dean J, Reid S, Roper S, Eisenschenck S, Gilmore R, Wilder B. Vagus nerve stimulation for epilepsy: A twelve-year experience.Epilepsia, 2003;44:123-124.

15. Ardesch JJ, Sikken JR, Veltink PH, Van Der Aa HE, Hageman G, Buschman HP. Vagus nerve stimulation for epilepsy activates the vocal folds maximally at therapeutic levels.Epilepsy Res., 2010;89: 227-31.

16. Grill WM, Mortimer JT. Neural and connective tissue response to long-term implantation of multiple contact nerve cuff electrodes. J Biomed Mater Res 2000;50:215-26.

17. Thil MA, Tran Duy D, Colin IM, Delbeke J. Time course of tissue remodelling and electrophysiology in the rat sciatic nerve after spiral cuff electrode implantation. J Neuroimmunol. 2006;185:103-14.

18. Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats.Epilepsia, 1990;31:S7-19.

19. Woodbury JW, Woodbury DM. Vagal stimulation reduces the severity of maximal electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording. Pacing Clin Electrophysiol, 1991;14:94-107.

20. Krahl SE, Senanayake SS, Handforth A. Destruction of peripheral C-fibres does not alter subsequent vagus nerve stimulation-induced seizure suppression in rats.Epilepsia, 2001;42:586-589.

21. Zagon A, Kemeny AA. Slow hyperpolarization in cortical pyramidal neurones - A possible mechanism behind vagus nerve stimulation therapy for refractory epilepsy? Journal of Physiology-London, 2000;526:59-60.

22. Agnew WF, McCreery DB. Considerations for safety with chronically implanted nerve electrodes.Epilepsia, 1990;S27-S32.

23. Evans DH, Murray JG. Histological and functional studies on the fibre composition of the vagus nerve of the rabbit. J. Anat., 1954; 88: 320-37.

24. Zalvan C, Sulica L, Wolf S, Cohen J, Gonalez-Yanes O, Blitzer A. Laryngopharyngeal dysfunction from the implant vagal nerve stimulator. Laryngoscope, 2003;113:221-225.

25. Shaffer MJ, Jackson CE, Szabo CA, Simpson CB. Vagal nerve stimulation: clinical and electrophysiological effects on vocal fold function.Ann Otol. Rhinol. Laryngol, 2005;14: 7-14.

26. Shaw GY, Sechtem P, Searl J, Dowdy ES. Predictors of laryngeal complications in patients implanted with the Cyberonics vagal nerve stimulator. Ann Otol. Rhinol. Laryngol, 2006;115:260-267.

27. Vonck K, Thadani V, Gilbert K, Dedeurwaerdere S, De Groote L, De Herdt V, Goossens L, Gossiaux F, Achten E, Thiery E, Vingerhoets G, Van Roost D, Caemaert J, De Reuck J, Roberts D, Williamson P, Boon P. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience.Journal of Clinical Neurophysiology, 2004;21:283-289.

28. De Herdt V, Vonck K, Verschelde H, Legros B, Hauman H, van Rijckevorsel K, Ceulemans B, Lagae L, Van Paesschen W, Sadzot B, Boon P. Vagus nerve stimulation for refractory epilepsy: Pooled results from epilepsy centres in Belgium.Epilepsia, 2006;47:205-6.

CHAPTER 8

Chapter 8

Chapter 8 bundles two different experimental vignettes. The first part describes an initial set up of experiments in which the goal was to identify vagal evoked potentials in the rat brain. The second vignette is an additional study we have performed in collaboration with the veterinary department. A pilot trial in one horse was carried out to test feasibility of VNS for functional laryngeal stimulation.

Chapter 8.1

The initial goal of this thesis was to identify an objective parameter reflecting stimulation of the vagus nerve. In an early phase of this work, efforts have been made to characterize vagal evoked potentials in anatomical sites of the rat brain to which the vagus nerve is known to project to. Characterization of a vagal evoked potential would have allowed to visualize stimulation of the vagus nerve and its effects in the brain. Moreover, recording of vagal evoked potentials could potentially have improved understanding of the mechanism of action of VNS. For example, questions such as whether fiber specific stimulation (i.e A, B or C) activates different anatomical sites could have been addressed.

The results of these initial experiments showed that the recorded signals, with use of our extracellular deep brain electrodes, were the result of far field potentials of another local generator. Further experiments have finally led to the conclusion that our results were reflection of activation of the recurrent laryngeal nerve and contraction of the larynx muscles (see chapter 7).

Chapter 8.2

Recurrent laryngeal neuropathy is a common disease in horses and may be treated by functional stimulation. In this feasibility study, one healthy horse was successfully implanted with a VNS model 102. Rheobase and chronaxie were calculated and values were presented as possible stimulation parameters for laryngeal stimulation in horses.

CHAPTER 8: EXPERIMENTAL VIGNETTES

8.1 Can a VNS evoked potential be recorded in the rat brain?

8.1.1 Introduction/Rationale

Complementary to recording of compound action potentials of the vagus nerve, we investigated whether VNS induces evoked potentials (vagal EPs) in different key projection sites of the vagus nerve. The effect of different stimulation paradigms on the activation of different anatomical structures which receive vagal nerve information may be examined using those vagal EPs.

Anatomically, the vagus nerve projects mainly to the insular cortex, but there is some evidence in literature that vagus nerve also projects to lateral portion of sensorimotor cortex (1,2). Moreover, it was shown that low intensities of VNS induce slow hyperpolarisation of parietal neurons of rat cortex (3). For this reason, we hypothesized that vagus nerve stimulation induces an evoked potential not only in the insular cortex, but also over the sensorimotor cortex. In a second stage we evaluated whether vagal evoked responses could be recorded in three main vagal afferent subcortical sites: (i)Nucleus Tractus solitaries (NTS) (ii)Locus coeruleus (LC) and (iii)Thalamus.

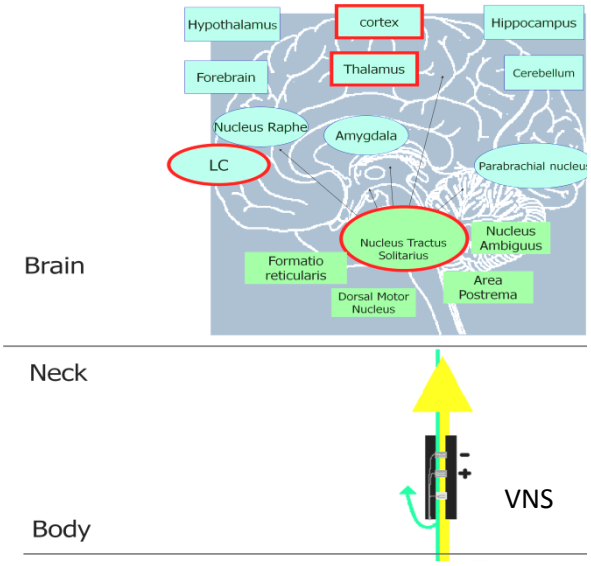


Fig. 1 Overview of vagal nerve projections into the brain. Red boxes are the anatomical sites which were explored for recordings of vagal EP's

8.1.2 Methods

Male Wistar rats (250-350gr) were implanted with stimulating self zing silicone cuff electrode around left cervical vagus nerve. In addition, a multi electrode (16 contacts) was manufactured (Fig. 2) to

record VNS induced extracellular fields of the sensori-motor cortex. A craniotomy exposing cortex from anterior secondary visual cortex to posterior motor cortex (M2) was performed. In other experimental setup, additional target sites (thalamus, LC, NTS) were implanted with deep brain electrodes that consisted of 2 to 4 stainless steel wires of 125 µm diameters, which were polyimide-coated and glued together. Vagus nerve was stimulated at 2 mA, 1Hz with a balanced charged rectangular pulse of 0.2 msec duration. Before the start of each experiment, a lead test was performed with the Cyberonics interrogation device to check impedance of stimulation electrodes.

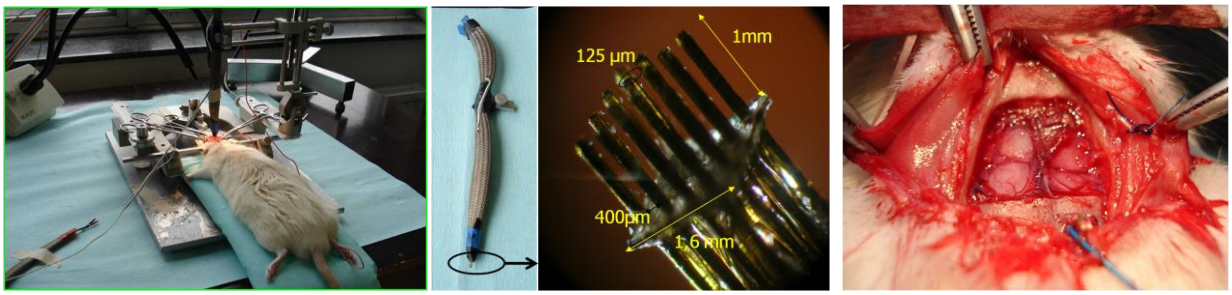


Fig 2: Multi-electrode for screening of VNS induced extracellular field potentials in sensori-motor cortex of rats

8.1.3 Results

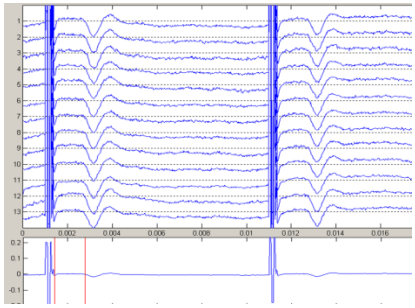


Fig 3: VNS induced extracellular field recordings of sensori-motor cortex

Signals recorded over the cortex appeared 2.7 msec after the end of stimulation artifact. Mean amplitude varied between 20 to 30 µV. Threshold to activate these responses was 100µA-200µsec, 500µsec. There were no latency differences between signals recorded in the neck and those recorded at surface of the cortex. Moreover, similar results were observed in all examined subcortical nuclei (thalamus, LC and NTS). In addition, no reversal of field recordings was observed, indicating a lack of local generators. Furthermore, in lesion experiments in which vagus nerve was sectioned, recorded signals in the brain disappeared, thus indicating a relationship to activation of the vagus nerve.

8.1.4 Discussion

As we could not detect any of latency differences between different recorded anatomical sites and peak latency was every time similar to the peak latency of signal recorded in the neck, we hypothesized that the recorded signals were derived from volume conduction of the activation of the cervical vagus nerve and/or recurrent laryngeal nerve and consequently did not correspond to VNS - induced evoked potentials.

This may partially be explained by the fact that the distance between stimulation in the neck and registration in the brain was very small. Amplitude of local field of activation of the vagus nerve may be much larger in comparison to very small local field potentials in the brain, rendering recording of evoked potentials more difficult.

Concerning LC and NTS recordings, an additional problem might have been the fact that these nuclei lack layered cell structures, which is known to facilitate extracellular field recording (4).

8.1.5 Conclusion

VNS-induced evoked potentials are not recordable with the techniques described in methods section. Recording on a smaller level such as multi- or single unit may be useful in characterizing upstream vagus nerve stimulation induced activity in the brain.

Reference List

1. Ito S. Electrophysiological evidence for projections of myelinated and non-myelinated primary vagal afferents to the rat insular cortex. Neurosci Lett 1994;179:29-32.

2. Ito S. Visceral region in the rat primary somatosensory cortex identified by vagal evoked potential. J Comp Neurol 2002;444:10-24.

3. Zagon A, Kemeny AA. Slow hyperpolarization in cortical neurons: a possible mechanism behind vagus nerve simulation therapy for refractory epilepsy? Epilepsia 2000;41[11], 1382-1389.

4. Johnston Daniel, Miao-sin Wu Samuel. Foundations of Cellular Neurophysiology. A Bradford Book, 1995.

8.2 Implantation of a VNS model 102 in a horse for recurrent laryngeal nerve stimulation

8.2.1. Introduction/Rationale:

Recurrent laryngeal neuropathy is a common disease in horses causing paralysis or paresis of the intrinsic laryngeal muscles innervated by the recurrent laryngeal nerve. Loss of abductor function of the dorsal cricoarytenoid muscle (CAD) leads to inadequate abduction of the ipsilateral arytenoid mostly during performances and consequently limiting capacities of racing horses (1,2). The use of functional electrical stimulation (FES) of the recurrent laryngeal nerve (RLN) is a possible novel treatment under research (3).

A feasibility study was performed in which a VNS model 102 was implanted around the left recurrent laryngeal nerve of one healthy horse.

8.2.2. Methods

Part 1

Measurements of recurrent laryngeal nerve diameters in cadavers lead to the assumption that the 3m inner diameter bipolar VNS electrodes should be appropriate in the normal adult horse.

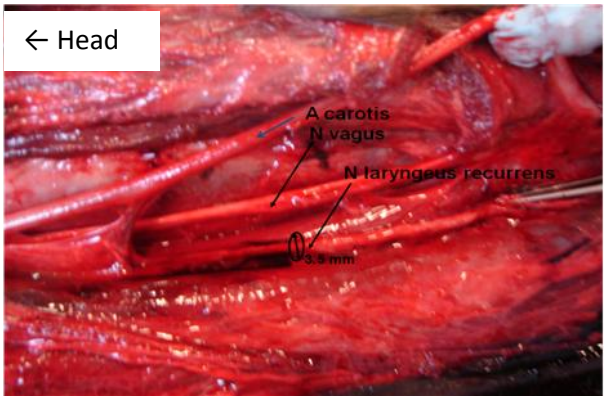


Fig 1: Dissection in a horse cadaver; determination of nervus laryngeus recurrens diameter

Part 2

A vagus nerve stimulation electrode was wound around the left RLN at the cervical level and was connected to a pulse generator. Three weeks after surgery, stimulus response characteristics were obtained by measuring stimulated arytenoid displacement endoscopically on the standing, non-sedated horse. A strength-duration curve of arytenoid abduction was determined based on the minimal intensity to evoke laryngeal muscle contraction using different parameters for pulse width

(1000, 750, 500, 250, 130 µs). Rheobase and chronaxie were calculated. In addition, stimulation parameters that evoke maximal sustained abduction were investigated.

8.2.3. Results

Implantation on VNS device was successful and no major problems occurred during surgery or in the follow-up period. Sustained abduction of the arytenoid was achieved when RLN was stimulated at a frequency of at least 25 Hz. Rheobase and chronaxie were calculated to be respectively 0.5 mA and 250 µs (Fig 2).

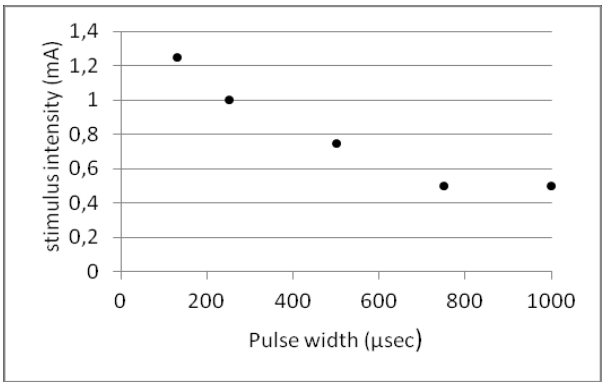


Fig 2 In-output curve of myelinated motor fibres of recurrent laryngeal nerve

8.2.4. Conclusion

Functional stimulation of the laryngeal nerve in the horse with a human VNS device (model 102) is feasible. To obtain a full and sustained abduction of the arytenoid in the normal horse a minimal stimulation frequency of 25 Hz is necessary.

Based on rheobase and chronaxie, respectively, an intensity of 1mA and pulse width of 250 µsec is postulated as parameters that can be used as guideline for RLN stimulation in horses.

Reference List

1. Dixon PM, Hahn CN, Barakzai SZ. Recurrent laryngeal neuropathy (RLN) research: where are we and to where are we heading? Equine Vet J 2009;41:324-7.

2. Hahn CN, Matiasek K, Dixon PM, Molony V, Rodenacker K, Mayhew IG. Histological and ultrastructural evidence that recurrent laryngeal neuropathy is a bilateral mononeuropathy limited to recurrent laryngeal nerves. Equine Vet J 2008;40:666-72.

3. Vanschandevijl K, Nollet H, Vonck K et al. Functional electrical stimulation of the left recurrent laryngeal nerve using an implanted vagus nerve stimulator in a normal horse. Vet. J.2010;578:290-6

CHAPTER 9

CHAPTER 9: CONCLUSION, DISCUSSION AND FUTURE PERSPECTIVES

9.1. Conclusion

From the performed studies the following conclusions can be made:

Animal experimental work

1. Implantation of a combined stimulation and recording spiral cuff electrode around the left cervical vagus nerve of rat is feasible.

2. Stimulation of the vagus nerve with parameters derived from clinical practice leads to activation of efferent A motor fibres of the laryngeal nerve which is a branch of the vagus nerve. Consequently a far field potential of contraction of the striated muscle (LCAMP) can be recorded with a single electrode around the vagus nerve.

3. When the vagus nerve is stimulated with commonly used clinical stimulation parameters with the newly developed electrode, no vagal nerve CAPs could be recorded. The recorded signals were merely the result of larynx muscle contraction.

4. LCAMP was successfully recorded chronically during follow-up of at least two months.

5. Latency and overall characteristics of the doses response curves of the LCMAP remained stable during the entire follow-up period.

6. The impedance of stimulation electrodes gradually increases over time.

7. LCMAP could be recorded immediately after surgery in 11/21 rats, while in the other 10/21 rats, a recovery period with an average of 25 days was required.

8. When the vagus nerve is stimulated with a balanced charged rectangular pulse of 0.2 msec duration and 2 mA intensity and depth electrodes of 125 µm stainless steel wires are implanted in different important anatomical brain projection sites of the vagus nerve (NTS, LC, thalamus and sensorimotor cortex), only a far field potential of the LCAMP could be recorded. In contrast, no vagal evoked potentials were measured.

9. Recording on a smaller level such as multi- or single unit may be useful in characterizing upstream VNS induced activity in the brain.

Human clinical work

1. Demipulse generators appeared to have similar tolerability compared to older generators, and no new side effects could be described. The main technical advances were the decrease in size and improved options for battery life follow-up.

2. The ADNS-300 is a new vagus nerve stimulator (Neurotech) which provides stimulation and recording of CAPs of the left vagus nerve and is transcutaneously rechargeable. Mean recharging time varied between 2 to 3 hours every week.

3. Three patients were implanted with this new device. Two of the three patients reported seizure reduction of 40% after 6 months of treatment, while the third patient became seizure free after seventh month of treatment in combination with AEDs adjustments. In this pilot study no new side effects were reported.

4. CAPs of the vagus nerve were recorded two to three weeks postoperatively in two of the three patients and consisted of an $N_1P_1N_2$ potential with the N_1 appearing 0.4 msec after the end of the stimulation artifact. Response threshold was low (100-150-µA and 250µsec), corresponding to activation of myelinated fibres.

5. CAPs could be recorded during ten months in patient 1, while in patient 2 serial doses response curves were performed during a follow-up period of 12 months.

6. The doses response curves revealed two groups of fibres, most likely corresponding to large and intermediate myelinated fibres. Full activation was obtained in the first group around 30mC (50 µA x 600 µsec), while for the second group the charge required increased to 200 mC (250 µsec X 800 µA).This kind of electrophysiological information may in the future guide clinicians in the choice of individual stimulation parameters.

7. Medical Implant Communication Service (MICS) communication between the implant and the main computer system could be improved, which would allow to record more CAPs per session. Online filtering of the recorded CAPs will permit clinicians to interpret the recorded signals directly at each visit. Optimisation of mechanical manufacture of the electrode will improve the quality of contact between lead and recording contacts.

9.2 Discussion

9.2.1 Introduction

Vagus nerve stimulation is a widespread adjunctive treatment for patients with refractory epilepsy. Although VNS was introduced many years ago, until now epileptologists do not fully understand its mechanism of action. The choice of parameters of stimulation is still based upon a number of experimental animal VNS studies in which VNS proved to induce a seizure reduction (1-3). The same parameters were applied in early clinical randomized controlled trials, such as the EO3 and EO5 studies in the nineties (4, 5), which ultimately lead to the introduction of VNS in the therapeutic arsenal for the treatment of refractory epilepsy. Surprisingly, throughout the early development and even after its widespread acceptance as an additional treatment for epilepsy, the stimulation parameters of VNS never have been derived from individual electrophysiological data obtained from the vagus nerve itself.

Optimizing and personalizing stimulation parameters according to the patient, is still a subject of research. In clinical practice, VNS is considered by some to be a last resort treatment, which is applied arbitrarily with little idea of how the vagus nerve reacts upon stimulation. Several questions in the domain of VNS remain unanswered. One of the main issues relates to why the responder rate varies between < 30% seizure reduction and > 50% seizure reduction, or even seizure freedom. There is little or no information about neither eventual predictive factors, nor any practical guidelines on how stimulation should be carried out most efficiently. This thesis focuses on objectifying stimulation of the vagus nerve by identifying an electrophysiological parameter that could guide VNS therapy for refractory epilepsy. Animal experimental work was carried out to characterize a parameter that reflects adequate VNS, which could be integrated in future VNS animal trials. Complementary to animal experimental work, human studies were performed to characterize an electrophysiological parameter of adequate vagus nerve activation.

9.2.2 Experiments

9.2.2.1. Animal experimental work

Determination of the activation of the vagus nerve after VNS can be arbitrarily divided into two parts. Firstly, how can the vagus nerve be activated adequately and secondly, how does stimulation and activation of the vagus nerve influence different projection sites in the brain. Activation of the nerve itself is necessary to produce any other beneficial brain effects.

In this context, our experiments had as a main goal to clarify activation of the vagus nerve by means of recording compound action potentials (CAPs) (2,6). CAPs are the result of many superimposed action potentials arising simultaneously in a large number of individual axons in the nerve and can be elicited by stimulating the vagus nerve with extracellular stimulating electrodes. It reflects the activation of different axons with different diameters and nerve conduction velocities (NCV's), which leads to a graded response whose magnitude increases with intensity of stimulation (7). Therefore, recordings of CAPs of the vagus nerve can help improve the understanding of which fiber types are activated at which amount of charge and subsequently more appropriately guide stimulation parameters in experimental settings. Ideally, the NCV of different CAP components can be measured by means of calculation of latency differences of different volleys at two different recording contacts at a distance from each other, which requires an electrode of sufficient length. In practice, such a stimulation and recording electrode is difficult to implant, as a small part of the vagus nerve can be dissected from the aortic sheet due to the risk of surgical damage to the nerve. This is the case not only in experimental rats, but also in humans. Researchers have often based NVC calculations on the basis of latency difference between cathode and only a single recording contact (2,6,8-10). In reality this represents an underestimation, as all fibre types need a certain time to be activated underneath the cathode and the first deflection of CAP that passes the recording contact is inevitably delayed. Attempts were made to implant electrodes with multiple recording contacts in rats, but postoperative success rate was low and consequently experiments were further continued with a single recording contact.

In previously published preclinical data, only one group has reported the use of hook and cuff electrodes for combined stimulation and recording of the vagus nerve in an epilepsy model (6). Woodburry and Woodburry performed several experiments in the PTZ and MES model in 1990 and 1991, in which they reported recording recorded several intra-and postoperative CAPs of the different vagus nerve fibre types (A,B,C)(2,6). CAPs of A- and B- fibres appeared 0.5 to 1 msec after stimulation artifact and were activated at threshold intensity between 30 and 70µA with a pulse width of 200µsec, while C-fibre activation occurred between 5 and 8 msec and required higher stimulation intensities, namely 500µA with pulse width of 250µsec. Calculation of NCV was based on the latency of the maximal CAP peak and the distance between cathode and first recording electrode, even though a second recording contact was available.

Presumably no difference in latency of the signal between both contacts was present, although authors did not elaborate on that question. NCV of A- and B-fibres was estimated to lie between 1.5 and 4 m/s, which is a large underestimation for described conduction velocities of mylelinated nerve

fibres in the literature (11). In addition, C-fibres were estimated to have a nerve conduction velocity of 0.5 m/sec, but the amplitude of the response was large namely 2 mV, in contrast to A and B components which were maximally 0.5 mV. No lesion experiments of the vagus nerve or its recurrent laryngeal branch were carried out. Moreover, the authors did not use any muscle paralysing agents to exclude that the large amplitude recorded signal appearing at 5 msec was not the consequence of a muscle artifact. Comparison with our results still remains difficult, as the authors used rather different stimulation and recording techniques. Woodburry and Woodburry performed their acute experiments with five stainless steel wire-hook electrodes (two cathodes and one middle anode combined with two recording electrodes) placed immediately above the nerve. The dissection pouch was filled with mineral oil until the nerve was covered to prevent drying out of the nerve and improve recording of small voltage differences. In contrast, our electrode was a silicone spiral cuff with a single anode and cathode and only one recording contact. The dissection pouch was moistened with a limited quantity of physiological water, which induces shunting of applied current and consequently renders recording more difficult. Later on, Woodburry's implanted a cuff electrode, allowing post operative recordings in unanesthetized animals (6). CAPs were measurable in four rats with a maximal recording time of 21 days for C fibres in one rat, while. A- and B-fibres tended to lose excitability after just a few days. They believed that this was caused by a lack of adequate blood supply as survival time of recorded signals increased when surgical procedure was performed more carefully. Autopsy findings of the nerve varied from a very swollen aspect to a more normal histological structure with identification of more (remaining) blood vessels supplying the nerve. However, the authors did not report any impedance measurements of stimulation contacts neither did they describe any histological details about the presence of gliosis. Hence it is not fully excluded that vagus nerve was not adequately stimulated due to growth of fibrous tissue between the stimulation contacts and the vagus nerve. In humans, revision of the stimulation electrode is sometimes indicated as the impedance may exceed a certain limit due to gliotic scaring (12,13). In those cases, the generator- although considered to be a constant current source may not deliver appropriate current as determined earlier by the neurologist.

Interestingly, in the studies of the Woodburrys higher charge values were needed to activate different fibres with the implanted cuff electrodes in anaesthetized animals compared to hook electrodes used in anesthetized rats, which pleads for the development of a different post-operative electrode-nerve interface. Besides the problem of increasing distance between stimulation contacts and vagus nerve due to formation of gliotic cells, an inflammatory reaction with release of toxic cytokines, may have further damaged the nerve and be at the basis of loss of its excitability. Indeed, it was shown that implantation of cuff electrodes around sciatic nerves of rats was followed by resumption of postoperative oedema which was replaced by a local inflammatory reaction with

release of toxic cytokines, such as TNFα (14, 15). TNFα is known to have inflammatory, demyelinating and degenerating properties (16-18) and moreover, was demonstrated to be up regulated during 1 month after cuff electrode implantation (15).This matches approximately the time span in which Woodburry and Woodburry were able to record VNS induced electrophysiological activity. In contrast, Woodburry and Woodburry postulated that recordings were restricted in time because of a low resistance shunt of fluid between nerve and cuff, which is contradictory to the idea that post-operative oedema is largely resolved after few weeks of implantation (14). In addition, strength-duration curves in freely moving rats were variable, as in the study performed in 1990 a pulse with a charge of 1500 nC was required to activate C fibres compared to a charge of 250 nC reported in 1991 (2,6), while no differences in technique were described. Whether or not the smallest diameter C fibres of the vagus nerve are activated and how this contributes to therapeutic effect, remains unclear.

First of all, the required current intensity to activate C fibre types with a diameter of about 1 µm is at least 100 times higher than the current intensity needed to activate fibre type with greater diameter such as for example 10 µm A-fibres, as intensity is inversely proportional to the squared diameter of nerve diameter (19). In practice, in the study of Woodburry and Woodburry values of at least 3 mA with long pulse widths of 500 µsec were necessary to recruit the smallest fibres, which was only the case in one of both studies (2). This amount of delivered charge is huge compared to size of the electrode-nerve interface in rats and is prone to activate other surrounding tissues, such as muscles of the neck region. In our hands, we were not able to record any C-fibre components with our designed electrode and choice of parameters.

Subsequently the Woodburry's implanted a cuff electrode and applied parameters that activated C-fibres in two kinds of epilepsy models, namely PTZ and maximum electroshock model (MES). VNS abolished the extensor component of the tonic phase in the MES model and shortened or prevented tonic seizures induced by PTZ. The anticonvulsant effectiveness of VNS was directly related to the fraction of vagal C fibres stimulated. The question whether activation of C fibres is necessary to obtain anti- seizure effects remains debatable, as Krahl has shown a few years later that selective destruction of C fibres with capsaicin did not alter the beneficial effects of VNS in the PTZ model (20). Moreover, reports in which patients treated with VNS and acutely stimulated with additional high charged pulses (750 µsec, 0.2-2.75 mA), which according to the authors should have activated C fibres, did not have any cardio respiratory effects and authors interpreted this as C fibres not being stimulated (21, 22).

In domains other than epilepsy, mainly in cardiovascular and to a lesser extent in gastro-intestinal applications, results of vagus nerve CAP are divergent. Results depend on the stimulation electrodes, distance between stimulation and recording sites, the species studied, or even how the vagus nerve was approached. Some studies provide information about a transected vagus nerve at the level of the nodose ganglion (23), in other studies an isolated heart with vagus nerve preparation was used (24). The situation is even more different when the nerve was isolated in situ and recordings were performed ex vivo (25, 26). Parameters such as the medium in which the nerve lies or temperature of experimental set-up, influence results in a meaningful way. Therefore, not all studies provide a clear explanation about how the nerve was stimulated, whether a constant voltage or current source was used, or what the exact stimulus charge was.

Our experiments had as a main goal to design a combined stimulation and recording electrode for VNS in experimental rats, to test its feasibility of implantation and to record VNS induced CAPs with parameters that are classically used in human epilepsy practice. Although we must keep in mind that extrapolating stimulation parameters from a human clinical situation to the experimental rat vagus nerve, most likely means stimulating the nerve with far greater charges than are really needed. In acute experiments, the vagus nerve was stimulated with a bipolar balanced shaped pulse. Threshold intensity was 65 µA for a pulse of 100 µsec duration, which yielded a very clear and easy-to-record signal at 3 msec away from the start of the stimulation artifact. This recorded signal had a very low NCV (as recording contact is located 2 mm away from the cathode), which is in accordance to the results of Woodburry and Woodbury and was far too slow to represent an activation of myelinated vagus nerve fibres.

At this point, we believed that recorded signals were not CAPs of the vagus nerve. Nevertheless, lesion experiments of the vagus nerve abolished the recorded signals indicating that results were VNS-dependent, which excluded the possibility of different kinds of artifacts such as contracting muscles in the neck, heart beating-or breath oscillations. Ruling out these artifacts was important, as Hammond et al performed experiments in humans in which they searched for a VNS-induced scalp EP and found out that their results were merely far field potentials of neck muscle activation (27).

As recorded VNS-dependent signals most likely did not represent CAPs of the vagus nerve, several hypotheses were suggested: (i) responses were far field potentials from VNS induced parasympatic contraction of smooth muscle of internal organs ;(ii) responses were a reflection of different possible upstream reflexes to the brainstem, such as blood pressure information from aortic baro-receptors which bring information to the NTS via the vagus nerve, and consequently stimulate vagal nuclei and activation of the parasympathetic nervous system. Another possible reflex is mediated by pulmonary stretch receptors present in the smooth muscle of the airways which respond to excessive stretching

of the lung during large inspirations and sent out information via the myelinated fibres of the vagus nerve to the medulla and pons to allow expiration; (iii) responses were CAPs of the recurrent laryngeal nerve; (iiii) responses were compound muscle potentials of the larynx striated muscle group innervated by the recurrent laryngeal nerve.

Lesioning of the vagus distal to the stimulation electrode but proximal to the aortic arch, abolished the recorded signal, while an afferent section of the vagus nerve cranially to the stimulation electrode, did not have such an effect. This suggested that VNS resulted in co-activation of the recurrent laryngeal nerve and consequently only two possibilities remained. Recorded signals were either CAPs of the nervus larengeus recurrens or muscle compound action potentials of the larynx musle (LCMAP). EMG recording of the larynx during VNS and abolishment of signals after injecting of vecuronium in the larynx, allowed us to show that the nature or our recordings was in fact LCMAP.

There are several possible reasons why LCAMPs were recorded in the absence of CAPs of the vagus nerve.

First of all, we initially used stimulation parameters that are classically used in clinical epilepsy practice, as the first idea was to objectify stimulation and characterize an electrophysiological parameter that could be applicable in experimental in VNS experiments.

Up till today, animal VNS experiments were performed with extrapolated stimulation paradigms, without taking into consideration of the fact that the electrode-nerve interface of rat is much smaller than that of a human. For example in our laboratory, the anti-seizure effect of VNS was demonstrated in the motor cortex model using high stimulation parameters (0.75 mA, 250µsec, 30 hz, 30 sec on/1.8 min)(28). From our point of view, no literature is reliable about which parameters are sufficient to allow real CAP recording of the vagus nerve and consequently no objective basis is available to adapt stimulation parameters to the needs of the vagus nerve in rats. Currently, new experiments being performed at our laboratory indicate that stimulation pulses must be reduced to 5 µsec to be able to appreciate CAP of the rat vagus nerve. Only when the specific parameters that activate the different fibres of the rat vagus nerve will be fully characterized, derivation of specific fibre stimulation in VNS experiments will be possible.

A second issue is the lack of space in the cervical region of rats, which prevents implantation of longer cuff electrodes in which recording contacts are sufficiently distant and adequately separated from stimulation contacts. Better configuration of electrodes and implantation in larger animals such as for example in rabbits, would limit the pick-up of huge stimulation artifacts by recording contacts and would permit observation of latency differences between recording contacts, thus allowing a more precise calculation of the NCV.

Our experiments which had as a goal objectifying VNS, yielded recordings of far field potentials of LCAMP. The designed electrode permitted to stimulate and record muscle activity without implanting an additional electrode on the larynx, which directly facilitates surgical manipulation and limits the amount of foreign material implanted. This is of particular interest, as several studies in humans in which the effect of VNS surgery on the larynx was investigated with video-laryngoscopy and laryngeal electromyography have demonstrated that 50 to 60% of implanted patients suffered from vocal fold paresis postoperatively due to manipulation of the vagus nerve. The paresis spontaneously recovers between 2 to 6 months after implantation (28, 29, 30). Implantation of a single electrode which combines both stimulation and recording of activation of Aα-fibres of the vagus nerve, therefore offers the opportunity to check stimulation of the recurrent laryngeal nerve in experimental VNS studies. It must be emphasized that the LCMAP merely represents **efferent** activation of the vagus nerve and does not provide any information of electrophysiological properties of vagal **afferent** fibres that provide anti seizure effects.

Consequently, the LCMAP can only be considered as a surrogate parameter for vagal nerve activation. On the other hand, largest myelinated Aα fibres are a type of fibres which are normally activated with the least amount of current compared to B- or C-fibres and consequently, if LCAMP is not recordable despite correct impedance values, the functionality of the vagus nerve must be questioned.

Interestingly, intra-operative VNS-induced vocal cord EMG performed in humans by Ardesh et al showed a very similar induced LCMAP (34). Human LCAMP had an identical shape, but characteristics such as latency and amplitude were longer and higher respectively, corresponding to the larger size of the vagus nerve in comparison with rats. Activation of the larynx in humans was obtained by applying a VNS pulse of 500 µA and 130 µsec, which is more than 10 times higher than the parameters used in our experiments.

Next, characterization of electrophysiological properties of Aα fibres and LCAMP in a chronically implanted vagus nerve, were analyzed over a follow-period of two months. Latency, intensity needed to activate a half maximal LCAMP amplitude ($I_{50\%}$) and slope factor of doses response curves remained stable over time, indicating that myelinated efferent fibres were adequately activated even though impedances of stimulation and of recording contacts gradually increased over time. An increase in impedance can be explained by natural the forming of gliosis between electrode contacts and nerve, which can have different consequences: on the one hand, in-growth of gliotic cells increases the distance between contacts and nerve, which in turn leads to higher required current values to activate the nerve. On the other hand, gliotic cells act as conductive tissue enhancing transmission of current. These two mechanisms probably compensate for each other to a certain

extent, “cancelling each other out”, and consequently $I_{50\%}$ remains rather stable over time. Post-mortem histological quantification of gliotic formation of the electrode nerve interface was not performed as we observed that dissection of the electrode and vagus nerve after death was a rather difficult procedure. The electrode was completely encapsulated with a fibrous cap and the nerve was barely identifiable.

An important observation was that half of implanted rats showed an average recovery period of 25 days before LCMAP could be recorded. If we consider that Aα fibres are the fibres that are recruited most easily, it is probable that other afferent fibres might have been damaged too.

Noteworthy is the fact that in clinical practice, beneficial effects of VNS are mostly assessed after 6 months to 1 year of therapy and no rational explanation for this fact has been given until today. In addition, laryngeal dysfunction has been reported after the implantation of vagus nerve stimulator and endoscopic evaluation documented return of vocal fold mobility 4 months after implantation (29, 31), supporting the hypothesis of a recovery period with functional loss after surgery. Our results further support the idea that the vagus nerve may require a recovery period in order to regain full functionality. Nevertheless, epilepsy patients start their VNS up-titration two to three weeks after surgery, but to date no investigation is available to check whether the nerve has recuperated sufficiently to initiate stimulation.

A next point of discussion, considers the efferent activation of the vagus nerve, despite the fact that the stimulation electrode around the left vagus nerve was oriented with the anode distally and the cathode cranially. Generally, this set-up is considered to preferentially activate afferent fibres, although in our experiments the principle of anodal block did not prevent activation of efferent motor fibres of the recurrent laryngeal nerve. In clinical practice, there exists a preconceived notion that stimulation must be directed towards the brain to avoid any side effects on heart rhythm. Several case-reports have described intra-operative bradycardia and ventricular asystole during testing of VNS device, but none of those patients experienced any cardiac side effects during their follow up with VNS (32, 33, 34). One of the possible hypotheses discussed in the literature regarding the underlying cause of cardiac side effects, is a reversal of electrodes counterbalancing the effect of anodal blocking and consequently activating more the vagal cardiac efferents. According to our animal experiments with implantation and stimulation of the vagus nerve with a cuff electrode, anodal blocking did not occur prominently, as VNS could systematically activate efferent Aα fibres and lead to recordable LCMAP in all our rats. Nevertheless, no conclusions can be made concerning activation of vagal cardiac efferents, as no continuous cardiac monitoring was effectuated. Interestingly, a recent study describing excitation properties of the right cervical nerve in dogs

demonstrated that electrode configuration did not affect the threshold or recruitment of the vagal nerve fibres (35).

Other more plausible explanations for the cause of cardiac side effects are possible variances among patients in anatomy of cardiac branches, which could consequently be more prone to be co-activated in certain patients. Different central vagal processing in the NTS of higher autonomic functions with an exaggerated effect on the AV node was also postulated as possible cause. Remarkably, negative effects on heart and lungs after VNS were mostly described in anesthetized patients and not in waking patients that already benefited from their treatment (32, 33, 34). In addition, it is also known that frequency of SUDEP in patients treated with VNS is not increased (36). In contrast, VNS is actually a new subject of research in the field of treatment for heart failure, as VNS has proved to improve left ventricular function in experiments with dogs, due to reduced heart rate, attenuation of sympathic overdrive and down regulation of the renin-angiotensin-aldosteron system (37).

9.2.2.2 Human clinical VNS studies

Our first study was a retrospective study in which we evaluated the efficacy, safety and practical improvements of the latest commercially available VNS device, the VNS Therapy Demipulse 103 Model (Cyberonics®, Houston, USA), for the treatment of patients with refractory epilepsy (38). Twenty patients were included with a mean follow-up of one year and an overall mean seizure reduction of 39% was reported. Besides equivalent efficacy compared to previous reported clinical VNS studies, Demipulse generators appeared to have similar tolerability as older generators, as no new side effects were described. The main technical advances were the decrease in size and improved options for battery life follow-up.

In next part of this thesis work, the ADNS-300, a new VNS device from Neurotech, was evaluated in a pilot trial (39). This new system provides stimulation and recording of compound action potentials of the human vagus nerve. In addition, the generator is transcutaneously rechargeable, extending battery life to an estimated 12 years.

The first three patients went through a pre-surgical evaluation for refractory epilepsy, and a discussion at a multidisciplinary epilepsy surgery meeting lead to the conclusion that patients were not good surgery candidates. Instead, patients were offered a treatment with VNS, more specifically with the ADNS-300 system. After 1 year of treatment, all patients experienced a more than 40 % reduction their seizure frequency and were able to reload their battery autonomously.

However, there has been a certain learning curve for patients as well as for the neurologist, in order to fully acquire all the technical skills relating to interrogation and programming of the generator, to recharge the battery adequately and finally to record CAPs of the vagus nerve. Moreover, the level of

recharging indicating that generator was fully reloaded had to be fine-tuned for of each patient to obtain efficient operation. The Medical Implant Communication Service (MICS) device acting as a link between the implant and the main computer system functioned well, although in the first implanted patient, the MICS device needed to be placed less than 1 meter from the patient to allow correct communication. This distance increased to 2-3 meters in the two following two patients, after improving the hardware. All three patients were satisfied with their VNS treatment. Reported side effects were stimulation-related hoarseness, especially during the ramping-up period and discomfort and light pain sensation in the left neck region. These side effects were already described in previously performed studies with Cyberonics devices (40-45).

Comparing both VNS systems, the most important differences are the following: first, the ADNS possesses a transcutaneous recharging system. For patients this opportunity may be an advantage, as replacements of generators still remain a (minor) surgical procedure. On the other hand life expectancy of the ADNS is extended to 12 years, so eventually younger patients will still need generator replacement(s) at some of time in their lives. Moreover, not all patients are able to charge their battery once or twice a week for about two hours. The antenna with the active coupling coil of the recharging system has to remain steady over the generator to permit maximum efficiency recharging some patients with intellectual or cognitive impairment may not be able to perform this procedure themselves.

Secondly, the electrode is not a helical, but a self-sizing spiral cuff structure in which a longitudinally arranged tripolar set of contacts provides the derivation of the compound nerve action potential. The longitudinal tripolar configuration can reduce EMG signal and improves the quality of nerve recordings, as recordings from peripheral nerves with extraneural electrodes are much smaller in magnitude than signals from surrounding muscle activation (46). In addition, cuff electrodes have a shielding effect and minimize current leakage to the surrounding tissues, which could provide more efficient stimulation of the vagus nerve and reduce muscle artifacts.

Thirdly, initial parameters of stimulation were programmed identically as per the Cyberonics models, with the exception of the possibility of an up-titration in steps of 0.2 or 0.25 mA, although added value of this possibility still needs to be examined in large patient groups.

Concerning other parameter settings, the possibility existed to program newer ones than those already used in the clinic, but considering the fact that this study was a first pilot trial, no further adjustments were carried out.

Other minor differences are the volume and shape of the ADNS-300, that has a more rectangular shape and a volume of 7 cc, while the latest Demipulse model 103 and 104 have a volume of 8/10 cc and a more rounded contour (47). In the surgical procedure of our first three implants, generator and

lead of the ADNS-300 were already connected, thus imposing a tunneling of the electrode in the direction of the head. In future implantations, both components will come separated and the technique of implantation will be very similar to the Cyberonics device implantation.

Within the framework of this thesis, the recording of human postoperative compound action potentials was a major advance in VNS research. CAPs were recorded in patient 1 and 2 at 2 to 3 weeks after implantation of the ADNS-300.

In two patients, the vagus nerve was stimulated with a pulse of 500 µA intensity and 250 µsec pulse duration, which resulted in recordings of vagal nerve CAPs ($N_1P_1N_2$), with a first peak appearing at 0.4 msec after the end of the stimulus. As mentioned earlier in this discussion, NVC is ideally based on latency differences between two distant recording contacts, which was not fulfilled by the ADNS-300 electrode configuration, due the evident problem of restricted length. Instead, it has a tripolar organisation in which the middle contact is considered to be the active recording contact referred to the outer two that are connected to each other (39). The first recording contact is situated 5 mm rostrally from the stimulation cathode, so NCV would be at least 12.5 m/sec, which falls in the range of velocities of myelinated (A and/or B) fibres (11). The threshold to obtain responses was quite low (100-150-µA and 250µsec), which is an additional support for the hypothesis of activation of myelinated fibres. The vagus nerve contains a number of functionally different fibres that contribute with different latencies and amplitudes to the CAP, but on the basis of our results, we only were able to conclude that CAP resulted from activation of myelinated fibers in general.

In the literature, only two authors have described recordings of intra-operative CAPs of the vagus nerve (48, 49), while our results date from a late acute stage of 2 to 3 weeks and a chronic situation at month 11 and 12 after implantation. After implantation, electrode-nerve environment evolves from a situation of postoperative oedema to a steady state with an encapsulated fibrous cap covering the electrode and nerve. The whole process most likely takes at least 2 to 3 weeks, although objective information on this topic is not available. Adaptation of the body to a foreign body influences nerve activation and consequently our results are probably not fully comparable to the intra operative CAPs reported by Evans et al and Koo et al.

Evans described three different fibre populations (A, Aδ and C), on the basis of the latency of each waveform from the stimulus artifact onset (48). Mean conduction velocities of 18.8, 9.5 and 2.1 m/sec corresponding respectively to the different fibre types of the vagus nerve were described. The initial component, consistent with the activation of myelinated fibres, occurred after 1 msec, while the recording contact was 2 cm away from the cathode, which is earlier then our first peak (N_1). Possible explanations were already mentioned above, i.e. our experiment settings might have been influenced by induction of an inflammatory reaction and beginning gliosis, which could have slowed

down NCV of myelinated fibres. Nevertheless, amplitudes of recordings by Evans were similar to our maximal amplitudes. Moreover, the threshold for A fibres in their study was 250 µA with a pulse of 130 µsec duration, which is in the same range of charge needed to activate the vagus nerve in our patients. Koo et al described very similar vagus nerve CAP with biphasic shape and NCV of 10.2 m/sec. One major difference between our study and results reported by Koo et al is the fact that a higher stimulus charge was required, namely 1mA and 100µsec, but this can easily be explained by the different type of electrode they used. Interestingly, important latency and threshold changes with age from childhood to adulthood were described. This indicates that an individual adjustment of the stimulus strength based on the observed nerve activation is absolutely necessary.

In addition, we performed a 12-month follow-up of one of the three patients and performed serial dose response curves of vagal nerve CAP. In the two other patients, due to technical problems (possibly caused by lead breakage), CAP recording was not possible in patient 3 right from the start of the study and in patient 2 at month 10. In these two last patients we have checked that therapeutic stimulation was administered correctly, by visualizing the stimulation pulse with use of specific hardware and software.

The patient received classical up titration scheme and stimulation paradigms namely 0.75 mA, 30 Hz, 500µsec, 30 sec on, 10 min off (43, 45). After 6 months, the patient did not experience any beneficial VNS effect and anti-epileptic drug (AED) treatment was adapted to his needs. The patient became seizure free starting from month 7. Therefore, it remains uncertain whether his seizure freedom resulted entirely from AED adjustments or was the consequence of a delayed effect of the VNS or even the combination of AED and VNS combined. Complementary, doses response curves of the vagal nerve CAP were successfully recorded over a time span of 1 year after implantation, indicating that CAPs of the human vagus nerve can be objectively recorded in response to an external electrical stimulus in a chronic way.

The recorded curves showed recruitment of two groups of fibres, most likely corresponding to large and intermediate myelinated fibres. Threshold activation was obtained in the first group at 50 µsec x 500 µA, while the second group necessary charge increased to 250 µsec X 150µA.

One of the drawbacks of our follow-up study was the fact that limited amount of date could be recorded at each visit, as communication between the implant and the main computer system for CAP recording required a substantial amount of battery charge, which limited the number of possible recordings. Subsequently, we have chosen to stimulate the vagus nerve with the shortest programmable pulse duration, i.e. 50 µsec, in addition to larger pulse duration compatible with a satisfactory CAP recording. Stimulation of the vagus nerve with longer pulse durations than 250 µsec

led to larger stimulation artifacts, obscuring early CAP components and saturating the recording system.

A second issue which needs further elaboration is the automatic filtering of data that are transmitted by MICS mode to the Neurotech computer. Indeed, in this pilot trial filtering was done off-line; whilst, in practice, the neurologist should at least be able to upload the chart showing the doses response curve of that day.

9.3. Future perspectives

9.3.1 In practice…

First of all, the important message of this work is the fact that individualized CAP recordings of the implanted vagus nerve may help to guide choice of stimulation parameters. The performed studies only provide information on choice of stimulation intensity or pulse width. The role of other parameters such as stimulation frequency or duty cycle was not examined.

In this context, we propose the following procedure:

Dose-response curves could be performed at each visit after surgery using maximal pulse width of 250µsec; higher pulse widths may interfere with recording of the early components of the CAP. Applying gradually increasing intensities from 0 to 1mA allows plotting of the CAP amplitude in function of the applied charge. The total amount of charge for which maximal amplitude is reached, may serve as an individual maximal output value. Hypothetically, the intensity or duration of pulse width would not be of further importance, as long as the applied parameters correspond to the postulated charge. This value may evolve along consecutive visits, correlating with a gradual nerve recovery and encapsulation of the electrode-nerve interface. If no CAP is recorded in the early stage of follow-up, starting stimulation would be preferably postponed, as stimulation in recovery phase of the nerve may not be suitable. On the other hand, functional stimulation is being applied in different medical areas, as for example FES of laryngeal nerve in horses with recurrent laryngeal nerve neuropathy (RLN), so this assumption may be incorrect.

With respect to the idea that the vagus nerve may need recovery time - especially myelinated fibres seem to be sensitive to surgical manipulation-careful up-ramping is still advised. The smallest intensity steps in our study were programmed at 0.2 or 0.25mA. These values may be used as a guideline. If a pulse duration of 250 µsec is selected, up-titration can proceed in steps of 0.25 mA every month until the optimal result is reached.

One may argue that applying this method to a large number of patients may yield enough biological information to deliver mean values applicable to all patients, but doing so, the value of checking real activation per patient would be lost, which might be a key element in explaining why some patients have better/earlier response than others. In this context, local inflammatory reactions after implantation may differ between individuals, emphasizing the importance of individual parameter choice in function of physiological vagus nerve recordings. This approach will reduce time for up-titration; avoid unnecessarily high outputs en save battery life.

9.3.2. Hypotheses concerning delay of effectiveness of VNS in responders and no effect in non responders: role of the vagal nerve CAP.

Implantation of larger groups of patients with the AND-300 will offer the opportunity to record vagus nerve CAPs on a larger scale than has so far been done in this domain. By measuring electrophysiological properties of the vagus nerve postoperatively and at regular intervals in the follow-up of VNS treatment, it may be possible to analyze whether local recuperation of the vagus nerve is an explanation for the delayed positive response to stimulation, i.e. 6 to 12 months are typically required to judge whether patient responds or not; i.e. whether or not the start of any beneficial VNS effect is linked with the successful recording of a CAP after a silent period and consequently be a partial explanation why non-responders to do not experience any benefit of VNS, if no CAP is recordable at all.

Several studies in vitro studies have implicated immunoregulatory cytokines such as IL-4, IL-13, IL-10, and transforming growth factor (TGF-β)], inflammatory cytokines IL-6 and tumor necrosis factor (TNFα) and chemokines (MCP-1) in the formation of the foreign body response (50-52). Moreover, as mentioned earlier, TNFα, which has inflammatory, demyelinating and degenerating properties, was shown to be upregulated during 1 month after cuff electrode implantation around the sciatic nerves of rats (15). In addition, certain cytokines that play a role in neural microvascular changes, such as Nitric oxide isoform I and II (NOSI and II) and vascular endothelial growth factor (VEGF) were analogously upregulated, especially in animals presenting functional deficit following electrode surgery (14, 15, 53).

Considering these data, we hypothesize that after implantation of a cuff electrode around the human vagus nerve, a local inflammatory response with several mediators takes place, before the formation of the dense fibrous cap around the electrode and nerve occurs. In this phase, the vagus nerve may not function adequately and components of the CAP could show increased latencies, reduced maximum amplitudes or could even not be recordable at all.

Therefore the research on the action mechanisms of VNS, should not only concentrate on the different effects of VNS in its different projection sites in the brain, but also be conducted in the field of local biological mechanisms altering electrode nerve interface after surgery and in the long term. The degree of postoperative oedema and the time needed for complete recovery in combination with the extent of the inflammatory reaction is most likely different for each individual, which might be one of the explanations why the response to VNS is so individually different.

In animal experimental work, no studies have been performed to sort out what the local inflammatory response exactly implies after vagal nerve implantation. Research on which cytokines play a prominent role in local demyelinisation after cuff implantation around the vagus nerve may not only lead to a better understanding of vagal nerve functioning, but could, in the future, also lead to enhanced stimulation electrodes and treatments. For example, in the cardiac domain, polymer-coated stents that are loaded with methylprednisolone have been described and reported to inhibit the severe foreign-body reaction induced by the combination of overstretch injury and the coating of metallic stents (54). In a similar way, localized and controlled delivery of an anti-inflammatory drug to the electrode-vagus nerve interface could counteract the inflammatory reaction and limit the tissue reaction around the nerve, aiming to improve electrode performance.

9.3.3. LCAMP

9.3.3.1 General

LCAMP reflects activation of efferent Aα and could function as a surrogate parameter of adequate vagus nerve stimulation, as we believe that applied stimulus travels in both directions in the nerve, namely efferent but also in afferent way. The main question is whether the dysfunctioning of the thickest myelinated efferent fibres may also imply some damage to the other types of fibres, especially to the afferent myelinated fibres that may provide beneficial anti-seizure effects. To evaluate the usefulness of LCAMP, new experiments in which rats are implanted with the combined stimulation and recording electrode and receiving VNS treatment in a specific epilepsy model must be performed. Experiments investigating whether rats in which LCAMP could be recorded immediately after surgery have a better outcome compared to rats with a delayed LCAMP could provide useful information. The most important requirement for the choice of the epilepsy model is that VNS has already proven to have anti-seizure effects in the particular model independently of LCAMP. Two possible models at our laboratory fulfil this criterion.

Firstly, VNS has proven to have seizure-suppressing effects in the intrahippocampal pilocarpine model for limbic seizures as a result of an increase in hippocampal noradrenalin concentration (55,

56). Secondly, VNS significantly increased motor seizure threshold in a cortical model for motor seizures (57). Stimulation paradigms were slightly different in the two models. Basically De Herdt V. applied 1 hour of VNS (0.75 mA, 30 Hz, 250 µsec; duty cycle = 7 sec on – 18 sec off), while Raedt R. et al continued VNS during thirteen 20 minute collections of CSF which corresponds to a total duration of more than 4 hours of VNS at 1 mA (other parameters settings were identical). In addition to VNS experiments in which outcome of *two postoperative* groups- namely a LCAMP group and a non LCAMP group are analyzed, it would be also interesting to repeat at several different time points the same experiment over a two-month period to investigate whether the time of *delay and recovery* inversely correlates with improved VNS response.

In line with our previous hypothesis regarding the importance of a local inflammatory reaction and dymelinisation of the vagus nerve, experiments in which LCAMP are monitored over time and different groups of rats are sacrified at different time stages for histological investigation of electrode and its embedded nerve could be interesting. Cytokines or vascular factors such as TNFα, TNFβ, NO or VEGF could be investigated with immunohistochemical methods. Hypothetically, rats which have altered Aα function do show a larger inflammatory response compared to rats in which LCAMP is effectively recorded.

9.3.3.2. Possible clinical implication of the LCAMP

In humans, larynx EMG is usually used to guide injections into the thyroarytenoid muscle in patients with adductor spasmodic dysphonia (58). Other medical applications are rather scarce and neurologists do not often use this investigation in their clinical practice. Only one author described intra-operative VNS induced larynx EMG in the context of epilepsy (59), but there are no reports of larynx EMG in patients with refractory epilepsy chronically treated with VNS, in whom a VNS pulse was the trigger for larynx activation. In practice, one possibility could be to activate the generator with the magnet to deliver an extra train of stimuli, while continuously recording EMG of the larynx. At the same time EEG scalp recording could provide an extra check when the VNS pulse was delivered as this causes a recognizable artifact on EEG. Characterization of VNS-induced larynx contraction in large of group of epilepsy patients could provide more information about co-activation of the recurrent laryngeal nerve and its induced muscle contraction during VNS.

Prospective analysis of VNS-induced larynx EMG after implantation and at regular intervals after initiation of therapy, might lead to more insight in the mechanism of recovery of the vagus nerve after surgery. This idea is extrapolated from animal experimental work and a comparison between

rats and human situation remains delicate. Nevertheless, VNS-induced larynx EMG may become an additional tool besides CAP recording to evaluate vagus nerve functionality

9.3.4. VNS induced evoked potentials

In the search of objectifying stimulation of the vagus nerve and complementary to VNS CAPs or VNS induced LCAMP, VNS-evoked potentials could be further explored. In this work, only far field potentials from local larynx contraction were measured in several brain targets. This was partially explained by the fact that VNS induces local fields that are likely to be very small and only measurable with single or multi-unit recordings. As the vagus nerve consists of different types of fibres, VNS-evoked potentials could help us understand whether some fibre types preferentially project to specific sites, such locus coerelus, thalamus or hypothalamus, which would clarify further the mechanism of action of VNS.

9.3.5. Functional stimulation of laryngeal nerve in horses with laryngeal nerve neuropathy

Our collaboration with the veterinary department lead to a successful implantation of the Cyberonics model 102 device for functional stimulation of the laryngeal nerve in one healthy horse. Further research in functional stimulation of laryngeal nerve in horses with pathological laryngeal nerve neuropathy and problems with the vocal cords will clarify whether the explored technique can offer a total or partial recovery of laryngeal function.

9.3.6. General possible future practical perspectives in identifying VNS responders on the basis of data in literature.

Progress was made in the domain of vagal evoked potentials after t-VNS at the inner side of the tragus, which may offer clinicians a non-invasive technique to evaluate the effect of VNS at the level of the brain (61, 62). Until now t-VNS has not yet been recognized as a good alternative for cervical stimulation.

Previous studies in a limited number of patients have shown that VNS influences the N_{13}-N_{20} component of the somatosensory evoked potential (62) or the P_3 amplitude of visual evoked potentials (63).

In the same line of interest and complementary to t-VNS EP's, better evaluation of VNS on these already commonly used EP's in a larger number of patients might be useful.

In addition to electrophysiological investigations, further research with neuro-imaging techniques such as PET and SPECT may clarify whether the role of thalamic activation consistently correlates

with positive seizure outcome, as this anatomical projection site appears to play a key role in different studies (65-67). Hypothetically, transcutaneous stimulation of the vagus nerve would permit to evaluate CBF changes in the thalamus prior to implantation, which might lead to more adequate selection of patients and consequently lead to higher responder rates.

Overall, VNS most likely induces its effect by recruiting various pathways in the brain and the resulting activation patterns probably interact in distinct ways, leading to an individually specific response. Researchers have gradually booked advances in unravelling small parts of this puzzle and a variety of investigations allow us to objectivise certain elements of it. Further research may lead to more adequate VNS, in which prediction of outcome and choice of stimulation paradigms will be derived from objective information in individual patients.

9.4 Further discussion related to VNS in general

9.4.1. Mechanisms of action of VNS in the brain

In addition to the study of electrophysiological characteristics of the vagus nerve, understanding the mechanism of action of VNS lies also in unraveling different effects of VNS in the brain. Different anatomical sites and neurotransmitters systems have been postulated to play a key role in the beneficial effect of VNS in patients with epilepsy.

9.4.1.1. Thalamus-cortex pathway

VNS induces increased synaptic activities in the thalamus and thalamo-cortical projection pathways bilaterally, leading to increased arousal and possibly to decreased synchrony of synaptic activities between end within cortical regions (64,67,68).

On cortical neurotransmitter level, the role of GABA has been pointed out in different studies. The development of post-traumatic seizures that occurs following brain injury may involve a loss of GABAergic cells and inhibitory tone in the brain. The role for the inhibitory neurotransmitter GABA in VNS mediated seizure suppression in humans undergoing VNS therapy is also suggested. At both high and low amplitude VNS, total GABA levels in the cerebrospinal fluid of VNS patients are significantly increased (40). Additionally, single photon emission computed tomography (SPECT) of GABA-A receptor density in the hippocampus following 1-year of VNS therapy showed a significant normalization of GABA-receptor density that correlated with seizure reduction (69). In the fluid percussion injury model VNS prevents the loss of GABA neurons within the cerebral

cortex, and possibly the hippocampal formation, which might facilitate the recovery of behavioral function (70).

9.4.1.2. LC-Hippocampus pathway

The vagus nerve projects directly to the raphe nucleus and indirectly to the LC. These nuclei are the major sources of serotonergic and noradrenergic neurons in the brain respectively (64). Both send direct projections to the hippocampus, a brain structure that is frequently involved in the generation of epileptic seizures in temporal lobe epilepsy (TLE) (71). Bilateral destruction of the LC has been found to reverse the seizure-suppressing effect of VNS in the maximal electroshock model (72). Single-unit recording experiments have shown that the activity of noradrenergic neurons in the LC is increased upon stimulation of the vagus nerve (73, 74). Enhancement of the activity of LC neurons, increases in extracellular noradrenaline concentration in projection areas of the LC such as the hippocampus and cortex in VNS-treated rats (75). In our laboratory, recent experiments have shown that VNS prevents the development of pilocarpine induced limbic seizures only in those rats in which hippocampal noradrenaline increased by at least 70%; and that selective a2-adrenoreceptor antagonism plays a key role in preventing seizures (56). The inter-subject variability of there response to VNS could be based on genetic variability and differences in external and/or internal environment could lead to differences in the number of noradrenergic neurons, their afferent and/or efferent projections and synaptic strengths within these noradrenergic neuronal networks. This intrinsic variation in the noradrenergic neural network could underlie the variable release of noradrenaline in response to VNS.

9.4.2 Cranial nerve stimulation for epilepsy: V, IX

In a first pilot trial in which Trigeminal nerve stimulation (TNS) was applied transcutaneously for patients with refractory epilepsy (76,77), TNS seemed to be well tolerated an 4 of 7 subjects who completed a 3 month follow-up period experienced a ≥50% reduction in seizure frequency. In a longer follow-up study of 1 year, the beneficial effect of TNS sustained over time (78). Importantly, trigeminal nucleus and vagal nuclei have extensive projections to the nucleus of the tractus solitarius and the locus coeruleus (LC), which may be an explanation for positive effects of TNS. Beside the common anatomical projections to NTS and LC, cranial nerve stimulation causes desynchronization of thalamic and cortical activity and in turn reduces seizure activity. This effect may be explained by the fact that stimulation activates midbrain reticular formation resulting in generalized arousal. In this context, seizure reduction effect of VNS can also be

achieved by stimulation of multiple cranial nerves that convey information to the reticular formation (76,77,78). In addition to trigeminal stimulation, stimulator of the Hering nerve (HN; a branch of cranial nerve nine) was shown to control epileptiform activity in a canine model study (79). Interestingly, Tubbs et al have demonstrated that the ability of HNS to reduce seizure activity in the rats is dependent on an intact dopamine system in the basolateral amygdala (80).

9.4.3 Profiling Vagus Nerve Stimulation Responders

9.4.3.1 Epileptogenic zone

A few studies have evaluated whether the lateralization of the epileptogenic zone influences the efficacy of VNS and showed only a non-significant trend towards a slightly higher rate of responders among patients with a right-sided epiletogenic zone(81, 82). Other small series have reported non-significant trends towards greater efficacy in patients suffering from temporal unilateral (83, 84), bitemporal (85,86) or frontal lobe epilepsy (81,87). Thus, at present there is no strong indication that the antiepileptic effect of VNS depends on the side or localization of the underlying epileptogenic zone.

9.4.3.2 Underlying Lesion

Some studies suggest that VNS is more effective in patients whose epilepsy is symptomatic of an underlying brain lesion, most notably malformation of cortical development (82, 88), including tuberous sclerosis (89).However, this issue remains controversial, with other series showing greater efficacy of VNS in patients with non-lesional epilepsies (90) or comparable findings in patients with and without abnormal findings on magnetic resonance imaging (91,92).

9.4.3.3 Function of Age

In three large pediatric trials, the 50% responder rate was found to be equal to or greater than that reported in adults, ranging from 46 to 83% after two years of follow-up (93-95).

9.4.3.4 Seizure types in generalized epilepsies

Several studies suggest that VNS is effective in drug-resistant idiopathic or symptomatic generalized epilepsy (81,82,96-100).The average reduction in seizure frequency appears comparable in these types of epilepsies (around 45% for follow-up ranging from three to 21 months) to that observed in

partial epilepsies (99,100) although a few studies suggest that higher responder rates could be observed in patients with symptomatic generalized epilepsy, specifically (81,82,97). VNS appears to be efficacious against all types of generalized seizures, including myoclonic jerks (96,100),tonic seizures (99), absences and generalized tonic-clonic seizures(81, 96). In Lennox-Gastaut syndrome, the average reduction in seizure frequency was found to be greater for atypical absences and tonic seizures (73 and 55%, respectively) than for partial seizures (23%) (101). Conversely, in infantile spasms VNS does not seem efficacious, with only two responders out of a series of 10 patients (102).

9.4.3.5 Concomitant AEDs

AED and VNS appear to have distinct mechanisms of actions. Sedative effects and impaired cognition are commonly observed with the use of AED's, which increase GABAergic inhibition or reduce repeated rapid interneural action potentials by limiting sodium conductance. These adverse events are not seen with VNS. In contrast, stridor of vocalization often occurs during activation of vagal efferents, but do not appear with AED. One study reported a beneficial effect on behavior (specifically alertness), when topiramate and VNS therapy were associated. This positive effect seemed not to be a consequence of solely seizure reduction, and possible synergistic effects were proposed. Consequently, the AED regimen per patient may induce a different 'brain status', which might influence response to VNS. Up till now, no studies really investigated this subject, although it is possible that according to the AEDs taken, a different VNS response is achieved.

Reference List

1. McLachlan RS. Suppression of interictal spikes and seizures by stimulation of the vagus nerve. Epilepsia 1993;34[5]:918-923.

2. Woodbury DM, Woodbury JW. Effects of vagal stimulation on experimentally induced seizures in rats. Epilepsia 1990;31(Suppl 2): S7-19.

3. Zabara J. Inhibition of experimental seizures in canines by repetitive vagal stimulation. Epilepsia 1992;33[6]:1005-1012.

4. Handforth A, DeGiorgio CM, Schachter SC et al. Vagus nerve stimulation therapy for partial-onset seizures: a randomized active-control trial. Neurology 1998;51[1]: 48-55.

5. A randomized controlled trial of chronic vagus nerve stimulation for treatment of medically intractable seizures. The Vagus Nerve Stimulation Study Group. Neurology 1995;45[2]: 224-230.

6. Woodbury JW, Woodbury DM. Vagal stimulation reduces the severity of maximal electroshock seizures in intact rats: use of a cuff electrode for stimulating and recording. Pacing Clin Electrophysiol 1991;14[1]:94-107.

7. Johnston Daniel, Miao-sin Wu Samuel. Foundations of Cellular Neurophysiology. A Bradford Book, 1995.

8. Bregeon F, Alliez JR, Hery G, Marqueste T, Ravailhe S, Jammes Y. Motor and sensory re-innervation of the lung and heart after re-anastomosis of the cervical vagus nerve in rats. J Physiol 2007;581:1333-40.

9. Tosato M, Yoshida K, Toft E, Nekrasas V, Struijk JJ. Closed-loop control of the heart rate by electrical stimulation of the vagus nerve. Med.Biol.Eng Comput. 2006;44[3]:161-169.

10. S.L.Chen, .Y Wu, Z.J.Cao et al. Subdiaphragmatic vagal afferent nerves modulate visceral pain. Am.J.Physiol Gastrointest.Liver Physiol 2008;294:1141-1449.

11. J Erlanger, H.S.Gasser. The action potential in fibres of slow conduction in spinal roots and somatic nerves. Am J Physiol 1929;92:43-82.

12. Ng WH, Donner E, Go C, Abou-Hamden A, Rutka JT. Revision of vagal nerve stimulation (VNS) electrodes: review and report on use of ultra-sharp monopolar tip. Childs Nerv Syst 2010;26:1081-1084.

13. Rychlicki F, Zamponi N, Cesaroni E et al. Complications of vagal nerve stimulation for epilepsy in children. Neurosurg Rev 2006;29:103-7.

14. Thil MA, Tran Duy D, Colin IM, Delbeke J. Time course of tissue remodelling and electrophysiology in the rat sciatic nerve after spiral cuff electrode implantation. J Neuroimmunol. 2006;185:103-114.

15. Vince V, Thil MA, Gerard AC, Veraart C, Delbeke J, Colin IM. Cuff electrode implantation around the sciatic nerve is associated with an upregulation of TNF-alpha and TGF-beta 1. J Neuroimmunol. 2005;159[1-2]:75-86.

16. Said G, Hontebeyrie-Joskowicz M. Nerve lesions induced by macrophage activation. Res Immunol 1992;143:589-99.

17. Uncini A, Di MA, Di GG et al. Effect of rhTNF-alpha injection into rat sciatic nerve. J Neuroimmunol 1999;94:88-94.

18. Hall SM, Redford EJ, Smith KJ. Tumour necrosis factor-alpha has few morphological effects within the dorsal columns of the spinal cord, in contrast to its effects in the peripheral nervous system. J Neuroimmunol 2000;106:130-136.

19. W.F.Brown, C.F.Bolton, M.J.Aminoff. Function and disease. Basic, clinical and electrodiagnostic aspects. Philadelphia: Saunders 2002.

20. Krahl SE, Senanayake SS, Handforth A. Destruction of peripheral C-fibres does not alter subsequent vagus nerve stimulation-induced seizure suppression in rats. Epilepsia 2001;42[5]:586-589.

21. Binks AP, Paydarfar D, Schachter SC, Guz A, Banzett RB. High strength stimulation of the vagus nerve in awake humans: a lack of cardiorespiratory effects. Respiration Physiology 2001;127: 125-33.

22. Banzett RB, Guz A, Paydarfar D, Shea SA, Schachter SC, Lansing RW. Cardiorespiratory variables and sensation during stimulation of the left vagus in patients with epilepsy. Epilepsy Res 1999;35[1]:1-11.

23. Ford TW, McWilliam PN. The effects of electrical stimulation of myelinated and non-myelinated vagal fibres on heart rate in the rabbit. J Physiol 1986;380:341-347.

24. De Neef KJ, Jansen JR, Versprille A. Attenuation of the negative chronotropic effect of myelinated fibres by nonmyelinated fibres in the vagus nerve of the rabbit. Circ Res 1983;52:508-14.

25. Benzon HT, Strichartz GR, Gissen AJ, Shanks CA, Covino BG, Datta S. Developmental neurophysiology of mammalian peripheral nerves and age-related differential sensitivity to local anaesthetic. Br J Anaesth 1988;61:754-60.

26. Schneider M, Datta S, Strichartz G. A preferential inhibition of impulses in C-fibres of the rabbit vagus nerve by veratridine, an activator of sodium channels. Anesthesiology 1991;74:270-80.

27. Hammond EJ, Uthman BM, Reid SA, Wilder BJ. Electrophysiologic studies of cervical vagus nerve stimulation in humans: ii. Evoked potentials. Epilepsia 1992;33[6]:1021-1028.

28. De Herdt, V, De Waele J, Raedt R et al. Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures. Acta Neurol Scand 2010;121:271-6.

29. Zalvan C, Sulica L, Wolf S, Cohen J, Gonalez-Yanes O, Blitzer A. Laryngopharyngeal dysfunction from the implant vagal nerve stimulator. Laryngoscope 2003;113:221-5.

30. Shaffer MJ, Jackson CE, Szabo CA, Simpson CB. Vagal nerve stimulation: clinical and electrophysiological effects on vocal fold function. Ann.Otol.Rhinol.Laryngol 2005;114:7-14.

31. Shaw GY, Sechtem P, Searl J, Dowdy ES. Predictors of laryngeal complications in patients implanted with the Cyberonics vagal nerve stimulator. Ann.Otol.Rhinol.Laryngol. 2006;115[4]: 260-267.

32. Asconape JJ, Moore DD, Zipes DP, Hartman LM, Duffell WH, Jr. Bradycardia and asystole with the use of vagus nerve stimulation for the treatment of epilepsy: a rare complication of intraoperative device testing. Epilepsia 1999; 40[10]:1452-1454.

33. Tatum WO, Moore DB, Stecker MM et al. Ventricular asystole during vagus nerve stimulation for epilepsy in humans. Neurology 1999;52[6]:1267-1269.

34. Ardesch JJ, Buschman HP, van der Burgh PH, Wagener-Schimmel LJ, van der Aa HE, Hageman G. Cardiac responses of vagus nerve stimulation: intraoperative bradycardia and subsequent chronic stimulation. Clin Neurol Neurosurg 2007;109[10]:849-852.

35. Mark A. Castoro , Paul B. Yoo , Juan G. Hincapie , Jason J. Hamann , Stephen B. Ruble ,Patrick D. Wolf , Warren M. Grill . Excitation properties of the right cervical vagus nerve in adult dogs Experimental Neurology 2011,227 62–68

36. Annegers JF, Coan SP, Hauser WA, Leestma J. Epilepsy, vagal nerve stimulation by the NCP I system, all-cause mortality, and sudden, unexpected, unexplained death. Epilepsia 1999;40:165.

37. Hanni N. S abbah, Itamar Ilsar, Asaph Zaretsky et al. Vgaus nerve stimulation in experimental heart failure. Heart Fail Rev 2011;16:171-8

38. El Tahry R, De Herdt V, Raedt R et al. Evolution in VNS therapy for refractory epilepsy, experience with Demipulse devices at Ghent University Hospital. Seizure 2010;19(9):531-5.

39. El Tahry R, Raedt R, Mollet L et al. A novel implantable vagus nerve stimutaion system (ADNS-300) forn combined stimulation and recording of the vagus nerve: pilot trial at Ghent University Hospital. Epilepsy Res. 2010 Dec; (2-3):231-9

40. Ben Menachem E, Manon-Espaillat R, Ristanovic R et al. Vagus nerve stimulation for treatment of partial seizures: 1. A controlled study of effect on seizures. First International Vagus Nerve Stimulation Study Group. Epilepsia 1994;35[3]:616-626.

41. DeGiorgio CM, Schachter SC, Handforth A et al. Prospective long-term study of vagus nerve stimulation for the treatment of refractory seizures. Epilepsia 2000;41[9]:1195-1200.

42. Handforth A, Ondo WG, Tatter S et al. Vagus nerve stimulation for essential tremor: a pilot efficacy and safety trial. Neurology 2003;61[10]:1401-1405.

43. De Herdt V, Boon P, Ceulemans B et al. Vagus nerve stimulation for refractory epilepsy: a Belgian multicenter study. Eur J Paediatr.Neurol 2007;11[5]:261-269.

44. Vonck K, Boon P, D'Have M, Vandekerckhove T, O'Connor S, De Reuck J. Long-term results of vagus nerve stimulation in refractory epilepsy. Seizure 1999;8[6]:328-334.

45. Vonck K, Thadani V, Gilbert K et al. Vagus nerve stimulation for refractory epilepsy: a transatlantic experience. Journal of Clinical Neurophysiology 2004;21[4]:283-289.

46. Dustin Jtyler, Kathararine H.Polasek. Electrodes for the Neural Interface. In: Elliot S.Krames, P.Hunter Peckham, Ali R.Rezai, eds. Neuromodulation. Academic Press, 2009:181-215.

47. Cyberonics I. Techical Guide for VNS. 2010. Cyberonics Europe, S.A./N.V.

48. Evans MS, Verma-Ahuja S, Naritoku DK, Espinosa JA. Intraoperative human vagus nerve compound action potentials. Acta Neurologica Scandinavica 2004;110[4]:232-238.

49. Koo B, Ham SD, Sood S, Tarver B. Human vagus nerve electrophysiology: a guide to vagus nerve stimulation parameters. Journal of Clinical Neurophysiology 2001;18[5], 429-433.

50. Brodbeck WG, Nakayama Y, Matsuda T, Colton E, Ziats NP, Anderson JM. Biomaterial surface chemistry dictates adherent monocyte/macrophage cytokine expression in vitro. Cytokine 2002;18:311-319.

51. Jones JA, Chang DT, Meyerson H et al. Proteomic analysis and quantification of cytokines and chemokines from biomaterial surface-adherent macrophages and foreign body giant cells. J Biomed Mater Res A 2007;83:585-596.

52. Rodriguez A, Meyerson H, Anderson JM. Quantitative in vivo cytokine analysis at synthetic biomaterial implant sites. J Biomed Mater Res A 2009;89:152-159.

53. Thil MA, Vince V, Veraart C, Delbeke J, Colin IM. Nitric oxide synthases II and III and vascular endothelial growth factor are up-regulated in sciatic nerve after spiral cuff electrode implantation29143. J Neuroimmunol. 2005;166[1-2]:158-166.

54. De Scheeerder I, Wang K, Wilczek K et al. Local methylprednisolone inhibition of foreign body response to coated intracoronary stents. Coron Artery Dis 1996;7:161-6.

55. Meurs A, Mollet L, Raedt R et al. Increased hippocampal extracellular noradrenalin concentration contributes to the anticonvuslant effect of VNS in the focal pilocarpine rat model for limbic seizures. Epilepsia 2009;50[suppl 11]:355.

56. Raedt R, Clinckers R, Mollet L, Vonck K, El Tahry R, Wyckhuys T, De Herdt V, Carrette E, Wadman W, Michotte Y, Smolders I, Boon P, Meurs A. Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation (VNS) in a limbic seizure model. J Neurochem. 2011;117:461-9

57. De Herdt, V, De Waele, J, Raedt, R, T. Wyckhuys, El Tahry R, Vonck K, Wadman W , P. Boon. Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures . Acta Neurologica scandinavica.2010 Apr; 121(4):271-276

58. Sataloff RT, Mandel S, Mann EA, Ludlow CL. Laryngeal electromyography: an evidence-based review. Muscle Nerve 2003;28:767-72.

59. Ardesch JJ, Sikken JR, Veltink PH, van der Aa HE, Hageman G, Buschman HP. Vagus nerve stimulation for epilepsy activates the vocal folds maximally at therapeutic levels. Epilepsy Res 2010;89:227-31.

60. Ventureyra ECG. Transcutaneous vagus nerve stimulation for partial onset seizure therapy – A new concept. Childs Nervous System 2000;16:101-102.

61. Fallgatter AJ, Neuhauser B, Herrmann MJ et al. Far field potentials from the brain stem after transcutaneous vagus nerve stimulation. J Neural Transm. 2003;110[12]:1437-1443.

62. Naritoku DK, Morales A, Pencek TL, Winkler D. Chronic Vagus Nerve-Stimulation Increases the Latency of the Thalamocortical Somatosensory Evoked-Potential. Pace-Pacing and Clinical Electrophysiology 1992;15:1572-8.

63. Brazdil M, Chadim P, Daniel P et al. Effect of vagal nerve stimulation on auditory and visual event-related potentials. Eur J Neurol 2001;8[5]:457-461.

64. Henry TR, Votaw JR, Pennell PB et al. Acute blood flow changes and efficacy of vagus nerve stimulation in partial epilepsy. Neurology 1999;52[6]:1166-1173.

65. Henry TR, Bakay RA, Pennell PB, Epstein CM, Votaw JR. Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. Prolonged effects at high and low levels of stimulation. Epilepsia 2004;45[9]:1064-1070.

66. Van Laere K, Vonck K, Boon P, Brans B, Vandekerckhove T, Dierckx R. Vagus nerve stimulation in refractory epilepsy: SPECT activation study. J Nucl.Med 2000;41[7]:1145-1154.

67. Vonck K, De Herdt V, Bosman T, Dedeurwaerdere S, Van Laere K, Boon P. Thalamic and limbic involvement in the mechanism of action of vagus nerve stimulation, a SPECT study. Seizure-European Journal of Epilepsy 2008;17:699-706.

68. Zagon A and Kemeny A. slow hyperpolarisation in cortical neurons: A possible Mechanism Behind Vagus nerve stimulation tTherapy for Refractory epilepsy. Epilepsia 2000;41:1382-1389

69. Marrosu F, Serra A, Maleci A, Puligheddu M, Biggio G, Piga M. Correlation between GABAA receptor density and vagus nerve stimulation in individuals with drug-resistant partial epilepsy. Epilepsy Res. 2003;55:59-70.

70. Steven L. Neese, Luke K. Sherill, Arlene A. Tan, Rodney W. Roosevelt, Ronald A. Browning, Douglas C. Smith, Andrea Duke, Rich W. Clough. Vagus nerve stimulation may protect GABAergic neurons following traumatic brain injury in rats: An immunocytochemical study. Brain research 2007;1128:157-163

71. Henry T. R. Therapeutic mechanisms of vagus nerve stimulation.Neurology2002; 59:S3–S14.

72. Castle M., Comoli E. and Loewy A. D. Autonomic brainstem nuclei are linked to the hippocampus. Neuroscience 2005; 134:657–669.

73. Groves D. A., Bowman E. M. and Brown V. J. Recordings from the rat locus coeruleus during acute vagal nerve stimulation in the anaesthetised rat. Neurosci. Lett. 2005;379:174–179.

74. Dorr A. E. and Debonnel G. (2006) Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. J. Pharmacol. Exp.Ther. 2006;318:890–898

75. Roosevelt R. W., Smith D. C., Clough R. W., Jensen R. A. and Browning R. A. (2006) Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res. 1119, 124–132.

76. Christopher M. De Giorgio, Alana Shewmon, Diana Murray and Todd Whitehurst. Pilot Study of trigeminal stimulation for epilepsy: a proof-of-concept trial. Epilepsia 2006; 47(7):1213-1215

77. Erika E fanselow, Ashlan P. Rei and Miguel A. L. Nicolelis. Reduction of Pentylenetetrazole-induced seizure acttivity in awake rats by seizure triggred trigeminal nerve stimulation The Journal of Neuroscience 2000;21:8160-68

78. Christopher M. DeGiorgio, Diana Murray, Daniela Markovic, et al. Trigeminal nerve stimulation for epilepsy. Long-term feasibility and efficacy. Neurology 2009;72;936

79. Tubbs RS, Patwardhan RV, Wellons JC 3rd, Oakes WJ. Cortical representation of Hering's nerve: a possible anatomical pathway for seizure cessation following electrical stimulation. Pediatr Neurosurg. 2002 Nov;37(5):235-9.

80. Tubbs RS, Wellons JC 3rd, Patwardhan RV, Oakes WJ, Wyss JM. The Effectiveness of Hering's Nerve Stimulation in Controlling Penicillin-Induced Seizures in the Rat Is Dependent on the Amygdala. Pediatr Neurosurg 2002;37:231-234

81. Ben-Menachem E, Hellstrom K, Waldton C, Augustinsson LE, Evaluation of refractory epilepsy treated with vagus nerve stimulation for up to 5 years, Neurology, 1999;52(6):1265–7.

82. Montavont A, Demarquay G, Ryvlin P, et al., Long-term efficiency of vagus nerve stimulation (VNS) in non-surgical refractory epilepsies in adolescents and adults, Rev Neurol (Paris), 2007;163(12):1169–77.

83. Casazza M, Avanzini G, Ferroli P, et al., Vagal nerve stimulation: relationship between outcome and electroclinical seizure pattern, Seizure, 2006;15(3):198–207.

84. Scherrmann J, Hoppe C, Kral T, Vagus nerve stimulation: clinical experience in a large patients series, J Clin Neurophysiol, 2001;18:408–14.

85. Alsaadi TM, Laxer KD, Barbaro NM, et al., Vagus nerve stimulation for the treatment of bilateral independent temporal lobe epilepsy, Epilepsia, 2001;42(7):954–6.

86. Kuba R, Brázdil M, Novák Z, et al., Effect of vagal nerve stimulation on patients with bitemporal epilepsy, Eur J Neurol 2003;10(1):91–4.

87. Koutroumanidis M, Binnie CD, Hennessy MJ, et al., VNS in patients with previous unsuccessful resective epilepsy surgery: antiepileptic and psychotropic effects, Acta Neurol Scand, 2003;107(2):117–21.

88. Janszky J, Hoppe M, Behne F, Tuxhorn I, Pannek HW, Ebner A. Vagus nerve stimulation: predictors of seizure freedom. Journal of Neurology, Neurosurgery and Psychiatry 2005;76[3]:384-389.

89. Parain D, Penniello MJ, Berquen P, et al., Vagal nerve stimulation in tuberous sclerosis complex patients, Pediatr Neurol, 2001;25(3):213–16

90. Wernicke J, Holder L, Keenan S, et al., Response predictors for VNS in the treatment of refractory partial epilepsy, Epilepsia 1992;33(Suppl. 3):S101.

91. Labar D, Vagus nerve stimulation for 1 year in 269 patients on unchanged antiepileptic drugs, Seizure, 2004;13(6):392-8.

92. Scherrmann J, Hoppe C, Kral T, Vagus nerve stimulation clinical experience in a large patients series, J Clin Neurophysiol, 2001;18:408–14.

93. Alexopoulos AV, Kotagal P, Loddenkemper T, et al., Long-term results with vagus nerve stimulation in children with pharmacoresistant epilepsy, Seizure, 2006;15(7):491–503.

94. Rychlicki F, Zamponi N, Trignani R, Ret al., Vagus nerve stimulation: clinical experience in drug-resistant pediatric epileptic patients, Seizure, 2006;15(7):483–90.

95. Murphy JV, Torkelson R, Dowler I, et al., Vagal nerve stimulation in refractory epilepsy: the first 100 patients receiving vagal nerve stimulation at a pediatric epilepsy center. Arch Pediatr Adolesc Med, 2003;157:560–64.

96. Kostov H, Larsson PG, Røste GK, Is vagus nerve stimulation a treatment option for patients with drug-resistant idiopathic generalized epilepsy?, Acta Neurol Scand Suppl, 2007;187:55–8.

97. Ng M, Devinsky O, Vagus nerve stimulation for refractory idiopathic generalised epilepsy, Seizure, 2004;13(3):176–8.

98. Labar D, Nikolov B, Tarver B, Fraser R, Vagus nerve stimulation for symptomatic generalized epilepsy: a pilot study, Epilepsia 1998;39(2):201–5.

99. Labar D, Murphy J, Tecoma E, Vagus nerve stimulation for medication-resistant generalized epilepsy. E04 VNS Study Group, Neurology, 1999;52(7):1510–12.

100. Holmes MD, Silbergeld DL, Drouhard D, et al., Effect of vagus nerve stimulation on adults with pharmacoresistant generalized epilepsy syndromes, Seizure, 2004;13(5):340–45.

101. Frost M, Gates J, Helmers SL, et al., Vagus nerve stimulation in children with refractory seizures associated with Lennox-Gastaut syndrome, Epilepsia, 2001;42(9):1148–52.

102. Fohlen MJ, Jalin C, Pinard JM, Delalande OR, Results of vagus nerve stimulation in 10 children with refractory infantile spasms, Epilepsia, 1998;39(Suppl. 6):170.

CHAPTER 10

Summary

Epilepsy is a neurological disorder characterized by recurrent aberrant electrical activity in the central nervous system which typically manifests itself as seizures. It is estimated that 1% of the population is affected worldwide. About 30% of these patients are considered refractory, meaning that they do not respond to anti-epileptic drugs. For these patients, alternative treatment modalities such as epilepsy surgery or neurostimulation (deep brain stimulation (DBS) or vagus nerve stimulation VNS) may be useful.

VNS is a less invasive treatment compared to DBS and is characterized by stimulation of the left vagus cervical nerve through electrical pulses delivered by a generator implanted under left clavicula. Pulses are determined by their intensity, pulse duration, frequency and duty cycle. Until today, more than 50.000 patients worldwide are treated with VNS. In general outcome of VNS can be divided into three groups. First group experiences a seizure reduction of more than 50%. A second group of patients has a seizure reduction between 30 and 50%. The last group, are those patients that only have a minor reduction in seizure frequency of less than 30% or do not respond to VNS. Unfortunately, there are no predictive factors to identify responders at earlier stage. In addition, there are no rational individual guidelines about how to up titrate VNS most efficiently or when to start stimulation after surgery. In epilepsy practice, patients are invited at the consultation two weeks after surgery to start VNS, which will be up-ramped in steps of 0.25 mA every 2 to 4 weeks, until seizure reduction is achieved or side effects appear.

The vagus nerve carries somatic and visceral afferents and efferents. Afferents compose about 80% of the cervical portion of the vagus and are mainly narrow-calibre unmyelinated C fibres, which predominate over faster conducting myelinated A and B fibres. Myelinitaed fibres need much lower charge densities to be activated comparing to unmyelinated fibres and are presumably the only fibres required to be activated to induce beneficial effects of VNS in epilepsy. Nevertheless, the mechanism of action of VNS remains poorly understood.

VNS research can be divided in two main domains. The first part concerns research about activation of the vagus nerve itself by applying electrical pulses with the use of extracellular electrodes.

Once the nerve is adequately activated, vagus nerve induces different effects at several projection sites in the brain. Characterization of those effects in the brain is a second branch of research and remains largely unsolved.

Choice of stimulation parameters in clinical practice is based on previously performed VNS animal experiments, but in none of these studies individual electrophysiological characteristics of the vagus nerve were considered. Therefore this thesis had as the main goal to objectify electrical stimulation

of the vagus nerve. For this purpose, a newly designed combined stimulation and recording electrode for the vagus nerve was developed for animal use.

Implantation of a large group of rats has resulted in recordings of VNS-induced signals that were too long in latency to be real compound action potentials (CAPs) of myelinated fibres of the vagus nerve. Experiments in which EMG recording of the larynx was performed and application of muscle paralyzing agents was applied, have confirmed that VNS co-activates the recurrent laryngeal nerve, a branch of the vagus nerve. As a consequence, contraction of the larynx could be consistently recorded as a far field muscle potential with a single electrode around the vagus nerve. In chronic experiments, we have performed daily dose-response curves and regular measurements of impedances of stimulation electrodes. Latency of larynx muscle compound action potential (LCAMP), intensity needed to activate a 50 % response and slope factor of dose-response curves remained stable over time. Impedances of stimulation electrodes gradually increased, consistent with the idea that a gliotic process encapsulates the electrode nerve interface.

In the follow-up of implanted rats, we observed that nearly half of the rats needed an average recuperation time of 25 days, indicating that the vagus nerve required a certain period to recover from surgery and implantation of an electrode. Interestingly, in epilepsy practice, evaluation of VNS responders is performed after at least 6 months to 1 year of therapy. Hypothetically, implantation leads to a local inflammatory reaction which could be at the origin of a temporary local demyelinisation of the nerve explaining a possible recovery time and delay in evaluation of responders. Performing more animal experiments, in which the LCAMP is applied in an epilepsy model in which VNS is tested, could offer new insights. Hypothesis would be strengthened if the outcome after VNS shows to be dependent on the actual recording of the LCAMP.

In addition, post-mortem immune histological analysis of electrode and vagal nerve could answer the question whether the inflammatory reaction is greater in those rats in which no LCAMP could be measured. LCAMP remains of course a surrogate parameter as it only reflects activation of **efferent** Aα fibres of the vagus nerve and does not give any information about myelinated **afferent** fibres which provide the anti-seizure effect of VNS.

Complementary to animal experimental work, we have tested in collaboration with Neurotech, a new VNS device (ADNS-300), which is capable of delivering therapeutic vagal nerve stimulation and offers the opportunity of recording CAPs of the human vagus nerve. In addition, the ADNS-300 possesses a transcutaneous recharging system, extending generator life up to 12 years.

Three patients were implanted for the first time with the ADNS-300. Two of the three patients reported a reduction of seizure frequency of 40%, while the third patient did not respond. For this patient, anti-epileptic drug treatment was adapted as he complained from medication-induced

somnolence, but parameters of his VNS remained unchanged. Thereafter the patient became seizure- free. Thus, positive evolution of the patient's seizure frequency could have been a direct consequence of the changed anti-epileptic drug treatment, but beneficial effect of VNS is cannot be excluded.

In same context of the animal experiments, we succeeded in recording CAPs of the vagus nerve in two of the three patients 2 to 3 weeks after surgery. The vagus nerve was stimulated with a pulse of 250 µsec duration and 500 µA intensity, which resulted in the recording of $N_1P_1N_2$ CAP, with N_1 appearing 0.4 msec after the end of the stimulation artifact. Minimum threshold was 250 µsec and 100-150µA. When pulse duration was shortened to 100 µsec, the threshold stimulus intensity increased to a value between 250 and 500 µA in patient 1 and 200 and 250 µA in patient 2.

Subsequently, we have carried out dose-response curves in patient 2 during a follow-up of 1 year. Our results show that two groups of myelinated fibres are recruited at different levels of charge density. The first group was fully activated at 25 mC (500 µA x 50 µsec), while the second group needed higher values, ie 37.5 mC (150 µA x 250 µsec). Stimulation parameters were kept stable the first year and were based on previous experience with Cyberonics devices. In the future, dose-response curves could allow clinicians to evaluate the maximum output charge to fully activated myelinated fibres and handle this value as a maximal stimulation level. This approach will reduce time for up-titration, avoid unnecessarily high outputs en save battery life

Further research on whether the beneficial effect of VNS is linked with the successful recording of a CAP after a silent period, could offer a partial explanation why non-responders to do not experience any benefit of VNS. Longitudinal follow-up studies in which seizure reduction is correlated with CAP recordings, might partially answer an important issue in clinical practice, namely why some patients respond to VNS and other do not.

Finally, implantation and recording of vagal nerve CAPs in a larger group of patients, will hopefully lead to better electrophysiological understanding of VNS.

Résumé

L'épilepsie fait partie des affections chroniques neurologiques les plus courantes et affecte 1 % de la population mondiale. Les crises épileptiques sont insuffisamment contrôlées dans le cas d'environ un tiers des patients, malgré un traitement antiépileptique adapté. Pour ces patients, une évaluation pré-chirurgicale s'impose, dans un centre d'épilepsie de référence. Seulement un petit pourcentage des patients réfractaires peut bénéficier d'une intervention, car les lésions épileptiques sont souvent situées au niveau du cortex éloquent. Pour ces patients, la neurostimulation constituerait alors un traitement alternatif valable.

La stimulation du nerf vague (SNV) se distingue de la neurostimulation profonde, car elle est moins invasive et stimule le nerf vague uniquement dans la région cervicale gauche. Le nerf est stimulé par des pulsations électriques, en provenance d'un générateur implanté en dessous de la clavicule. Les paramètres de la stimulation sont l'intensité, la durée des pulses, leur fréquence, ainsi que le cycle de charge. Actuellement, plus de 50.000 patients sont traités par SNV.

Généralement, une réduction de la fréquence des crises de plus de 50 % est observée dans un tiers des cas, tandis qu'elle n'atteint que 30 à 50 % pour une même proportion des patients traités. En ce qui concerne le tiers restant, la fréquence est réduite de moins de 30 % ou aucune amélioration n'est relevée. Il n'existe actuellement pas encore de facteurs prédictifs, ni de recommandations claires quant à l'utilisation de la SNV.

En pratique, le patient consulte deux semaines après l'implantation du générateur. Une stimulation progressive, intensifiée par paliers de 0,25 mA, est appliquée jusqu'à la réduction de la fréquence des crises ou une éventuelle apparition d'effets secondaires, comme une sensation douloureuse au niveau de la région cervicale gauche.

Le nerf vague est composé de trois types de fibres différentes. La majorité sont des fibres non myélinisées (C), une minorité est représentée par les fibres myélinisées (A, B). Il est à noter que les fibres myélinisées nécessitent des stimulations d'intensité bien moins importante par rapport aux fibres C, qui sont moins facilement activées. Selon les données issues de la littérature, seule l'activation des fibres A et B semble important pour la réduction de la fréquence des crises.

Les recherches visant à comprendre le mode d'action de la SNV portent sur deux aspects distincts de la problématique.

Premièrement, l'activation du nerf s'effectue via des pulsations électriques qui génèrent un potentiel d'action composé. Ce dernier dépend de la fonctionnalité du nerf, d'autant plus lorsqu'une électrode de stimulation a été préalablement implantée. Ensuite, un second intérêt de recherche réside dans

les effets de la stimulation du nerf vague sur les différents sites de projection au niveau du système nerveux central.

Cette thèse de doctorat vise à présenter une méthode innovante de stimulation du nerf vague, permettant un enregistrement de l'activité électro-physiologique résultant de sa stimulation.

Dans un premier temps, une électrode, combinant les fonctions de stimulation et d'enregistrement, a été conçue et implantée chez un groupe de rats dans l'objectif d'évaluer la faisabilité et la fonctionnalité de l'implant.

Cependant, l'enregistrement de l'activité électro-physiologique du nerf vague ne s'avérait pas être des actions potentielles évoquées (CAPs), mais bien la contraction du muscle laryngé (LCAMP) suite a l'activation d'une branche du nerf vague, notamment le nerf laryngé récurrent. En conséquence, des courbes de recrutement du LCAMP ont été effectuées durant une période de deux mois. Les caractéristiques du LCAMP comme la latence, l'intensité requise pour activer 50 % des fibres, l'amplitude maximale et le facteur de pente sont restés stables pendant toute la durée du suivi. L'impédance des contacts de stimulations mesurée s'accroît graduellement, ce qui peut s'expliquer par l'apparition de gliose. Néanmoins, la moitié des rats ont eu besoin d'une période de récupération de 25 jours en moyenne, indiquant clairement que l'implantation de l'électrode induit un dommage fonctionnel au niveau du nerf vague. Cette dernière observation est importante, car une attente d'au moins 6 mois avant l'évaluation des résultats de la SNV est conseillée chez l'homme. Cet intervalle pourrait donc correspondre à une période de récupération, subséquente à l'implantation, lors de laquelle les œdèmes résultant d'une réaction inflammatoire locale se résorbent.

Afin de confirmer cette hypothèse, le LCAMP doit être utilisé dans un modèle d'épilepsie chronique qui implique l'usage de la SNV comme traitement antiépileptique. Ces résultats seraient alors probants dans le cas où l'enregistrement de LCAMP ou les délais de cet enregistrement seraient corrélés avec les données fournies par la SNV.

Par ailleurs, l'examen immunohistochimique post-mortem effectué sur les rats pourvus d'une électrode de stimulation et d'enregistrement pourrait mettre en évidence une éventuelle réaction inflammatoire plus importante pour le groupe présentant un délai d'enregistrement LCAMP.

Cependant, le LCAMP étant une mesure de la stimulation des fibres efférentes du nerf vague, les résultats obtenus ne correspondent en aucun cas à l'activation des fibres myélinisées afférentes, qui traduirait un effet antiépileptique de la SNV.

Afin de compléter le travail de recherche expérimentale effectué, un nouveau stimulateur (ADNS-300) du nerf vague, combinant la stimulation thérapeutique avec la possibilité d'enregistrer des CAPs, a été implanté pour la première fois chez trois patients.

Ce générateur étant rechargeable par voie transcutanée, son temps de vie est prolongé d'une durée qui peut atteindre 12 ans. Dans le cas de deux des patients traités, une diminution des crises de 40 % a été observée après 6 mois de traitement par SNV. En ce qui concerne le troisième patient, aucune réponse au traitement antiépileptique (AED) n'a pu être relevée au terme de cette étape du suivi médical. En conséquence, son traitement a été adapté en tenant compte des effets secondaires de somnolence observés. Depuis lors, le patient n'a plus manifesté de crises épileptiques. L'effet bénéfique obtenu pourrait résulter des modifications apportées à ces AED, de la SNV, ou encore d'une action synergique de ces deux thérapies.

Pour deux patients sur les trois traités, des CAPs du nerf vague ont été enregistrés pour la première fois dans les 2-3 semaines qui ont suivi l'opération. Pour se faire, le nerf vague a été stimulé via des pulsations d'une durée de 250 µsec et de 500 µA d'intensité. De cette façon, un $N_1P_1N_2$ CAP, avec un N_1 apparaissant 0,4 msec après la fin de l'artifact de stimulation, a pu être enregistré.

L'intensité minimale des pulsations requise afin d'activer le nerf se situe dans une gamme de 100 à 150 µA et leur durée est de 250 µsec. Lorsque la durée des pulsations est fixée expérimentalement à 100 µsec, l'intensité nécessaire à l'activation du nerf atteint des valeurs comprises entre 200 et 250 µA en ce qui concerne le premier patient et entre 250 et 500 µA pour le deuxième.

Les enregistrements de CAPs ont été poursuivis chez un des patients sur une durée de 12 mois. Au terme de chacune des sessions, des courbes doses-réponses ont été obtenues. Les résultats ont démontré que deux groupes distincts de fibres myélinisées peuvent être recrutées. Dans le cas de l'un, les fibres sont activées à 25 mC (500 µA x 50 µsec), tandis que pour l'autre, des paramètres fixés à 37.5 mC (150 µA x 250 µsec) sont requis.

Dans un futur proche, une courbe dose-réponse pourrait être mise à la disposition du neurologue au cours de chaque visite. L'amplitude de la charge maximale ou l'amplitude du CAP n'augmente plus, pourrait correspondre à une valeur maximale de stimulation qui indiquerait alors qu'un accroissement de l'intensité au-delà de ce seuil n'apporterait pas plus de gains. Au contraire, elle causerait davantage d'effets secondaires et le générateur serait alors utilisé de manière inadéquate. En conséquence, cette nouvelle approche pourrait signifier un important gain pour les couts et avantages de la SNV. De plus, des études additionnelles pourraient investiguer si l'enregistrement du CAP est corrélé avec la response therapeutique, ce qui pourrait partiellement expliquer l'existence de « non-responders ». Ce dernier aspect du traitement étant un élément très discuté et insufisssamment examiné jusqu'a présent. En effet, une réponse à la SNV défavorable peut résulter, non seulement du manque d'effets de ce traitement sur le cerveau, mais également d'un problème local lié à la stimulation du nerf vague, comme, par exemple, une inflammation subséquente à l'implantation.

En conclusion, l'implantation du dispositif ADNS-300 chez un plus grand nombre de patients épileptiques permettrait une meilleure compréhension du fonctionnement electrophysiologic de la SNV.

Samenvatting

Epilepsie is één der meest frequent voorkomende chronische neurologische aandoening met een prevalentie van 1%. Bij ongeveer 30% van de patiënten reageren de aanvallen niet op de klassieke anti-epileptische behandeling en wordt de diagnose van refractaire epilepsie gesteld. Een doorverwijzing naar een epilepsie referentiecentrum is op dat ogenblik aangewezen. Slechts een klein deel van deze patiënten komt in aanmerking voor een chirurgische behandeling, daar de epileptogene zone zich vaak in de eloquente cortex bevindt. Voor deze patiënten zijn andere behandelingsmogelijkheden een optie, met name diepe hersenstimulatie (DBS) of nervus vagus stimulatie (VNS). VNS onderscheidt zich van DBS, daar het een minder invasieve behandeling betreft. De nervus vagus wordt buiten de hersenschedel, nl. ter hoogte van de linker hals, gestimuleerd. Deze stimulatie bestaat uit elektrische pulsen die gegenereerd worden door een generator die onder het sleutelbeen wordt geïmplanteerd. Parameters van stimulatie worden bepaald door de intensiteit, de pulsbreedte, de frequentie en duty cycle.

Tot op heden worden er ruim 50.000 patiënten behandeld met VNS. Algemeen stelt men dat één derde van deze patiënten goed respondeert en een aanvalsreductie kent van meer dan 50%. Een tweede groep reageert minder goed en ervaart een aanvalsreductie tussen 30 en 50%. Ten slotte ondervindt een laatste groep patiënten een aanvalsreductie van minder dan 30% of helemaal geen reactie op VNS. Tot op heden bestaan er geen goede predictieve factoren om de respons op VNS op voorhand in te schatten. Daarenboven bestaan er geen duidelijke, wetenschappelijk gebaseerde richtlijnen voor titratie en keuze van stimulatie parameters. In de praktijk wordt bij de patiënt een VNS systeem geïmplanteerd, waarna hij twee weken later terugkomt op consultatie en de stimulatie gestart wordt. Vervolgens wordt de stimulatie met 0.25 mA per keer verhoogd, tot er een aanvalsreductie optreedt. Indien er vroeger nevenwerkingen ontstaan, kan een verdere opdrijving van de output worden gestaakt.

De nervus vagus bestaat er drie verschillende soorten vezeltypes: de niet-gemyeliniseerde dunne C-vezels en de dikkere gemyeliniseerde en intermediaire A- en B-vezels. De gemyeliniseerde vezels vereisen kleinere hoeveelheid lading om geactiveerd te worden, dit in tegenstelling tot de niet-gemyeliniseerde vezels. Uit de literatuur blijkt dat stimulatie van gemyeliniseerde vezels volstaat om bij VNS een aanvalsreductie te bekomen.

Onderzoek naar het werkingsmechanisme van VNS kan ingedeeld worden in twee grote luiken. Het eerste luik bestaat uit onderzoek naar adequate activatie van de zenuw en een onderdeel hiervan wordt bepaald door het registreren van “Compound Action Potentials“(CAPs). Deze meting is uiteraard sterk afhankelijk van de functionaliteit van de zenuw, die na een operatie en implantatie

van een cuff elektrode, mogelijks kan veranderen. Een tweede luik bestaat erin de effecten van VNS op de verschillende en talrijke projectieplaatsen in de hersenen te onderzoeken.

Dit doctoraatswerk heeft als doel de stimulatie van de nervus vagus te objectiveren, door een VNS geïnduceerde elektrofysiologische parameter te identificeren die adequate activatie van de nervus vagus weergeeft. Hiervoor werd getracht vagale CAPs te karakteriseren. Met dit doel voor ogen, werd in de eerste plaats een nieuwe cuff elektrode voor dierexperimentele doeleinden ontworpen om de zenuw te stimuleren en tegelijk ook de CAPs te registreren.

Implantatie bij een grote groep ratten heeft aangetoond dat de implantatie en registratie van VNS geïnduceerde activiteit met een dergelijke elektrode haalbaar is. Met de gebruikte methode werd echter ook aangetoond dat opgemeten signalen geen CAPs van de nervus vagus waren, maar wel het gevolg zijn van co-activatie van de nervus laryngeus recurrens, die aanleiding geeft tot contractie van gestreepte larynx spieren (Larynx compound muscle action potential of LCAMP)

Vervolgens werden de LCAMP metingen chronisch opgevolgd gedurende een periode van twee maanden. Hierbij werden er dagelijks dosis respons curven en wekelijks impedantie van de stimulatie-elektroden opgemeten. De kenmerken van de LCAMP, zoals latentie, de intensiteit nodig om een 50% maximale respons te bekomen en de hellingsgraad van de dosis respons curven, bleven stabiel doorheen de tijd. Impedantie van de stimulatie elektroden steeg geleidelijk over 8 weken, in overeenstemming met het concept dat na de implantatie, er een gliotisch proces ontstaat waardoor de geïmplanteerde elektrode en zenuw volledig worden ingekapseld.

Bij opvolging van de ratten bleek de helft van de groep een recuperatietijd nodig te hebben van gemiddeld 25 dagen, wat een indicatie geeft dat de heelkunde en implantatie van de elektrode een zekere zenuwschade oplevert. In de klinische praktijk wordt er tevens aangeraden minstens 6 maanden te behandelen alvorens de uiteindelijke respons op VNS te beoordelen. Hypothetisch ontstaat er lokaal een inflammatoire reactie met demyelinisatie tot gevolg, waardoor de nervus vagus enige hersteltijd nodig heeft. Om de hypothese van lokale inflammatie en herstel verder te ondersteunen zijn bijkomende experimenten noodzakelijk. Eerst en vooral zou de LCAMP moeten worden toegepast in een chronisch epilepsiemodel waar bij VNS wordt getest. Dit experiment zou nagaan of de positieve outcome van VNS gerelateerd is met het al dan niet onmiddellijk of later registreren van de LCAMP. Daarnaast zou men met post-mortem immunohistologische coupes kunnen nagaan of de inflammatoire reactie groter is bij de non-LCAMP.

LCAMP blijft echter wel een surrogaat parameter, aangezien deze enkel de weerspiegeling is van de activatie van efferente Aα-vezels van de nervus vagus en geen informatie geeft over het al dan niet functioneel zijn van de gemyeliniseerde afferente vezels die het anti-epileptisch effect van VNS verzorgen.

Aanvullend bij dit dierexperimenteel werk, werd er in samenwerking met Neurotech, een nieuwe nervus vagus stimulator ontwikkeld (ADNS-300) en getest. Deze stimulator levert zowel therapeutische stimulatie als de mogelijkheid om CAPs van de nervus vagus te meten bij de mens. Daarnaast is hij ook transcutaan oplaadbaar, waardoor de levensduur van de generator wordt verlengd tot 12 jaar.

Na 6 maanden behandeling rapporteerden twee van de drie geïmplanteerde patiënten een aanvalsreductie van ongeveer 40%. De derde patiënt toonde op dat ogenblik nog geen respons op zijn VNS. Zijn anti-epileptische medicatie werd aangepast daar hij belangrijke medicamenteus geïnduceerde somnolentie vertoonde. Parameters van VNS bleven ongewijzigd. De patiënt werd kort nadien echter wel aanvalsvrij. Deze gunstige evolutie kan dus zowel het gevolg zijn van de veranderingen van zijn medicatieschema als van zijn VNS behandeling, of combinatie van beiden.

Samenlopend met het uitgevoerde dierexperimenteel werk, hebben we bij twee van de drie patiënten 2 à 3 weken postoperatief, CAPs van de nervus vagus geregistreerd. Hiervoor werd de zenuw gestimuleerd met een puls van 250 µsec lang en 500 µA sterk, waardoor een $N_1P_1N_2$ CAP kon worden opgemeten. N_1 verscheen 0.4 msec na het einde van het stimulatie artefact. De minimum vereiste lading om een CAP te kunnen registreren bedroeg een puls van 250 µsec lang en tussen 100 en 150 µA sterk. Wanneer de pulsduur werd verkort tot 100 µsec, lag de drempelwaarde intensiteit tussen 250 en 500µA bij patiënt 1 en tussen 200 en 250 µA bij patiënt 2.

Vervolgens is er bij één patiënt (patiënt 2) een follow-up studie uitgevoerd, waarbij op regelmatige tijdstippen dosis respons curven van de CAP werden geregistreerd. Uit deze metingen bleek dat er twee groepen gemyeliniseerde vezels gerekruteerd kunnen worden. Een eerste groep werd geactiveerd bij een stimulatiepuls van 25 mC (500µA x 50µsec), terwijl de tweede groep een hogere lading nodig had, nl 37.5 mC (150µAx250 µsec). Parameters van stimulatie werden het eerste jaar ingesteld op gelijkaardige wijze als bij eerder uitgevoerde studies met Cyberonics stimulatoren.

Het gebruik van dosis respons curven in klinische praktijk zou de behandelende neuroloog toelaten een maximale stimulatie output te hanteren, waarbij hogere stimulatie intensiteiten enkel zou leiden tot inefficiënt batterij verbruik en toename van het risico op optreden van bijwerkingen. In deze context kunnen de CAP metingen een belangrijke bijdrage vormen voor het kosten-baten aspect van deze behandeling. Daarenboven kunnen toekomstige longitudinale studies, waarbij zowel aanvalsreductie als CAP registraties worden uitgevoerd, mogelijks een antwoord bieden waarom bepaalde patiënten al dan niet gunstig reageren op de behandeling.

Implantatie en registratie van CAPs van de nervus vagus bij een grotere groep patiënten, zal hopelijk leiden tot een beter begrip van het werkingsmechanisme en elektrofysiologische aspect van VNS.

DANKWOORD

Uit goede bron heb ik vernomen dat het “Dankwoord” blijkbaar het eerste hoofdstukje is dat elke lezer doorneemt ☺

Daarom begin ik alvast met volgende uitdrukking: “Repetitio mater scienta est, herhaling is de moeder van de wetenschap”. Met volle overtuiging zal ik ieder van jullie ook bevestigen dat dit echt een treffer van een uitspraak is, die ik met vallen en opstaan gedurende vier jaar levendig heb meegemaakt.

Hierbij wil graag mijn promotor, Prof P. Boon, bedanken voor hartelijk ontvangst uit Brussel en mij de kans te geven om mijn onderzoeksdroom tot werkelijkheid te brengen. Prof K. Vonck, Prof W Wadman, Prof. Dr A Meurs en Prof. Dr. R. Raedt vormen een sterk epilepsie team. Elke kritische opmerking werd opgevangen en verwerkt: bedankt voor het begeleiden van de proeven en nalezen van wetenschappelijke output.

Robrecht, hier een speciaal woordje voor je: ik denk dat ik alles, of toch heel veel van jou heb geleerd. Het beginsel dat elk soldeerwerk de basis vormt voor een goed experiment is me nog steeds bij gebleven. If you do it, do it good, and all rubbish goes the trash in.

Dr. J Delbeke, U bent voor mij een speciale leermeester geweest. De wereld van elektroden en stimulatie werden me door U geïntroduceerd. Voor mij bent U ongeveer de slimste mens ter wereld, maar misschien kan Wytse jou wel tippen ☺

Graag had ik alle leden van het Referentiecentrum voor Refractaire epilepsie bedankt: Dr. Lut Goossens, Dr. Michel Van Zandycke, Prof. Dirk Van Roost, Dr. Frank De Waele, Prof Erik Achten, Prof. Karel Deblaere, Prof Guy Vingerhoets, Dr. Marijke Miatton, Prof Evert Thiery, Prof Ingeborg Goethals en Prof Luc Defreyne. Nog een speciaal woordje voor Corine Heyse, zij zorgde er steeds voor dat alles netjes verliep…

Graag had ik al mijn collega’s uit het labo bedankt: Tine, Lies, Ine, Bregt, het was echt leuk om met jullie te mogen samenwerken. Mijn eerste jaar in de kliniek was zeer aangename ervaring en daarom een woord van dank voor alle assistenten neuro: Veerle, Annelies, Hanne-Laure, Stefanie, Deborah, Eva, Evelien, Jelle en Pieter.

Hierbij ook een dankwoord voor alle nieuwe supervisoren uit de kliniek. Prof L. Crevits was diegene die mijn leergierigheid nog een extra puls gaf. Samen met Prof P. Santens, Prof J. de Bleecker, Prof K. Paemeleire en Dr. D. Hemelsoet en Dr. A sieben vormen zij een zeer verscheiden en sterk neurologie team, waarbij ik graag mijn opleiding als neuroloog verder bij voltooi.

Ik dank alle leden van de examencommissie, leescommissie en begeleidingscommissie van deze thesis, de decaan van onze faculteit en Dhr. Christian Maes voor hun bijdrage.

Bedankt aan alle secretariaat medewerkers, in het bijzonder Corine, die er steeds voor zorgde dat alles netjes in orde kwam. Dank je wel Selfet en Els voor jullie hulp en tips bij het opstellen van de lay-out deze thesis.

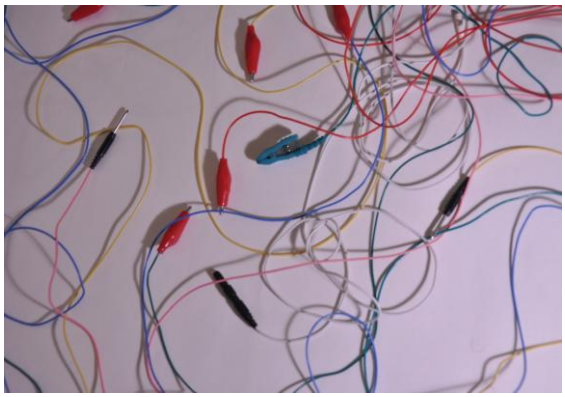
Zonder de bereidwillige medewerking van de patiënten was dit onderzoek niet tot stand gekomen!

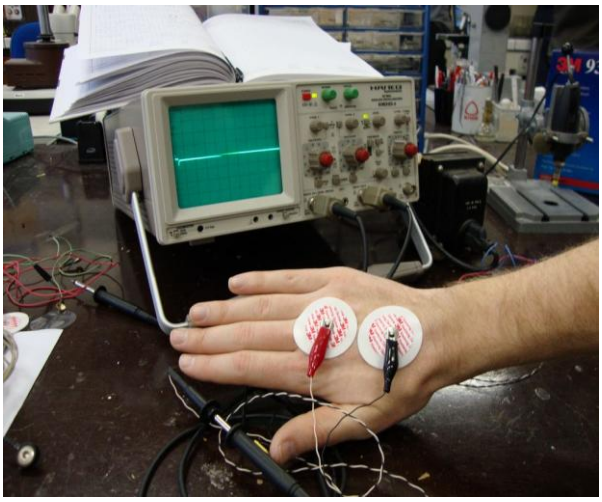
Dit werk kwam tot stand dankzij de financiële steun van het Bijzonder Fonds voor Wetenschappelijk onderzoek van de Universiteit Gent. Dank ook aan volgende bedrijven: Cyberonics, Neurotech, Glaxo-SmithKline.

En dan mijn ouders, dat zijn de mensen met het grootste uithoudingsvermogen ooit… Zij hebben me de weg geleid, van Brussel naar Gent, hebben me getoond hoe je een opdracht tot een goed einde brengt. Mams, jij bent diegene die uiteindelijk meer weet over VNS dan ikzelf, je kent elk verhaal door en door en heb me telkens de uitweg getoond, waar ik dacht dat er geen was. Daddie, I'm just so proud today to tell you that I have finally got that pHd. Broerke, jij was er steeds, vooral toen ik achter mijn pc af en toe op skype kwam piepen.

Gelukkig waren er ook super goede vrienden, ik bedank jullie allemaal voor het geduld maar vooral voor alle fijne momenten die we samen beleefd hebben: Sophie en Constantijn, Ineke en Alex, Els en Tommy, An en Kristof, Jan en Kim, Heidi en Miel, Valerie en Maarten, Wendy en Panna, Margot, Barbara, Bram en David (L). Frederik, jij deed me lachen en zingen op elk ogenblik, het is me een waar genoegen jou als beste vriend te mogen hebben. Nolwenn, un grand merci pour ta touche artistique ☺

Hieronder nog enkele “pics” uit het labo, voor interpretatie vatbaar ☺



CURRICULUM VITAE

Curriculum Vitae

Personal information:

Name: Riëm El Tahry

Adress: Frans De Mildreef 8, 9050 Ledeberg (Ghent)-Belgium

Birthdate: Brussels, 29th November 1981

Tel: 0032-498 57 62 90

Email: Riem.eltahry@ugent.be

Nationality: Belgian

Graduate studies:

Master in Medicine and Health Sciences, obtained at Vrije Universiteit Brussels, July 2006 with greatest distinction

Post graduate Courses

Transcranial Doppler training course Ghent University hospital July 2007

Basis course in animal sciences, October 2008

Introduction to Good Clinical Research Practice (GCP) training course", November 2008

Postgraduate position:

Junior Researcher (BOF-grant), august 2006-september 2010

Reference center for refractory epilepsy, Laboratory for Clinical and Experimental Neurophysiology,

Department of Neurology, Ghent University Hospital, De Pintelaan 185, B-9000 Gent

Director: Prof. Dr. P Boon

Clinical Trainee in neurology since September 2006

Department of Neurology, Ghent University Hospital

De Pintelaan 185, B-9000 Gent

Director: Prof. Dr. P Boon

Publications international peer-reviewed journals

Evolution in VNS therapy for refractory epilepsy, experience with Demipulse devices at Ghent University Hospital.
El Tahry R, De Herdt V, Raedt R, Van Dycke A, Meurs A, Dewaele F, Boon P, Van Roost D, Vonck K.
Seizure. 2010 Nov; 19(9):531-5.

A novel implantable vagus nerve stimulation system (ADNS-300) for combined stimulation and recording of the vagus nerve: pilot trial at Ghent University Hospital
El Tahry R, Raedt R, Mollet L, De Herdt V, Wyckuys T, Van Dycke A, Meurs A, Dewaele F, Van Roost D, Doguet P, Delbeke J, Wadman W, Vonck K, Boon P
Epilepsy Res. 2010 Dec; (2-3):231-9

Recruitment curves of compound action potential human vagus nerve recorded with the ADNS-300
El Tahry R, Raedt R, Mollet L, De Herdt V, Wyckuys T, Van Dycke A, Meurs A, Dewaele F, Van Roost D, Doguet P, Delbeke J, Wadman W, Vonck K,Boon P
In preparation

Recording of larynx compound muscle action potentials as an objective electrophysiological parameter for vagus nerve activation using a self-sizing cuff electrode around the vagus nerve in experimental rats
El Tahry R, Mollet L, Raedt R, De Herdt V, Wyckhuys T, Hemelsoet D, Meurs A, Delbeke J, Vonck K, Wadman W, Boon P.
Journal of Neuroscience Methods 2011 Jun 15;198(2):287-93

Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation (VNS) in a limbic seizure model.
Raedt R, Clinckers R, Mollet L, Vonck K, El Tahry R, Wyckhuys T, De Herdt V, Carrette E, Wadman W, Michotte Y, Smolders I, Boon P, Meurs A.
J Neurochem. *2011 May;117(3):287-93*

Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures De Herdt, V; De Waele, J; Raedt, R, T. Wyckhuys, R. El Tahry, Vonck K, Wadman W , P. Boon.
Acta Neurologica scandinavica.2010 Apr; 121(4):271-276

Predictive factors for outcome of invasive video-EEG monitoring and subsequent resective surgery in patients with refractory epilepsy
Carrette E, Vonck K, De Herdt V, Van Dycke A, El Tahry R, Meurs A, Robrecht Raedt, Goossens L, Van Zandijcke M, Van Maele G, Thadanid V, Wadman W, Van Roost D, Boon P.
Clin Neurol Neurosurg. 2010 Feb; 112(2):118-26.

Increased rat serum corticosterone suggests immunomodulation by stimulation o the vagal nerve
De Herdt, V; Puimege, L; De Waele, J, Raedt R, Wyckhuys T, El Tahry R, Libert C, Wadman W, Boon P, Vonck K.
J Neuroimmunol. 2009 Jul 25; 212(1-2):102-5

Serial day rapid kindling is an effective tool in screening the anti-epileptic properties of topiramate De Smedt T, De Rouck S, Raedt R, Wyckhuys T, Waterschoot L, De Herdt V, Van Dycke A, EL Tahry R, Vonck K, Boon P.
Seizure. 2007 Oct;16(7):620-6.

A novel implantable vagus nerve stimulation system (ADNS-300): pilot trial at Ghent University Hospital
El Tahry R, Raedt R, Mollet L, De Herdt V, Wyckuys T, Van Dycke A, Meurs A, Dewaele F, Van Roost D, Doguet P, Delbeke J, Wadman W, Vonck K,Boon P
Epilepsia 2010; 51 (suppl: s4):185

In vivo measurements of vagus nerve compound action potentials in experimental rats
El Tahry, R, Robrecht R, De Herdt V, Van Dycke A, Wyckhuys T, Meurs A, Vonck K, Wadman W, Boon P. Epilepsia 2009; 50 (suppl: 11):346

Identification of a brainstem evoked potential following cervical vagus nerve stimulation in rats
El Tahry R; Raedt R, De Herdt V, Waterschoot L, Wyckhuys T,. Van Dycke A; Meurs A; Vonck K; Wadman W, Boon P.
Epilepsia 2009; 50 (suppl 4):179

The identification of prognostic factors prior to invasive video-EEG monitoring
Carrette E, Vonck K, De Herdt V, Van Dycke A, El Tahry R, Dewaele I, Goossens L, Raedt R, Van maele G, Meurs A, Van Roost D, Wadman W, Boon P.
Epilepsia 2009; 50 (suppl 4):261

Beneficial effect of add-on levetiracetam in patients treated with vagus nerve stimulation for refractory epilepsy
De Herdt V, Vancaester E, Van Dycke A, Dewaele I, El Tahry R, Meurs A, Boon P, Vonck K
Epilepsia 2009; 50 (suppl 4):195

Increased hippocampal extracellular noradrenalin concentration contributes to the anticonvulsant effect of VNS in the focal pilocarpine rat model for limbic seizures
Meurs A, Mollet L, Raedt R, El Tahry R, De Herdt V, Vonck K, Smolders I, Michotte Y, Boon P
Epilepsia 2009; 50 (suppl 11):355

Anti-epileptic effects of vagus nerve stimulation in the focal pilocarpine model
Raedt R, Mollet L, Meurs A, Wyckhuys T, El Tahry R, Vonck K , Michotte Y, Boon P., Smolders I
European journal of Neurology 2009; 16 (suppl 3):141

Effect of acute and long-term vagus nerve stimulation in fully kindled rats
Raedt R, Waterschoot L., Wyckhuys T; De Herdt V, El Tahry R, Delbeke J, Vonck K, Wadman W, Boon P
Epilepsia 2009; 50 (suppl 4):93

Epilepsy surgery: diagnosis, techniques, outcome
Carrette E, Vonck K, De Herdt V, Van Dycke A,El Tahry R, Goossens L, Readt R, Meurs A,Van Roost D, Wadman W, Boon P.
Epileptic disorders 2008; 10(4):382

Vagus nerve stimulation supresses pilocarpine-induced limbic seizures and increases hippocampal extracellular noradrenalin concentration
Meurs A, Clinckers R, Raedt R, El Tahry R, De Herdt V, Vonck K, Smolders I, Michotte Y, Boon P
Epilepsia 2008; 49 (suppl 7):350

Local delivery of adenosine has an anti-epileptic effect in rats with spontaneous seizures
Van Dycke A, Raedt R, Dauwe I, Wyckhuys T, De Herdt V, El Tahry R, Meurs A, Vonck K, Wadman W, Boon P.
Epilepsia 2008; 49 (suppl 7):382-383

Neuropsychological outcome of bilateral hippocampal stimulation for refractory temporal lobe epilepsy
Van Dycke A, Vonck K, Miatton M, De Herdt V, El Tahry R, Dewaele I, Carrette E, Vingerhoets G, Van Roost D, Boon P.
European Journal of Neurology 2008; 15 (suppl 3):86-87

Vagus nerve stimulation induced changes in corticosterone in rats
De Herdt V, Libert C, De Waele J, De Smedt T, Waterschoot L, Wyckhuys T, El Tahry R, Van Dycke A, Raedt R, Vonck K, Wadman W, Boon P.
Eur. J. Neurol 2007; 14 (suppl 1):83

Serial day rapid kindling is an effective tool in screening the anti-epileptic properties of topiramate
De Smedt T, De Rouck S, Raedt R, Wyckhuys T, Waterschoot L, De Herdt V, Van Dycke A, El Tahry R, Vonck K, Boon P
Eur. J. Neurol 2007; 14 (suppl 1):208-209

An overview of 50 patients who underwent epilepsy surgery at Ghent University Hospital
Dewaele I, Carrette E, de herdt V, van Dycke A, El Tahry R, Hemelsoet D, Vingerhoets G, Miatton M, Thiery E, Achten R, Goossens L, Goethals I,Van zandycke M, Vonck K, Van Roost D, Boon P
Eur. J. Neurol 2007; 14 (suppl 1):83

Book chapters

Book Title: Seizure prediction in Epilepsy, from basic mechanisms to clinical applications
Chapter: Vagus nerve and hippocampal stimulation for refractory epilepsy
Boon P, De Herdt V, Vonck K, Van Dycke A, Wyckhuys T, El Tahry R, Van Roost D, Readt R, Wadman W. Wiley Weinheim 2008; 283-298

Abstracts published in national journals

Clinical added value of MEG in patients with refractory partial epilepsy and non-localizing conventional pre-surgical evaluation
Carrette, E; De Tiège, X; Vonck K, De Herdt V, Van Dycke A, El Tahry R, Goossens L, Readt R, Meurs A, Van Roost D, Wadman W, Boon P.
Acta Physiologica 2009 ; 197 (suppl 674):1

Local infusion of neuropeptide Y has anti-epileptic effects in fully kindled rats
Dauwe I, Raedt R, van Dycke A, Meurs A, Wyckhuys T, El Tahry R, Vonck K, Wadman W, Boon P.
Acta Physiologica 2009; 195 (suppl 670):11

Anti-epileptic effects of vagus nerve stimulation in the focal pilocarpine model
Mollet L, Raedt R, Meurs A, Wyckhuys T, van Dycke A, El Tahry R, Vonck K, Wadman W, Michotte Y, Smolders I, Boon P.
Acta Physiologica 2009; 195 (suppl 670):10

Adenosine secretion by neural stem cells isolated from ADK-/- mice
Van Dycke A, Raedt R, Boison D; Verstraete A, Meurs A, Wychuys T, El Tahry R, Vonck K, Wadman W, Boon P.
Acta Physiologica 2009; 195 (suppl 670):13

Effect of hippocampal deep brain stimulation on blood perfusion evaluated by SPECT Van Nieuwenhuyse B, Wyckhuys T,Raedt R, Meurs A, Van Dycke A, El Tahry R, Vonck K, Wadman W, Deleye S, Staelens S, Boon P.
Acta Physiologica 2009; 195 (suppl 670):12

Efficacy of vagus nerve stimulation for refractory epilepsy among patient subgroups
De Herdt V, Vonck K, Van Dycke A, El Tahry R, Carrette E, van Roost D, Boon P. Thadani V
Acta neurologica Belgica 2008; 108 (suppl 1):85

Oral presentations

Ghent University Hospital- Neurology Department May 29, 2008: “Vagus nerve induced evoked potentials”

VUB October 24, 2009: Belgian Society of fundamental and clinical physiology and pharmacology
“In vivo measurements of vagus nerve compound action potentials in experimental rats”

Ghent 21 -22 -May 2010: International Epilepsy workshop: new approaches for epilepsy treatment in Europe: back to the future. “Compound action potentials of the vagus nerve in humans and experimental rats”

ULB October 16, 2010: Belgian Society of fundamental and clinical physiology and pharmacology
“A novel implantable vagus nerve stimulation system (ADNS-300) for combined stimulation and recording of the vagus nerve: pilot trial at Ghent University Hospital”

Congrescentrum "Lamot" te Mechelen”, Herfstvergadering Vlaamse Vereniging voor Neurologie (VVN), "Nieuwe onderzoekstechnieken in de Neurologie:cost-benefit", November 20,2010: **“**A novel implantable vagus nerve stimulation system (ADNS-300) for combined stimulation and recording of the vagus nerve: pilot trial at Ghent University Hospital”

Kempenhaeghe- Landelijke Werkgroep Nervus Vagus Stimulatie (NL), wetenschappelijke VNS vergadering September 15, 2011: “VNS & Evoked potentials”

Teaching and students

Practica Neurofysiologie 2006-2007: ‘Reflexen, electromyografie en zenuwgeleiding' (Prof. Dr. L. Leybaert)- 1ste jaar geneeskunde

Karakterisatie van het corticale stimulatie model. Voorbereiding onderzoekstage-1ste Master Biomedische wetenschappen -Ine Buffel

Thesis: Karakterisatie van het corticale stimulatiemodel, 2de master Biomedische wetenschappen- Ine Buffel