The neuromodulatory effect, safety and effectiveness of Vagus Nerve Stimulation

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Cover illustration by Ylva Rydenhag

"Freedom is absolutely necessary for the progress in science and the liberal arts."

Baruch Spinoza (1632–1677)

To my beautiful and beloved wife and to my dear parents

ABSTRACT

Background

Vagus nerve stimulation (VNS) is an adjunctive palliative neuromodulatory treatment for drug resistant epilepsy (DRE) and chronic depression. It has also been proposed as a treatment for many other conditions such as chronic pain, heart failure, and Alzheimer's disease. Vagal Blocking Therapy (VBLOC) was recently approved for the treatment of obesity. However, the mechanisms of action still remains unclear and its long-term safety and efficacy in combination with antiepileptic drugs (AEDs) needs to be further evaluated. The aim of this thesis was to study the action of VNS on hippocampal neurogenesis as a possible mechanism of action on depression, to evaluate VBLOC as a new treatment model for obesity, and to study the long-term safety and effectiveness of VNS.

Patients and methods

In Study I rats were implanted with VNS and different stimulation parameters were compared to sham, in order to evaluate the effect of VNS on hippocampal progenitor proliferation. The number of Bromodeoxiuridine (BrdU) positive cells was compared between groups. In Study II rats were implanted with VBLOC, and leads were placed around the gastric portion of the vagus nerve. Body weight, food intake, hunger/satiety, and metabolic parameters were monitored and compared between control and sham-stimulated animals. In Study III all patients that had been implanted with VNS between 1990 and 2014 were analyzed for surgical and hardware complications. In Study IV data from 130 consecutive patients implanted with VNS between the years 2000 and 2013 was analyzed for seizure frequency and AEDs prior to VNS implantation as well as at 1, 2, and 5 years postoperatively. Study III and IV were retrospective cohort studies.

Results

VNS at the output current of 0.75 mA for 48 hours showed a significant increase in progenitor cell proliferation. VBLOC reduced food intake and body weight, and was associated with increased satiety but not with decreased hunger. Complications occurred in 8.6 % of all VNS surgeries in patients with DRE. The most common complications, all with an occurrence rate of about 2 %, were postoperative hematoma, infection, and vocal cord paralysis. Hardware related complications occurred in 3.7 % of all implanted VNS systems, and significantly less lead associated complications occurred during 2000–2014 compared to 1990–1999. There was a significant seizure reduction overall (all $p<0.001$) regardless of AED regimen, and VNS efficacy increased with time from 22.1 % at 1 year to 43.8 % at 5 years.

Conclusions

VNS induces stem cell proliferation in the rat hippocampus, which supports the notion that hippocampal plasticity is involved in the antidepressant effect of VNS. The mechanism of action of VBLOC as a treatment for obesity could be regulated by inducing satiety through vagal signaling, leading to reduced food intake and loss of body weight. The treatment was well tolerated in rats. VNS is a safe palliative neuromodulatory treatment for DRE, and the 25 years of follow-up to study safety is of great strength considering that VNS can be a life-long treatment with repeated surgeries. VNS efficacy increased with time, with improvements seen up to 5 years, and did not differ between patients that had altered or remained on the same AEDs throughout the study period.

SAMMANFATTNING PÅ SVENSKA

Vagusnervstimulering (VNS) är framför allt en tilläggsbehandling vid svårbehandlad epilepsi där mediciner inte har tillräcklig effekt mot upprepade krampanfall. Behandlingen är också godkänd mot kronisk och recidiverande depression. VNS innebär att man med hjälp av en inopererad elektrod på halsen stimulerar den vänstra vagusnerven (även kallad den 10:e kranialnerven, n. X). Elektroden kopplas till en stimulator som placeras under huden nedanför vänster nyckelben. Elektriska impulser skickas via vagusnerven till hjärnan och det hela kan liknas vid en slags pacemaker. På så sätt försöker man minska antalet epileptiska anfall eller behandla svår depression. VNS har också testats som behandling mot exempelvis hjärtsvikt, kroniska smärttillstånd och Alzheimers sjukdom. Nyligen godkändes en ny typ av VNS mot övervikt, så kallad VBLOC. Trots omfattande forskning och trots att VNS använts kliniskt sedan början av 1990-talet känner man fortfarande inte till de exakta verkningsmekanismerna. När det gäller operationsrelaterade komplikationer och behandlingseffekt i kombination med antiepileptiska mediciner över lång tid finns det begränsade data.

Målet med denna avhandling var att studera en möjlig verkningsmekanism för VNS på stamceller i råttans hippocampus, vilket skulle kunna utgöra en del i den antidepressiva effekten. Det var även att utvärdera en ny möjlig behandlingsmetod mot övervikt samt att studera risken för komplikationer i samband med VNS-inläggning och effektiviteten av VNS över en längre tidsperiod.

VNS effekt på cellnybildning i hippcampus och dess möjlighet att minska födointag undersöktes i två separata studier på råtta. Samtliga råttor fick VNS inopererade och därefter jämfördes stimulerade och icke-stimulerade råttor. I två andra studier insamlades data för samtliga patienter som opererats med VNS vid neurokirurgiska kliniken på Sahlgrenska Universitetssjukhuset mellan åren 1990 och 2014. Komplikationspanorama, komplikationsfrekvens samt effekten av VNS, och då även i förhållande till förändringar i antiepileptisk läkemedelsbehandling, utvärderades.

VNS-stimulerade råttor hade ett signifikant större antal nybildade celler i hippocampus jämfört med icke-stimulerade råttor men effekten var dosberoende. VBLOC-stimulerade råttor hade mindre födointag och minskade i vikt jämfört med kontrollgruppen. Komplikationer till VNS-inläggningar och batteribyten inträffade i 8,6 % av samtliga fall. Blödning, infektion och stämbandspares var de vanligast förekommande komplikationerna. I 3,7 % av fallen uppkom tekniska fel i den inopererade utrustningen. Oavsett antiepileptisk medicinering så minskade antalet epileptiska anfall signifikant med VNS. VNS-effekten ökade över tid från 22,1 % till 43,8 % över 5 år. Dock minskade inte läkemedelsanvändningen hos mer än ett fåtal patienter, och överlag ökade den signifikant.

Studierna i avhandlingen visar att ökningen av antalet celler i hippocampus kan vara en effekt av VNS, vilket är en möjlig antidepressiv verkningsmekanism. VBLOC tolererades väl av försöksdjuren och tycktes påverka mättnad, vilket resulterade i viktminskning. Försöken talar för att VBLOC är en metod som kan användas som behandling mot övervikt. Kliniskt tillämpad VNS är en säker behandlingsmetod med förhållandevis låga risker även ur ett långtidsperspektiv. Dessutom ses en ökad behandlingseffekt över tid.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I.   Révész D, Tjernstrom M, Ben-Menachem E, Thorlin T. Effects of vagus nerve stimulation on rat hippocampal progenitor proliferation. Experimental Neurology. 2008 Dec;214(2):259-65.

II. Johannessen H, Révész D, Kodama Y, Cassie N, Skibicka KP, Barrett P, Dickson S, Holst J, Rehfeld J, van der Plasse G, Adan R, Kulseng B, Ben-Menachem E, Zhao CM, Chen D. Vagal Blocking for Obesity Control: a Possible Mechanism-Of-Action. Obesity Surgery. 2017 Jan;27(1):177-185.

III. Révész D, Rydenhag B, Ben-Menachem E. Complications and safety of vagus nerve stimulation: 25 years of experience at a single center. Journal of Neurosurgery Pediatrics. 2016 Jul;18(1):97-104.

IV. Révész D, Rydenhag B, Ben-Menachem E. Estimating long-term VNS efficacy: Accounting for AED regimen changes. *Submitted*

TABLE OF CONTENTS

ABBREVIATIONS

AED	Antiepileptic drug
BrdU	Bromodeoxiuridine
DG	Dentate gyrus
CNS	Central nervous system
DRE	Drug resistant epilepsy
EEG	Electroencephalogram
GCL	Granule cell layer
LC	Locus coerulius
MDD	Major depressive disorder
NA	Noradreanline
NTS	Nucleus tractus solitarius
VBLOC	Vagal blocking therapy
VNS	Vagus nerve stimulation
5-HT	Serotonin

INTRODUCTION

Definition and brief history

Therapeutic neuromodulation is defined by the International Neuromodulation Society as "the alteration of nerve activity through targeted delivery of a stimulus, such as electrical stimulation or chemical agents, to specific neurological sites in the body"[1]. By stimulating the nervous system with non-invasive or minimally invasive neuromodulating devices, modulation in neural signaling and function can be achieved, possibly resulting in molecular, physiological, and behavioral alterations. Neuromodulatory treatments for disorders such as epilepsy, depression, chronic pain, Parkinson's disease, tremor, as well as restoration of bowel and bladder control have been developed in the last decades. The first described use of neuromodulation occurred in about 15 A.D. when a man suffering from gout accidentally stepped on an electric fish, and after the shock noticed much less pain[2]. Several pioneering discoveries in the fields of electricity and neurophysiology by Galvani, Fritsch and Hitzig, and Bartholow amongst many others in the early 18th and 19th century, laid the groundwork for the understanding and further development of modern neurostimulators, such as vagus nerve stimulation (VNS), deep brain stimulation (DBS), and spinal cord stimulation (SCS)[3]. Today neuromodulation is a rapidly developing field with new and more targeted treatments and therapies for many different conditions.

The vagus nerve – anatomy and function

The vagus nerve (the tenth cranial nerve, n. X) is the longest of the cranial nerves. As a consequence of its long and complex course from the brain stem to the abdomen, its name is derived from the Latin word for wanderer[4]. It is a mixed nerve containing both efferent and afferent fibers that are attached to multiple rootlets in the medulla. Approximately 20 % of the vagus nerve fibers are efferent and originate from the dorsal motor nucleus and the nucleus ambiguus in the medulla oblongata. These fibers provide parasympathetic innervation to essentially all the thoracic and abdominal organs as well as project motor neurons to striated muscles of the pharynx and larynx[5]. As for all parasympathetic nerves, the fibers do not innervate

peripheral organs directly but via parasympathetic ganglia close to or in the walls of the organs. The postgangliotic neurons are then connected to the cardiovascular, respiratory, and gastrointestinal organs.[6] The remaining 80 % of the vagus nerve fibers are afferent. Most of the neurons contributing afferent input to the cervical vagus nerve, found in the carotid sheath, have cell bodies located in the jugular and the nodose ganglion, which are situated at and immediately below the jugular foramen. Nerve fibers from these ganglia transmit visceral, somatosensory, and taste sensations to the brain via medullary nuclei. The nerve consists of myelinated A-, B-, and unmyelinated C-fibers, and conduction velocities are proportional to their sizes. Large myelinated A-fibers carry mostly somatic afferent and efferent information, and small myelinated A-fibers primarily transmit visceral afferent information, B-fibers provide efferent and parasympathetic preganglionic innervation, and small unmyelinated C-fibers primarily carry afferent visceral information[4,6]. At the cervical level most vagus nerve fibers (60-80 %) are afferent C-fibers[7]. The vast afferent direct and indirect projections from the vagus nerve to multiple higher deep, subcortical, and cortical brain centers, mainly via the nucleus tractus solitarius (NTS), are considered to play an important role in the mechanism of action of VNS in treating epilepsy and mood disorders[6]. Other vagal sensory afferent projections from the external auditory meatus, the posterior fossa meninges, the larynx, and the upper esophagus are received in the spinal nucleus of the trigeminal nerve and relayed to the sensory cortex via the thalamus[8]. The vagus nerve is also known to play an important role in mediating vital digestive reflexes and influence digestive behavior[9]. Nutritional and metabolically relevant information is conveyed to the brain by gut-produced hormones and the vagus nerve[10]. To sum up, the extensive spread of afferent projections from the vagus nerve has raised the question if VNS can be developed into a successful treatment in other areas such as heart failure, Alzheimer's disease, obesity, and pain.
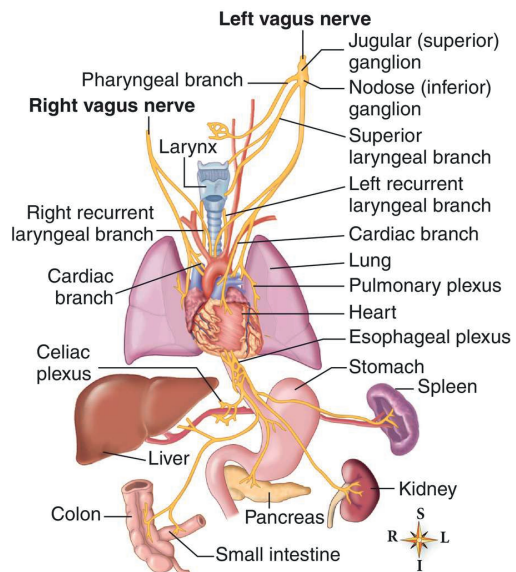

Figure 1. Illustration of the vagus nerve anatomy and its connection to visceral organs (retrieved from http://medical-dictionary.thefreedictionary.com).

Vagus nerve stimulation (VNS)

VNS is a neuromodulatory treatment for drug resistant epilepsy (DRE) and chronic or refractory depression. A bipolar electrode is placed around the left cervical vagus nerve, and direct electrical modulatory access to subcortical brain areas are generated by a pulse generator placed in the chest wall. Because of the nerve's location it provides a unique entrance to the brain[11]. In the early 1990's the Neurological and Neurosurgical Department at Sahlgrenska University Hospital was one of the first centers involved in implanting VNS when clinical trials were initiated[12,13]. VNS has since then been approved for the treatment of refractory epilepsy since 1994 in Europe and 1997 in the USA. It was also approved for treating chronic and recurrent depression in 2001 in Europe and 2005 in the USA. Even though more than 100.000 devices have been implanted in around 80.000 patients worldwide, its mechanism of action still remains unclear, and its clinical safety and efficacy is frequently debated. As VNS can be a life-long treatment, it is of great importance to have long-term follow-up data regarding efficacy and tolerability.

Historically in the late 19th century, the American neurologist Corning suggested that stimulating the vagus nerve, by compressing the carotid arteries and at the same time stimulating the vagus nerve electrically, could interrupt epileptic seizures by reducing cerebral blood flow. Corning, amongst other scientists, believed that seizures were associated with alterations of cerebral blood flow, and by creating a crude and external VNS in the 1880s, he lowered the number of seizures in patients treated with cardioinhibitory vagus stimulation[14]. However, it was not until 1952 that Zanchetti et al showed that vagal afferent stimulation directly affected cortical activity. Epileptic activity in their animal model could be suppressed depending on stimulation frequency[15]. In the 1980s, Zabara began to analyze the effect of VNS on chemically induced seizures in dogs, and the results were remarkably positive[16]. Since that time VNS has become a globally accepted form of treatment for epilepsy.

VNS implantation

VNS implantation is considered to be a minimally invasive surgical procedure. Nevertheless, the implantation should be performed by a surgeon with detailed familiarity with the procedure. Surgery is performed under general anesthesia and prophylactic antibiotics are administered to minimize the risk of infection. Via a transverse incision lateral to the thyroid cartilage

on the neck, the left vagus nerve is carefully dissected in its carotid sheath between the carotid artery medially and the internal jugular vein laterally. At least 3 cm of the nerve has to be exposed in order for the wrapping of the bipolar electrodes. A silicone lead consisting of a helical anchor, anode, and cathode is wrapped around the nerve. The negative electrode (cathode) is placed cranially, and the positive electrode (anode) caudally. The lead is then tunneled subcutaneously to an incision below the left collarbone, where a subcutaneous pocket is made. To avoid tension on the lead, it is looped in a gentle curve and sutured to soft tissue adjacent to the nerve. The lead is then connected to a pulse generator and placed in the subcutaneous pocket over the left major pectoral muscle[17,18]. The stimulator can also be placed in a submuscular (subpectoral) pocket[19]. If no adverse event occurs, the patients are usually discharged from the hospital the day after surgery.
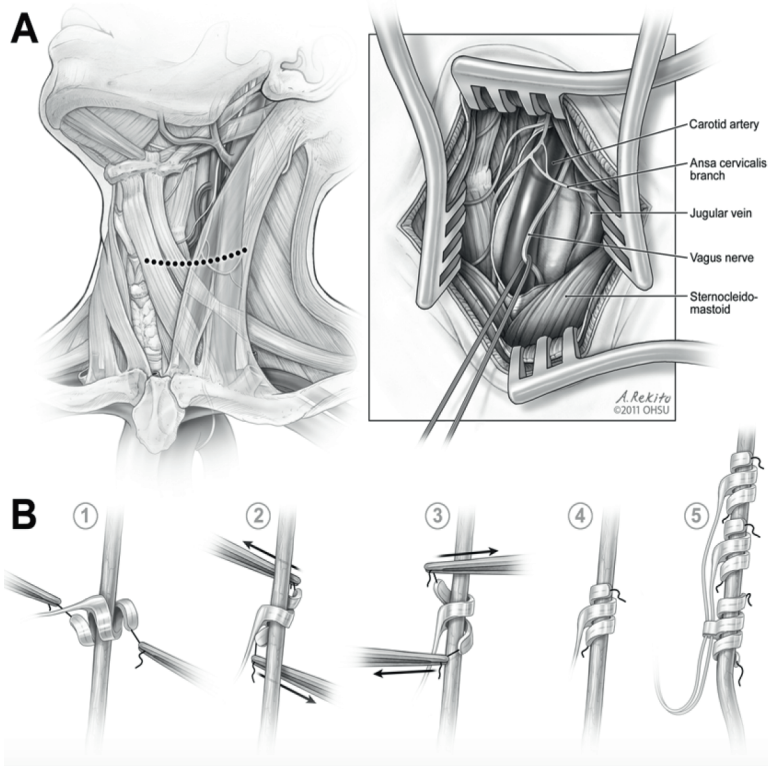
Figure 2A. Showing a schematic drawing of the skin incision on the left side of the neck and the cervical anatomy. 2B. Illustrating the wrapping technique of the lead electrodes around the vagus nerve. (Courtesy of Journal of neurosurgery Pediatrics and the artist Andrew Rekito)

When the pulse generator is about to be depleted a battery replacement is made, usually under local anesthetics with prophylactic antibiotics administered. If no adverse event occurs, the patients are discharged from the hospital the same day.
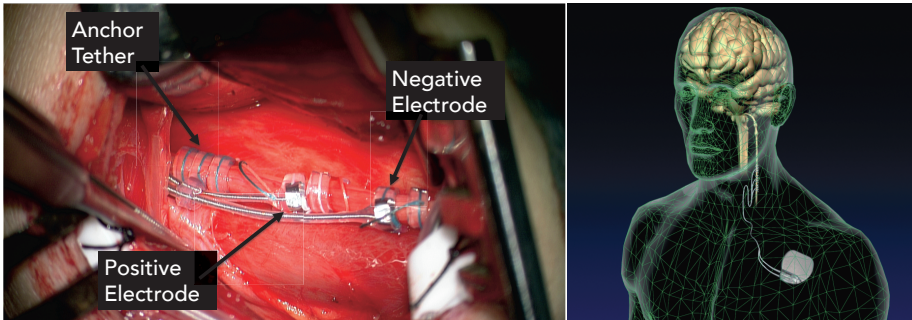
Figure 3. Showing the VNS lead in place with the negative electrode cranially and the anchor tether caudally and a schematic picture of the lead and stimulator in place. (Courtesy of Daniel Nilsson and Lancet neurology)

In Studies I and II, rat VNS implantation was made in a similar fashion. Because the vagus nerve of the rat is substantially thinner than the human, the surgical procedure was always made under microscopical control in order to decrease mechanical nerve manipulation. The VNS electrode was also modified to better fit the smaller and more delicate nerve in the rat. For details see the materials and methods section.

Stimulation parameters

VNS can be administered with a range of at least five different use parameters (intensity, frequency, pulse width, on-time, and off-time). The pulse generator is initiated 2 weeks after implantation. The rationale for the time delay is the concept of nerve swelling following surgery[20]. The device is started at 0.25 mA, 30 Hz, and 500 µsec pulse width for 30 seconds on and 5 minutes off. If patients complain of vocal side effects, the frequency is reduced to 20 Hz and pulse width to 250 µsec. To improve efficacy, the settings are adjusted in some patients to 30 seconds on and 1.8 or 3 minutes off. Rapid cycling has been tried in some patients (7 seconds on and 21 seconds off), but because of shortened battery life and lack of improved efficacy[21], all patients were reverted back to 30 seconds on and 1.8–5 minutes

off. There is also a possibility to initiate extra stimulation using a magnet that comes with the VNS system. The most common use of the magnet is when a seizure is anticipated or in progress, and the idea is to try to abort a seizure at the initial phase[22]. Invariably, the magnet settings are one notch higher than the regular stimulation. The stimulation parameters are increased gradually with 0.25 mA to the highest tolerable setting. Maximum output current is 3.5 mA, and frequencies of 50 Hz, since higher current and frequencies may cause major irreversible damage to the vagus nerve[23]. VNS parameters can easily be adjusted by the treating physician or nurse with a programming wand placed over the pulse generator.

For stimulation parameters and programming in the rat, see the materials and methods section.

Surgical complications

Surgical complications can be divided into complications as a result of the actual invasive procedure e.g. vocal cord paralysis and postoperative hematoma, and into hardware complications such as lead break/fractures or lead malfunction. The most common complication to VNS implantation is postoperative infection followed by postoperative hematoma and vocal cord paralysis, but other more uncommon complications such as jugular vein puncture and pneumothorax have also been reported[18,24,25]. Arrhythmia, including asystole and bradycardia, is an important but rare complication that has been reported in the literature[26-30]. Because of anatomical differences between the left and right vagus nerve, where the right vagus nerve carries most of the parasympathetic fibers that more densely innervate the sinoatrial node, stimulation of the left vagus nerve, innervating the atrioventricular node, is favored to avoid affection of the cardiac rhythm[31]. Most long-term safety data ranges from 1 to 5 years and are summarized in Table 1. VNS implantation in children has been carried out since 1994. The complication panorama is of the same kind as for adults except for a higher infection rate in the pediatric population[32,33]. Generally, if a postoperative wound infection occurs, it is most often initially treated with oral, and in some cases intravenous antibiotics. If the infection persists, which is usually the case with deep wound and pocket infections, the stimulator needs to be removed in order to treat the infection[32,34].

For surgical complications in the rat, see the materials and methods section.

Table 1. Occurrence of complications to VNS related surgeries in the literature

	Adults	Children
Surgical complications		
Hematoma	0.2 – 1.9 %[18,24,25]	
Infections	2.0 – 7.0 %[18,24,25,32,35-40]	2.9 – 12.5 %[34,41-43]
Vocal cord paralysis	1.0 – 5.6 %[18,24,25,35,38,39]	1.4 %[34]
Facial palsy	0.2 %[18]	
Pain and sensory related	1.1 – 2.0 %[18,36,38,40]	2.4 %[34]
Bradycardia	0.7 – 9.95 %[24,39]	
Puncture of jugular vein	2.1 %[24]	
Large cutaneous nerve cut off	0.7 %[24]	
Aseptic reaction	0.2 – 2.9 %[18,36]	
Cable discomfort	0.2 %[18]	
Surgical cable break	0.2 %[18]	
Oversized stimulator pocket	0.2 %[18]	
Battery displacement	0.2 %[18]	
Technical complications		
Lead fracture/lead malfunction	2.9 – 11.9	0.0 – 20.8 %[34,41-43]
Spontaneous VNS turn on	0.2 – 1.4 %[18,24]	
Lead disconnection	0.5 – 2.8 %[18,24,39]	

Stimulation tolerability

The most common stimulation related side effects are voice alterations, hoarseness, cough, dyspnea, paresthesia or tingling sensation, headache, and pain[45]. Usually they are mild to moderate and most often improve with time[46]. Stimulation related side effects can also be reduced or avoided by altering stimulation parameters, usually by reducing the output current, but also by lowering stimulation frequency and decreasing pulse width[47]. Hoarseness and voice alterations are the results of efferent stimulation of the recurrent laryngeal nerve with its innervation of the striated muscles of the larynx which branches off distally to the implanted electrodes[48,49]. Paresthesia or tingling sensation is believed to be a result of secondary stimulation of the superior laryngeal nerve, which supports the laryngeal mucosa with sensory nerve fibers and branches off from the vagus nerve proximally to the implanted electrodes[50]. The possible cardiac side effects of VNS, such as bradycardia, ventricular asystole, and complete heart block, are constantly under discussion from a safety perspective. They mainly occur in the operating room during initial device testing, and rarely emerge years after VNS implantation[27-30]. Possible reasons for this phenomenon could be polarity reversal of the leads during implantation casing efferent instead of afferent stimulation[51], indirect stimulation of the cervical cardiac nerves,

technical malfunction of the implanted device, differences in anatomical innervation, or accidental over-manipulation of the nerve when placing the leads around it. Implantation of the electrodes can be more or less challenging and time consuming due to anatomical variations in the implantation area. Only sporadic cases of late VNS related cardiac side effects have been reported in the literature[52-54]. Another possible effect of stimulating efferent fibers of the vagus nerve is hypersecretion of gastric acid due to its efferent innervation of the visceral mucosa. However, no significant side effects on gastrointestinal vagus nerve function, such as gastric ulcers, have been reported in both short- and long-term studies[13,55]. Unique side effects such as drooling and increased hyperactivity have been reported in children[56].

Epilepsy

Epilepsy is the second most common neurological illness after cerebrovascular disease, with a prevalence of approximately 0.5–1.0 %. An estimated 50 million people suffer from epilepsy worldwide.[57-59] In Sweden there are about 60.000 people suffering from epilepsy[60]. The causes of epilepsy are heterogeneous, ranging from genetic defects, structural abnormalities, metabolic diseases, infections of the central nervous system (CNS), neurodegenerative disorders, brain injury, stroke, to brain tumors[61]. Classification of epileptic seizures are divided into focal onset seizures that are conceptualized as originating at some point within networks limited to one hemisphere, and generalized seizures that are conceptualized as originating at some point within one hemisphere and rapidly engaging bilaterally distributed networks. Focal seizures can manifest themselves differently and vary in severity depending on the area of onset. They may occur with or without affecting the patient's consciousness or awareness, and can also spread to become a bilateral seizure. Genetic generalized seizures are subdivided into tonic-clonic, absence, myoclonic, clonic, tonic, and atonic seizures. These are further subdivided according to the clinical and electroencephalographic (EEG) features[62]. Patients are evaluated with respect to medical history, semiology, imaging (magnetic resonance tomography, MRI), and EEG.

Epilepsy is a disease of the brain defined by any of the following conditions:[59]

1. At least two unprovoked (or reflex) seizures occurring > 24 h apart

2. One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years

3. Diagnosis of an epilepsy syndrome

Antiepileptic drugs (AEDs) are by far the most common and usually the first-line treatment for epilepsy. The mechanisms of action of AEDs at a molecular level are not yet fully understood, and in some cases seem to have multiple molecular targets[63]. The main mechanisms, however, are involved in restoring the cellular firing equilibrium within the brain through blockage of voltage-gated ion channels. Thus, potentiating inhibitory neurotransmitters and modulating excitatory transmission[64]. Most patients can successfully be treated with one or multiple AEDs, but despite the continuing development of new AEDs, and the rapid progress of diagnostic techniques, 20–30 % of patients with epilepsy do not respond to treatment sufficiently[65,66]. This may have a devastating effect on both patients and their families, as DRE can cause major individual suffering and poor quality of life. DRE can be defined as a seizure frequency exceeding one per month and failure of more than two AEDs[67,68]. If complete seizure control is not achieved with trials of two appropriate AEDs, the likelihood of success with subsequent regimens is much reduced and drops to about 5 %[69,70]. Adverse effects from AEDs such as somnolence, dizziness, and cognitive impairment are common and can become intolerable even if the drug itself is effective in treating the epilepsy. It may necessitate either discontinuation or dose reduction if symptoms are felt to be unbearable[71]. Other treatment options available are participating in clinical trials of newly developed AEDs, epilepsy surgery, dietary treatments, immunological treatments, and neuromodulation[72].

VNS is usually used as a palliative antiepileptic treatment for patients that have been evaluated with respect to possible epilepsy surgery, or have been subjected to failed epilepsy surgery. In Sweden it is the consensus that patients with DRE must first be evaluated for the possibility of resective epilepsy surgery before being offered VNS.

VNS mechanism of action in epilepsy

Today the most common and widely used neuromodulatory treatment for DRE is VNS[73]. Since its introduction as a non-pharmacological treatment for epilepsy, numerous studies have been conducted to explain and understand its mechanisms of action. Despite extensive research no single mechanism of action has been shown to mediate the antiepileptic effect of VNS, hence the mechanism is believed to be complex and multifactorial. It is feasible that the VNS effect in humans is primarily mediated by afferent A- and B-fibers[74,75]. Selective destruction of C-fibers with capsaicin does not affect VNS-induced seizure suppression in rats[76]. Moreover, therapeutic VNS appears to be sub-threshold for C-fibers[48].

Functional imaging studies have shown that unilateral VNS affects both cerebral hemispheres via projections from the NTS to higher cerebral nuclei[77,78]. Widespread VNS-induced metabolic changes occurred in brain regions involved in seizure generation including the thalamus, cerebellum, orbitofrontal cortex, limbic system, hypothalamus, and medulla[79]. Initially it was hypothesized that the main mechanism of action of VNS consisted of desynchronization of neuronal activity, since epilepsy is considered to be a disease of cortical origin as well as the fact that epileptic seizures are characterized by highly synchronized EEG activity. VNS has also been shown to alter EEG activity in animal studies[80-84]. Furthermore, experimental animal studies have demonstrated that VNS reduces cortical excitability and decreases interictal epileptiform EEG discharges[15,85-88]. Brain structures that have been shown to play a role in regulation or generation of seizures, such as the amygdala, the hippocampus, and parts of the thalamus, are directly and indirectly connected to the vagus nerve via the NTS in the brain stem and could cause desynchronization as a result of VNS[89-91]. There is increasing evidence that these afferent polysynaptic pathways from the NTS to cortical regions mediate its antiepileptic action through an increased synaptic activity in the thalamus and thalamo-cortical projection pathways, and through a decreased synaptic activity in the limbic system[11].

A modulated release of several neurotransmitters have also been linked to the antiepileptic effect of VNS. VNS have been shown to decrease the levels of excitatory neurotransmitters in cerebrospinal fluid[92-94]. VNS also induces increased levels of the inhibitory neurotransmitter GABA[92]. It has been hypothesized that VNS is effective because it affects the ratio between the excitatory neurotransmitter glutamate, which is extensively released during seizures, and GABA[95]. Moreover, locus coerulius (LC), the most important source of noradrenaline (NA) in the brain, seems to be crucial for the

antiepileptic effects of VNS since the seizure-suppressive effects of VNS were averted by lesioning the LC[96]. The vagus nerve both directly and indirectly projects to the LC and raphe nuclei[4,5,97,98], and basal firing rate of serotoninergic neurons in the dorsal raphe nucleus of the rat have also been shown to increase after chronic VNS[99-101]. Furthermore, there is growing evidence suggesting that nitric oxide and acetylcholine release can be mediated by VNS[102,103]. Hippocampal plasticity may also play a role in the antiepileptic action of VNS, possibly mediated via an increased NA concentration[104,105]. Moreover, VNS has been shown to induce an increase in the extracellular hippocampal concentrations of NA, and at the same time decrease seizures in an animal model[106]. Interestingly, in this study there was also a "responder-rate" similar to observations in clinical trials. Growing evidence suggests that VNS has a neuroimmunomodulatory effect, which is believed to be another antiepileptic mechanism of VNS[107-110].

Depression

Major depressive disorder (MDD) is a life-threatening disease with increased risk of mortality and severe human suffering for the affected individuals. The lifetime prevalence is reported to be as high as 16–17 %[111,112]. Depression is a global illness found in all races, cultures, and socioeconomic groups. The World Health Organization estimates that MDD will be the second largest cause of global disease in the world by 2020 after ischemic heart disease[113]. The prevalence of the depressive disease has been continually increasing in recent years with an alarming trend of increasingly younger people being afflicted. The one-year prevalence in Sweden is estimated to be 5–8 %[114] costing society approximately 35 billion Swedish kronor per year[115]. The cause of depression is multifactorial, but there are two main hypotheses for the development of depression in humans, the monoamine and the neural plasticity hypotheses. The monoamine hypothesis proposes that depression is caused by a deficiency in monoaminergic levels and transmission, mainly serotonin (5-HT) and NA[116-118], and current pharmacological treatments are mainly focused on restoring this chemical imbalance in different ways by increasing the levels of monoamines in the CNS[119]. Although antidepressants produce a rapid increase in extracellular levels of NA and 5-HT, the onset of an appreciable clinical effect usually takes at least 3 to 4 weeks, and this delay suggests that slow neurochemical and structural changes take place within the limbic target areas of monoaminergic projections[120]. More recently a new hypothesis for the development of depression in humans has been formed which includes neurogenesis as a factor of importance in the depressive disease. The hypothesis states that reduction of neurogenesis in the hippocampus is a causality factor in the generation of depression, and that

stimulated neurogenesis is part of the recovery process from the depressive state[121,122]. This theory was proposed when the birth of new neurons from neuronal stem cells, the process called neurogenesis, was discovered and observed in the adult brains of both rodents and humans[123,124]. This process of neurogenesis is especially prominent in the hippocampus, a brain area and part of the limbic system known to be involved in mood and memory functions[125].

Over the last three decades antidepressant pharmacological treatment have been successfully developed, and new medications are continuing to emerge on the market. Despite this, up to 20 % fail to respond appropriately to antidepressant treatment[126] and, furthermore, the risk of recurrence and chronic depression increases with failure to reach full clinical remission[127]. Some of the chronic and recurrent cases can be successfully treated with electroconvulsive therapy, although usually temporarily. Thus, it is important to find new non-pharmacological well-tolerated treatments with mild to moderate side effects. VNS treatment can be an adjunctive long-term treatment for depression, and it can be offered to patients with chronic or recurrent depression who are experiencing a major depressive episode and have not had an adequate response to four or more antidepressant treatments[128]. Although approved in the USA as well as in Europe, the Swedish National Board of Health does not recommend the use of VNS as an adjunctive antidepressant treatment, as they still consider the evidence for clinical efficacy to be limited[129].

VNS mechanism of action in depression

Early on, positive effects on mood were reported in patients treated with VNS for epilepsy[130], even regardless of the effects on seizure frequency[131,132]. Several clinical trials have later shown beneficial effects of VNS on depression[133-135]. However, the underlying mechanism of action of VNS on depression is still not fully known. As described earlier hippocampal plasticity seems to be affected by VNS, and a possible mechanism is increased levels of NA and 5-HT in the hippocampus. Earlier studies in rat have shown an increased firing rate of neurons in both the LC and the dorsal raphe nucleus as a result of VNS[99,100,105]. This could increase the progenitor cell proliferation and possibly facilitate adult neurogenesis, the production of new and fully functional neurons within the brain of an adult organism. Adult neurogenesis is primarily restricted to the subventricular zone and the subgranular zone of the dentate gyrus (DG) of the hippocampus[123]. These neurons are generated from neural stem and progenitor cells in the subgranular zone and migrate into the granular cell layer, where they differentiate into neurons[136]. These cells are then integrated into the

hippocampal circuitry[137,138]. Hippocampal volume-loss in patients with MDD has been reported in several studies. A large amount of data suggests that increased levels of cortisol results in neuronal death in the hippocampus in animal models[139,140]. Hypercortisolism is known to be an important factor in stress-induced depression[141], and is a strong inhibitor of neurogenesis[142]. About 40-60 % of medicine-free depressed patients exhibit pathologically high levels of cortisol[143,144]. It has been shown that decreased hippocampal volume may be a sensitive marker of underlying brain pathology in MDD[145-147], and that hippocampal volume may predict clinical outcome in major depression[148]. Several studies have shown that antidepressants induce hippocampal volume increase in both animal models and humans with depression compared to healthy controls[149-151]. MRI studies show smaller (10–20 % reduction) hippocampal volumes in depressed patients[152,153], which could indicate a decreased neurogenesis or increased neuronal apoptosis in this brain structure. Recent studies have shown that hippocampal volume increase after electroconvulsive therapy in patients with depression, suggesting a dynamic response to the treatment[154,155]. In animal studies, inhibition of hippocampal neurogenesis by irradiation impairs antidepressant efficacy[120].

Short-term VNS in rats showed an increase in progenitor cell proliferation in the DG after 48 hours, suggesting a rapid effect of VNS; however, the survival of the progenitor cells was not affected by VNS[156]. Similarly, another study in rats, using short-term (3 hours) and long-term (1 month) VNS, found increased progenitor cell proliferation only in the short-term experiments[157]. On the other hand, chronic VNS induced a long-lasting increase in the expression of brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF), important modulators of hippocampal plasticity and neurogenesis[104,157]. Electroshock therapy has also been shown to stimulate cell proliferation in the hippocampus more rapidly than antidepressant drugs in animal studies[158].

Taken together, there is convincing data supporting VNS effect on hippocampal plasticity and depression. However, these studies are mainly performed on animals, and there is still need for further clinical trials in evaluating the efficacy of the actual treatment.

VNS and obesity

Obesity is an increasingly growing problem in the developed and the developing world[159]. In some countries such as the USA the prevalence is reaching epidemic proportions[160]. A major concern is the increased risk of accompanying comorbidities, such as diabetes, cardiovascular disease, and cancer[161]. So far only bariatric surgery (weight loss surgery) has demonstrated long-term therapeutic effects, and therefore the use of surgery to treat obesity is on the rise[162,163]. Nevertheless, this elective surgery carries risks for considerable morbidity and potential mortality. A large meta-analysis of >22,000 patients reported the mortality rate for gastric bypass at 0.5 %[164], with different studies publishing a mortality range of 0 % to 1.5 %[165-168]. Considering the risk for surgical complications and high surgery-related costs, it has been proposed that the development of minimal invasive procedures to treat obesity is urgently needed[169]. The central role of the vagus nerve in the regulation of food intake and energy expenditure, the vagal afferent activity or the so-called gut-brain axis, is activated by mechanoreceptors and chemoreceptors in the gut and converge in the NTS of the brainstem. Neuronal projections from the NTS, in turn, carry signals to brain areas such as the hippocampus and hypothalamus.[10] The hypothalamus and the brainstem are the main CNS regions responsible for the regulation of energy homeostasis[170]. The NTS governs the responses of the organs responsible for energy and metabolic control through the dorsal motor nucleus of the vagus nerve and its efferent fibers. This makes the vagus nerve an ideal target for new less or noninvasive procedures to treat obesity. VNS has been shown to have a positive effect on weight reduction in experimental studies[171,172]. Conversely, it did not affect body weight in VNS treated patients with epilepsy[173,174]. Recently, the Food and Drug Administration (USFDA) has approved vagal blocking therapy (VBLOC), by which an intra-abdominal electrical device with leads is placed laparoscopically around the vagus nerve, as a new treatment for obesity[175,176]. It is hypothesized that VBLOC activates the vagal signaling to the brainstem and hippocampus and blocks the vagal signaling to the gut, leading to increased satiety, reduced food intake, and eventually loss of body weight. However, the mechanism of action is unclear and remains to be further elucidated.

Effectiveness of VNS in epilepsy

In the USA, VNS is currently approved as adjunctive therapy for partial onset seizures in patients over 12 years of age, with medically intractable partial seizures who are not candidates for potentially curative surgical resections such as lesionectomies or mesial temporal lobectomies. In Europe, VNS is approved at any age for patients with refractory epilepsy. In the recently updated guideline from the American Academy of Neurology (AAN), it is stated that: "VNS may be considered for seizures in children, for Lennox-Gastaut syndrome-associated seizures, and for improving mood in adults with epilepsy. VNS may also be considered to have improved efficacy over time. Especially children should carefully be monitored for site infection after VNS implantation"[177]. In general, ≥50 % seizure reduction rate is considered as an effective VNS treatment. Since its introduction, several meta-analyses and reviews regarding treatment efficacy has been presented, including a Cochrane review on VNS for partial seizures (updated in 2015)[178]. As is the usual scenario, the randomized-control trials were all short-term studies and did not account for long-term follow-up concerning efficacy and safety[12,131,179,180]. Many of the registry and retrospective long-term studies investigating the efficacy of VNS show that there is an approximately 50 % seizure frequency reduction in about 40–60 % of the implanted patients[25,41,181]. However, when analyzing the majority of these studies, there is a widespread variation in follow-up time, number of patients included, and duration of study. Data from 48 long-term follow-up studies (mean ≥12 months) are presented in Table 2. Three of the studies are prospective open label studies without randomization. One with exclusively a pediatric population, one with no AED changes during its 18 months of follow-up, and one that comprises of patients with low IQ. Only one additional study has investigated the efficacy of VNS without changing AEDs for 12 months[182]. Approximately half of the studies take into account what kind of AEDs the patients are treated with, but only a limited number of the studies describes the actual changes in AEDs and the effect on seizure frequency[46]. So far, there have been no studies attempting to match the number of AEDs with the individual seizure frequency reduction. Usually the variation in the number of AEDs is presented for the cohort as a whole.

Different evaluation scales have been used to determine the efficacy of VNS. Thus, comparing results can be challenging since different studies use different protocols and evaluation methods. The Engel classification was originally suggested as a standard outcome scale after resective epilepsy surgery[183], but has been modified and used in VNS studies. The McHugh classification was, however, proposed as an evaluation scale for patients

treated specifically with VNS[184]. The McHugh classification has "magnet use only" as a single parameter, but only a small number of studies account for the magnet use.

Considering that a large number of patients treated with VNS have multiple medications both prior and after VNS implantation, it is important to analyze how these medications might influence both VNS and patient outcomes. Since many patients with DRE also suffer from comorbidities, there may be other medications and possible interactions to consider as well[185]. Only a small number of studies take into account other measures, such as quality of life, improved life situation, activity of daily life, cost-benefit analyses, and number of hospital admissions[186-188]. The ongoing discussion about efficacy and safety should in the future include an overall effectiveness evaluation, including quality of life and possible AED alterations.

Table 2. Long-term VNS studies in the literature

Study	No. cases	Seizure type	Notes	Follow-up (months)	No. of centers	% resp-onders	AED changes reported
Pakdaman et al., 2016[189]	44	Mixed		60	Single	11	Yes
Majoie et al., 2005[190]	19	Mixed	Children Prospective	24	Single	21	.No
Lund et al., 2011[191]	48	Mixed	Learning disabilities	16-143 mean 55	Single	25	Yes
Ardesch et al., 2007[192]	19	Focal		24-72 mean 48	Single	25 (6 yrs)	Yes
Huf et al., 2005[193]	40	NR	Adults, low IQ Prospective	24	Single	28	Yes
Galbarriatu et al., 2015[194]	59	Mixed		6-102 mean 39	Single	34	No
Vale et al., 2011[188]	37	Mixed	QoL assessm.	18-120 mean 60	Single	35*	Yes
Buoni et al., 2004[195]	13	Mixed		8-36 mean 22	Single	38	No
Spanaki et al., 2004[196]	26	Mixed		60-84 mean 67	Single	38	Yes
Hui et al., 2004[197]	13	Mixed		18-71 mean 47	Single	40	No
Menascu et al., 2013[198]	44	Mixed	QoL assesm.	18	Single	43	Yes
Benifla et al., 2006[199]	41	Mixed	Children	6-72 mean 31	Single	43	No
Orosz et al., 2014[200]	347	Mixed	Children	24	Multi	44	Yes
Morris et al., 1999[46]	440	Mixed		36	Multi	44	Yes
Ben-Menachem et al., 1999[201]	64	Mixed		3-64 mean 20	Single	45	No
Murphy et al., 2003[43]	96	Mixed		12-108 mean 32	Single	45	Yes
Arhan et al., 2010[202]	24	Mixed	Children	6-100 mean 41	Single	45	No
Scherrmann et al., 2001[21]	95	Mixed		6-36 mean 16	Single	45	No
Tanganelli et al., 2002[40]	47	Mixed		6-50 mean 26	Single	47	No
Majkowska-Zwolinska et al., 2012[203]	56	Mixed	Children	12-48 mean 35	Single	50	Yes
Saneto et al., 2006[204]	43	Mixed	Children	7-40 mean 18	Single	51	No
Coykendall et al., 2010[42]	28	Mixed	Children	3-96 mean 41	Single	52 (1yr)	No
Vonck et al., 2008	27	NR		19-71, mean 42	Single	52	Yes
You et al., 2007[205]	28	Mixed	Children	12-79 mean 31	Single	54	No
Vonck et al., 2004[206]	118	Mixed	Prospective	6-94 mean 33	Multi	55	Yes

Study	No. cases	Seizure type	Notes	Follow-up (months)	No. of centers	% responders	AED changes reported
Kabir et al., 2009[36]	69	Mixed	Children	6-120 mean 46	Single	56 (Engel class I-III)	No
Alexopoulos et al., 2006[41]	46	Mixed	Children	12-48 med. 24	Single	59	Yes
Aburahma et al., 2015[207]	28	Mixed	Children	36-78 mean 62	Multi	59	No
De Herdt et al., 2007[181]	138	Mixed		12-120 mean 44	Multi	59	Yes
Uthman et al., 2004[208]	48	Focal		4-144 mean 38	Single	60	Yes
Qiabi et al., 2011[209]	34	Mixed		24-46 mean 30	Single	60 (3 yrs)	Yes
Choi et al., 2013[210]	20	Mixed		48	Single	60 (4 yrs)	Yes
Ryzi et al., 2013[211]	15	Focal	Children	24 and 60	Single	60 (2 yrs) 60 (5 yrs)	Yes
Chavel et al., 2003[212]	29	Focal		mean 20	Single	61	No
Arcos et al., 2014[213]	37	Mixed		mean 39	Single	62	No
Garcia-Navarrete et al., 2013[214]	43	Mixed	Prospective	18	Single	63	Yes
Nagarajan et al., 2002[215]	16	Mixed	Children	6-47 mean 25	Single	63	Yes
Serdaroglu et al., 2016[216]	56	Mixed	Children	60-186 mean 87	Single	63	Yes
Kuba et al., 2009[38]	90	Mixed		66-82 mean 79	Multi	64 (5 yrs)	Yes
Meng et al., 2015[217]	94	Mixed		6-65 mean 42	Multi	64	No
Elliott et al., 2011[25]	400	Mixed		3-132 mean 59	Single	64	Yes
Elliott et al., 2011[218]	141	Mixed	Children	1-137 mean 62	Single	65	Yes
Vonck et al., 1999[219]	15	Mixed		12-48 mean 29	Single	67	Yes
Kawai et al., 2002[220]	13	Focal		48-91 med. 56	Single	69	Yes
Rychlicki et al., 2006[44]	34	Mixed	Children	3-36 mean 31	Single	71	Yes
Bodin et al., 2016[221]	29	Mixed	Children	24 and 60	Single	75	Yes
Al Said et al., 2015[222]	26	Mixed		24	Single	78	Yes
Elliott et al., 2011[223]	65	Mixed		120-139 mean125	Single	91	Yes

*AED = antiepileptic drugs, responders = patients with ≥50 % seizure reduction, * responders = >30 % seizure reduction*

VNS and other treatments

Regarding the widespread afferent and efferent innervation of the vagus nerve to multiple inner organs, and its projections to multiple higher subcortical and cortical brain centers, VNS has been evaluated in the treatment of several other disorders. Most of the theories behind using VNS as an alternative treatment for other illnesses have developed after case reports from patients receiving VNS for epilepsy, such as the positive mood effects noticed early on[130]. Several studies on the possible neuroprotective effects of VNS have been conducted showing a possible effect on cognition[224,225]. These cognitive effects are believed to be mediated via an increase of noradrenergic levels in the hippocampus[226]. A small study on Alzheimer's disease was performed where patients were evaluated with neuropsychological tests. The results suggested a positive impact on the disease and VNS was well tolerated in this older age group[227]. However, further studies are needed to evaluate the mechanisms and effects of VNS treatment on Alzheimer's disease and cognition.

There are an increasing number of studies supporting the use of VNS for multiple sustained pain conditions such as chronic pelvic pain, fibromyalgia, trigeminal allodynia, as well as chronic headaches and migraine. The mechanisms of action are still unclear, but there is increasing evidence suggesting anti-inflammatory effects working in conjunction with both central and peripheral pain pathways[228].

In chronic heart failure, reduced vagus nerve activity is associated with increased mortality[229] and is characterized by an autonomic imbalance with increased sympathetic activity[230]. VNS has been shown to be beneficial in chronic heart failure in both experimental and clinical studies with improved left ventricular hemodynamics and decreased mortality[230-232]. However, in the recently published multicenter randomized INOVATE-HF trial, there was no reduction in the rate of death or heart failure events in chronic heart failure patients[233].

In recent years there has been increasing evidence that VNS is neuroimmunomodulatory. This could possibly reduce the inflammatory response to brain ischemia and decrease the extent or improve the recovery after stroke. Conceivably it could also suppress inflammation in rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.[234-237]

In summary, with its widespread connections to the CNS, stimulation of the vagus nerve can potentially affect the entire human body. It has been suggested that an adequate name for the vagus nerve should be "the great wandering protector" considering its involvement in autonomic, cardiovascular, respiratory, gastrointestinal, immune, and endocrine systems[4].

AIMS

The overall aim of this thesis was to contribute to the understanding of the mechanisms of action of VNS and to evaluate VNS therapy from a safety and effectiveness perspective.

The specific aims were:

- To determine if a possible mechanism of action of VNS is the proliferation or increased survival of hippocampal progenitor cells. (**Study I**)

- To study the feasibility of a novel treatment for obesity in a rat model. (**Study II**)

- To describe the panorama of surgical and hardware complications to VNS implantation since its introduction in clinical trials at a single center. (**Study III**)

- To investigate the efficacy of VNS in combination with pharmacological therapy in a longitudinal study at a single center. (**Study IV**)

MATERIALS AND METHODS

Methods when studying progenitor cell proliferation and survival in rat (study I)

Animals

The study was approved by the Gothenburg Regional Ethics Committee for Animal Experiments.

All animals were male Sprague-Dawley rats weighing 200–300 g. Animals were kept and handled in accordance with the regulations of the Swedish Animal Welfare Agency. Animals were housed two per cage under standard conditions, including ad libitum access to food and water in a 12 h light/dark environment. All procedures were performed during the light period. In total, a number of 52 rats that were implanted with a VNS pulse generator. Six were excluded from the study because of adverse events, most commonly edema at the generator skin pocket. Approximately one-third of the rats developed a left sided postoperative ptosis, which persisted throughout the study period.

Surgery

All surgeries were performed under general anesthesia. To decrease postsurgical pain, the animals received subcutaneous buprenorphine preoperatively (0.03 mg/kg) and subcutaneous sterile saline (10 ml/kg) postoperatively. A ventral midline incision was made on the neck. The skin and muscles were retracted, and the left carotid artery and vagus nerve was identified. The left cervical vagus nerve was dissected from the carotid artery and the lead electrodes were wrapped around the nerve under microscopic control. The lead was then tunneled under the skin and the VNS therapy pulse generator NCP (Neuro Cybernetic Prosthesis) Model 102 (Cyberonics, Inc.; Houston, Texas) was connected and implanted in a subcutaneous pocket on the back of the rats, after which the wounds were closed.

Study protocol

After VNS surgery, rats were randomly assigned to either VNS, with output currents of 0.25 mA, 0.75 mA, 1.5 mA, or sham stimulation. All output currents were delivered at 20 Hz, 250 µsec pulse width, and duty cycle 30 s on and 5 min off. Twenty-four hours after surgery, the impedance of the stimulating electrodes was tested, and VNS was initiated for the rats assigned to the stimulation group. In the progenitor proliferation experiment, VNS

was delivered for 48 h after which the rats were sacrificed, and in the progenitor survival experiment, VNS was delivered for 2 weeks. Stimulator parameters were set according to an earlier study investigating VNS in rat and hippocampal NA increase[105].

Bromodeoxyuridine (BrdU) labeling was used to detect proliferating cells. BrdU is a thymidine analogue that is being incorporated into new DNA, which is formed during the cell division process. To detect the proliferation of progenitor cells, the rats received intraperitoneal injections of BrdU (200 mg/kg) at 7:00 a.m. and 7:00 p.m. on day 2, and at 7:00 a.m. at day 3 of the experiment. The last injection was given 7 h before sacrifice. In this manner BrdU-incorporation into dividing cells occurred during several time points of the VNS treatment, enabling the evaluation of possible changes in proliferation rate in VNS-treated rats as compared to sham-operated animals.

To analyze the effect of VNS on progenitor survival, BrdU was administered before the VNS stimulation was started[149,238]. BrdU (75 mg/kg) was administered 4 times with 2 hours in between to VNS implanted rats with devices turned off. VNS was then started 24 h after the last BrdU-injection. After two weeks of stimulation the VNS device was turned off, and the animals were left without stimulation for 2 weeks before sacrifice. Experimental paradigms are presented in Figure 4.
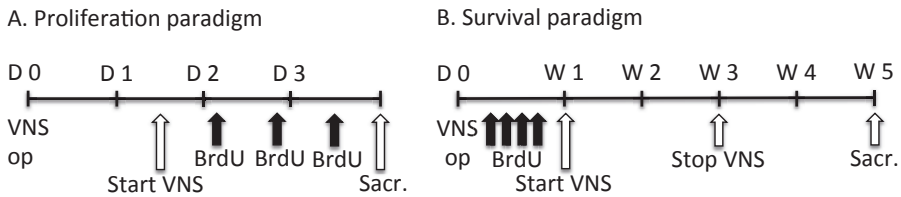
Figure 4. Experimental paradigms. (A) Proliferation paradigm. VNS electrodes and stimulators were implanted and activated at day 1 in half of the rats, whereas the other half were sham treated rats identically handled but with no activation of the implanted VNS devices. On day 2 and day 3 BrdU-injections were given every 12th hour (3 injections in total) and the animals were sacrificed 7 h after the last BrdU-injection. (B) Survival paradigm. To examine the influence of VNS treatment on the survival of BrdU-labeled cells, BrdU was given to the animals before initiating VNS treatment. Rats were then sacrificed 28 days after BrdU-injection (14 days after the last VNS treatment). The longer treatment time was used to ensure that even a small effect on survival would be detected.

Tissue preparation

Rats were anesthetized and decapitated. The brains were removed and fixed in 5 % buffered formaldehyde for 24 h and thereafter kept in 30 % sucrose solution at 4 ºC. Sectioning was performed on a freeze microtome. The immunohistochemistry experiments were performed on free-floating sections. In brief, the sections were denatured, endogenous peroxidase activity was removed, and sections were incubated with a monoclonal mouse anti-BrdU IgG antiserum. Sections were washed, incubated with biotinylated secondary horse anti-mouse IgG antiserum and allowed to react with avidin-peroxidase. Finally, cells incorporated with BrdU were visualized with a detection solution.

Data collection

In each rat, the number of BrdU-positive cells in the granule cell layer (GCL) (including the subgranular layer) was determined in 12 immunoperoxidase-stained, 40-µm-thick coronal sections per DG taken 240 µm apart. The number of BrdU-positive cells was counted within the GCL and two cell diameters below the GCL, ignoring the cells in the uppermost focal plane and focusing through the thickness of the section to avoid errors caused by oversampling[239]. Microscopy images were obtained with a Nikon Diaphot microscope equipped with a CCD camera. The results are expressed as mean BrdU-positive cells per section.
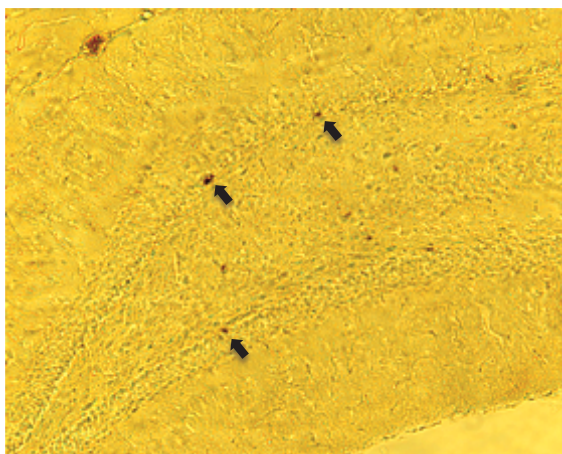
Figure 5. BrdU-positive cells in the hippocampus identified as black dots representing single cells or clusters of 3–10 cells (arrows). Most of the BrdU-positive cells showed irregularly shaped nuclei and coarse patterns of BrdU-staining, which are features of immature and dividing cells. (Courtesy of Journal of experimental neurology)

All samples were assigned a code that obscured the identity of samples for the examiner. A limited number of VNS devices were available for the experiments, and this forced the extensive dose-response experiments to be performed at two separate time points.

Methods when analyzing VBLOC in rat and its possible mechanism of action (study II)

Animals

The study was approved by the Norwegian National Animal Research Authority (Forsøksdyrutvalget, FDU).

Animals were either male Sprague-Dawley rats (pilot experiment) or female Long Evan rats (second experiment). All applicable institutional and national guidelines for the care and use of animals were followed. All animals were housed three to four together under standard conditions with free access to tap water and standard rat pellet with a 12 h light/dark cycle. In total a number of 25 rats were implanted with a VNS pulse generator.

Surgery

All surgeries were performed under general anesthesia. Atropine was given at a dose of 0.04 mg/kg subcutaneously 20 min before anesthesia. All animals were injected with buprenorphine subcutaneously (0.05 mg/kg) postoperatively and one day after surgery when needed. Sterile saline (10 ml) was administered subcutaneously after surgery to keep the animals hydrated.

VBLOC implantation was performed through a midline abdominal incision. The subdiaphragmatic vagus nerve was dissected from the esophagus and two electrodes (Lead Model 302, Cyberonics, Houston, TX) were wrapped around both the anterior and posterior nerve. The lead was then tunneled under the skin and connected to the VNS therapy pulse generator NCP (Neuro Cybernetic Prosthesis) Model 102 (Cyberonics, Inc.; Houston, Texas), which was implanted in a subcutaneous pocket on the back of the rats, and the wounds were closed. The sham and VBLOC rats underwent the same surgical procedure. Implantation of the µVBLOC device followed the same method. In the gastric acid secretion experiment, a gastric fistula was implanted in the stomach. Before this procedure the animals were fasted overnight, and after surgery they were given water immediately and food the same evening.

Study protocol

The paradigm of VBLOC between 0.5 and 2.0 mA with 30 Hz, 500 µsec pulse width, 30 s on, and 5 min off was chosen according to the safety, tolerance, and possible efficacy assessments in response to the long-term VBLOC.

Short-term VBLOC

In the first experiment, rats (Sprague-Dawley) received VBLOC (2 mA, 30 Hz, 500 µsec pulse width, 30 s on, and 5 min off) while they were subjected to electrophysiological recordings in the hippocampus. In the second experiment, rats (Sprague-Dawley) that had implanted gastric fistulae were acclimatized to Bollman cages for 3 h at three separate occasions before and after VBLOC and gastric fistula implantation, and then subjected to gastric acid secretion measurement (at baseline and after pentagastrin stimulation). After gastric acid output measurement, VBLOC was turned off. Three days after, rats received VBLOC (2 mA, 30 Hz, 500 µsec pulse width, 30 s on, and 5 min off) for 48 h while eating behavior and metabolic parameters were measured in the Comprehensive Laboratory Animal Monitoring System (CLAMS)[240].

Long-Term VBLOC

In the first experiment (Sprague-Dawley rats), VBLOC was started 4 weeks after implantation. The device was then constantly on while the current was gradually increased during 6–8 weeks. Each rat was placed in CLAMS at four time-points for measurements of eating behavior and metabolic parameters 3 weeks after VBLOC implantation (before stimulation baseline), at 0.5 mA, at 1 mA, and at 2 mA stimulation. The settings were 30 Hz, pulse width 500 µsec, and the on and off time were 30 s and 5 min. The VBLOC and sham groups consisted of nine and four rats per group.

In the second experiment (Long Evan rats), we developed smaller VBLOC devices (µVBLOC) due to device-size-related irritation. The µVBLOC and sham groups consisted of four rats per group, and µVBLOC was continuously on at 2 mA, 30 Hz, 500 µsec, 30 s on, and 5 min off for 2 weeks. The sham group was implanted with the stimulators only, and no electrodes were used.

Tissue preparation

In the short-term experiment, rats were euthanized immediately after 48 h of VBLOC and brain samples were collected for in situ hybridization. Plasma was collected for radioimmunoassay. After euthanization in the first long-term experiment, brain samples were taken for Taqman array analysis and RNA sequencing. Plasma was collected for radioimmunoassay as well in the second long-term experiment.

Data collection

Rats were placed in the CLAMS, with free access to standard rat powder food and tap water. This system is composed of a four-chamber open circuit indirect calorimeter designed for continuous monitoring of individual rats. Eating behavior and metabolic parameters were recorded automatically. In

all studies performed, food intake was higher and satiety ratio lower during nighttime than daytime for all animals at every time-point.

Methods when compiling data on VNS safety and long-term efficacy (Studies III and IV)

The study was approved by the Gothenburg Regional Ethics Committee for Human Research.

In study III data from 247 consecutive patients implanted with a VNS device (Cyberonics Inc. or BioControl Medical) between January 1990 and December 2014 were compiled from patient journals and analyzed retrospectively. Apart from primary implantation, all stimulator replacements due to battery depletion were analyzed to determine the overall occurrence of surgical and hardware complications. In total 497 procedures were performed over the time period.

In study IV data from 130 consecutive patients implanted with a VNS device between the years 2000 and 2013 were analyzed. Seizure frequency and AED treatments were recorded prior to VNS implantation as well as at 1, 2, and 5 years after implantation.

Statistical analyses

Study I

All data are presented as means ± standard error of mean (SEM). Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for group comparisons in experiments that included more than two groups. For experiments involving two groups, the unpaired two-sided Student's t-test was used. Statistical significance was set at $p < 0.05$.

Study II

The results are expressed as mean ± SEM. Statistical comparisons were performed using independent t-test between the surgical groups. ANCOVA with Sidak test was performed for energy expenditure statistics, while ANOVA with Tukey's test was performed to determine eating behavior and metabolic parameters. A p-value of < 0.05 was considered statistically significant. The data analysis was performed in SPSS version 15.0 and 20.0 (IBM Corp., Armonk, NY, USA).

Study III

Continuous variables are presented both as a mean with standard deviation (SD) and as a median with first and third quartile (Q1, Q3). Chi-square test was used for the comparison of distributions within groups. A p-value below 0.05 was considered to be statistically significant. All statistical calculations were performed with SPSS version 22 (IBM Corp., Armonk, NY, USA).

Study IV

None of the continuous parameters were normally distributed (tested with Kolmogorov-Smirnov test and plotted in a histogram as well as a Q-Q-plot); thereby, data is presented as median with first and third quartile (Q1, Q3). Non-parametric tests (Mann Whitney U test and the paired Wilcoxon signed rank test) were used. Categorical parameters are presented with absolute numbers and percentage. For group comparisons, Fischer's Exact test was used. Paired tests were used to evaluate changes in seizure frequency and numbers of AEDs at the different follow-up times. All significance tests were two-sided and conducted at the 5 % significance level. IBM SPSS Statistics, version 23 (IBM Corp, Armonk, NY, USA) was used for statistical analysis.

RESULTS

Study I

In the progenitor proliferation study, a significantly greater number of BrdU-positive cells were seen in the 0.75 mA group compared to the sham control group, with 22.6 ± 2.1 respectively 14.6 ± 2.0 BrdU-positive cells per hippocampus slice (p=0.04). Regarding the dose-response rate, 0.75 mA stimulation current presented a significant increase in BrdU-positive cells compared to sham (with a 54 % relative increase, p=0.004), which was not shown for 0.5 mA and 1.5 mA (23 %, p=0.34, and 19 %, p=0.36, respectively). The greatest effect on progenitor proliferation was at the 0.75 mA output current (ANOVA-Bonferroni correction; sham control n=15).
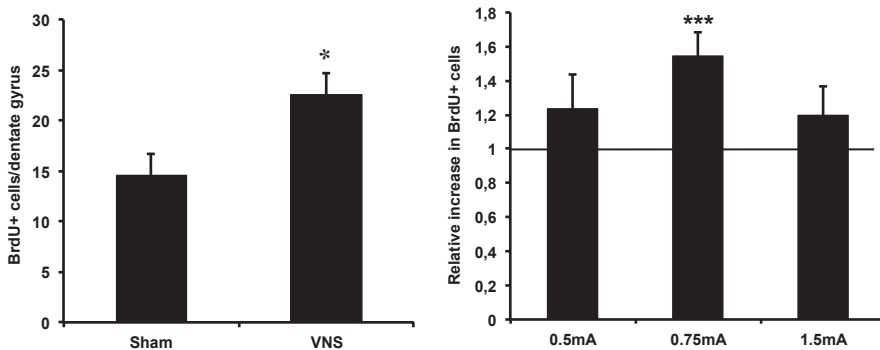
Figure 6. The number of BrdU-positive cells in the adult rat hippocampus was increased after VNS treatment for 48 h with 0.75 mA output current (mean ± SEM number of BrdU-positive cells; No. VNS=8, No. Sham=7). VNS treatment for 48 h also increased BrdU-positive cells in the adult hippocampus dose dependently. Output currents of 0.75 mA (No.=8), but not 0.5 mA (No.=7) or 1.5 mA (No.=6), significantly increased the number of BrdU-positive cells. (Courtesy of Journal of experimental neurology)

In the progenitor survival study, there was no significant difference in the number of BrdU-positive cells with 8.3 ± 1.3 and 6.7 ± 1.4 (± SEM) BrdU-positive cells per hippocampus slice in sham and VNS-treated animals respectively (p=0.420, Student's t-test, n=5.5). No difference in weight gain was observed between the sham and VNS-treated animals.
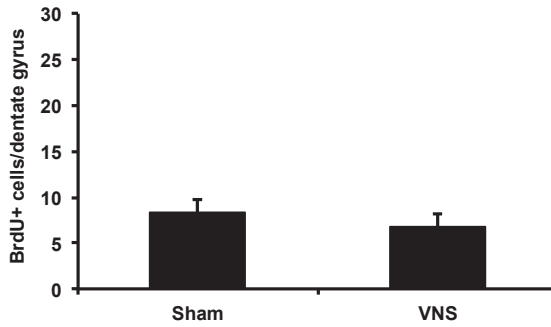
Figure 7. No significant effects were seen from VNS on survival of BrdU-positive cells in the dentate gyrus after a 4 week survival paradigm (mean ± SEM number of BrdU-positive cells; No. VNS=5, No. Sham=5). (Courtesy of Journal of experimental neurology)

Study II

In the short-term VBLOC study (48 h at 2mA current), no changes in body weight, food intake, eating behavior, or metabolic parameters including energy expenditure were observed.

In the long-term VBLOC, in which the current was gradually increased (from 0.5 to 2 mA), the body weight and food intake were reduced as the current increased, reaching reductions of 10 % (p>0.05) and 30 % (p<0.05), respectively. Energy expenditure (kcal/h/body weight) was reduced compared to baseline values. Satiety ratio, particularly during nighttime, was increased, but the number of meals ("hunger index") was unchanged. When the current was started at 2 mA, a 10 % body weight reduction was achieved within 1 week (p<0.05). Additional parameters of eating behavior and metabolism were unchanged after long-term VBLOC.

Study III

In total there were 37 surgical complications and 16 hardware complications in 47 different patients. The median follow-up time was 11.8 years (Q1 6.9, Q3 17.7), and the mean follow-up time was 12.0 years (SD 6.5). Fifty-five of the patients were children (age range 4–17). Table 3 shows a summary of the 497 surgical procedures performed. All complications are summarized in Table 4. Complications related to surgery occurred in 8.6 % of all procedures performed and the hardware complications rate was 3.7 %. The most common surgical complications were infection (2.6 %), postoperative hematoma (1.9 %), and vocal cord paralysis (1.4 %), and the most common

hardware-related complication was lead fracture/malfunction (3.0 %). The infection rate was 4 times higher between 2000 and 2014 compared to between 1990 and 1999 (3.3 % vs. 0.8 %, p=0.057). The rate of lead breaks was decreased 3.2-fold (2.3 % vs. 7.3 %, p=0.049) during the same time periods.

Table 3. All VNS related procedures performed between 1990 and 2014

Summary of surgical procedures	All	Children	Adults	1990-1999	2000-2014
VNS implantation	247	55	192	89	158
Plain stimulator replacement in one session	161	29	132	29	132
System replacement in one session	8	2	6	2	6
Plain lead replacement in one session	9	1	8	4	5
Reimplantation of stimulator	4	1	3	0	4
Explantation of stimulator	45	9	36	14	31
Lead exploration	5	0	5	4	1
Wound revision	5	0	5	1	4
Explantation of lead	4	1	3	0	4
Intentional stimulator replacement discont.	1	0	1	0	1
Intentional lead replacement discontinued	1	0	1	1	0
Reconnection of lead to stimulator	2	0	2	0	2
Repositioning of lead	1	0	1	1	0
Reposition of stimulator	2	0	2	1	1
Removal of postoperative hematoma	2	0	2	0	2
Total surgical procedures	**49**	**98**	**399**	**146**	**351**

Table 4. All VNS surgery related complications between 1990 and 2014

Complications	All	Children	Adults	1990-1999	2000-2014
Surgical complications (total)	37 (8.6 %)	6 (6.8 %)	31 (9.1 %)	11 (8.9 %)	28 (9.2 %)
Hematoma	8 (1.9 %)	1 (1.1 %)	7 (2.1 %)	2 (1.6 %)	6 (2.0 %)
Post primary implantation	7 (2.8 %*)	1 (1.8 %*)	6 (3.1 %*)	2 (2.2 %*)	5 (3.2 %*)
Post stimulator replacement	1 (0.6 %^)	0 (0 %^)	1 (0.7 %^)	0 (0 %^)	1 (0.7 %^)
Infections (all)	11 (2.6 %)	4 (4.5 %)	7 (2.1 %)	1 (0.8 %)	10 (3.3 %)
Post primary implantation	8 (3.2 %*)	4 (7.3 %*)	4 (2.1 %*)	0 (0 %*)	8 (5.1 %*)
Post stimulator replacement	2 (1.1 %^)	0 (0 %^)	2 (1.4 %^)	1 (2.9 %^)	1 (0.7 %^)
Post stimulator reimplantation	1 (% n. a.)	0 (% n. a.)	1 (% n. a.)	0 (0 %)	1 (0.3 %)
Vocal cord palsy (all)	6 (1.4 %)	0 (0 %)	6 (1.8 %)	3 (2.4 %)	3 (1.0 %)
Post primary implantation	5 (2.0 %*)	0 (0 %*)	5 (2.6 %*)	2 (2.2 %*)	3 (1.9 %*)
Post lead explant./replacement	1 (% n. a.)	0 (% n. a.)	1 (% n. a.)	1 (% n. a.)	0 (% n. a.)
Pain and sensory related	6 (1.4 %)	1 (1.1 %)	5 (1.5 %)	2 (1.6 %)	4 (1.3 %)
Technical complications (total)	16 (3.7 %)	0 (0 %)	16 (4.7 %)	9 (7.3 %)	7 (2.3 %)
Lead fracture/malfunction	13 (3.0 %)	0 (0 %)	13 (3.8 %)	8 (6.5 %)	5 (1.6 %)
Spontaneous VNS turn on	1 (0.2 %)	0 (0 %)	1 (0.3 %)	0 (0 %)	1 (0.3 %)
Lead disconnection	2 (0.5 %)	0 (0 %)	2 (0.6 %)	1 (0.8 %)	1 (0.3 %)

*Percentage of all n=247 / children n=55 / adults n=192 / female n=125 / male n=122 / 1990-1999 n=89 / 2000-2014 n=158 VNS primary implantations

^Percentage of all plain stimulator replacement in one session, system replacement in one session (stimulator and lead), plain lead replacement in one session, or reimplantation of stimulator (all n=174 / children n=33 / adults n=141 / female n=73 / male n=101 / 1990-1999 n=35 / 2000-2014 n=147)

Study IV

There was a significant seizure reduction overall (all p<0.001), and VNS efficacy increased with time. The responder (≥50 % seizure frequency reduction) rate increased from 22.1 % to 43.8 % between the first and fifth year, with the largest increase between the first and second year (22.1 % – 38.1 %), for the cohort as a whole and regardless of AED changes. Out of 76 patients with no seizure frequency reduction after 1 year, 12 patients (15.8 %) had a ≥50 % seizure frequency reduction and 2 patients (2.6 %) had a 1–49 % seizure frequency reduction at 2 years. Furthermore, out of 55 patients with no seizure frequency reduction after 2 years, 5 patients (9.1 %) had a ≥50 % seizure frequency reduction and 2 patients (3.6 %) had a 1–49 % seizure frequency reduction at 5 years. One out of 27 patients (3.7 %) who reported a ≥50 % seizure reduction after 1 year returned to baseline seizure frequency after 2 years, and 4 out of 43 patients (9.3 %) who reported a ≥50 % seizure reduction after 2 years returned to baseline seizure frequency after 5 years. Only 2 patients became seizure free.

VNS efficacy did not differ between patients who altered or remained on the same AEDs. Patients were treated with a median of 3 AEDs throughout the study but the number of AEDs significantly increased after 2 (p=0.007) and 5 (p=0.001) years. Table 6 shows the use and variation in AEDs during the study period.
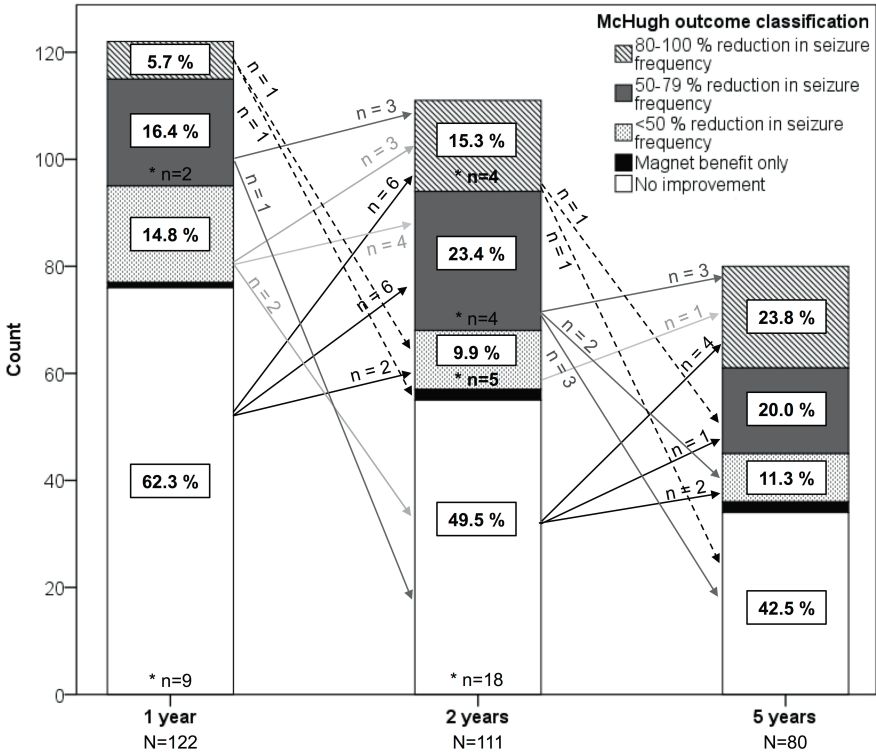

Figure 8. Seizure control outcomes at 1, 2, and 5 year follow-up with McHugh Outcome Classification. Arrows indicating changes in VNS efficacy between follow-ups. (*Patients lost to follow-up)

Table 5. Seizure frequency at follow-up

Table 4. Comparison of seizure frequency and number of AEDs as well as proportion of patiens with ≥50 % seizure reduction at the different follow-up times when stratified into subgroups (daily, weekly, and monthly seizures before VNS implantation).

1 year follow-up

	No.	Seizure frequency Before Median (Q1, Q3)	Seizure frequency 1 year Median (Q1, Q3)	p*	≥50% effect N (%)	Number of AEDs Before Median (Q1, Q3)	Number of AEDs 1 year Median (Q1, Q3)
Daily							
All	66	2.5/day (1.0, 5.0)	1.8/day (1.0, 4.6)	<0.001	13 (19%)	3.0 (2.0, 3.0)	3.0 (2.0, 4.0)
Altered AEDs	15	2.5/day (1.0, 5.0)	2.5/day (1.0, 5.0)	0.141	3 (19%)	3.0 (3.0, 3.0)	4.0 (3.0, 4.0)
Same or less AEDs	50	2.5/day (1.0, 5.0)	1.6/day (1.0, 4.1)	<0.001	10 (19%)	3.0 (2.0, 3.3)	3.0 (2.0, 3.0)
Weekly							
All	35	2.8/week (1.4, 3.5)	1.6/week (0.9, 3.0)	<0.001	11 (32%)	2.5 (2.0, 3.0)	2.5 (2.0, 3.0)
Altered AEDs	9	1.5/week (1.1, 3.2)	1.5/week (0.8, 3.2)	0.109	3 (33%)	3.0 (2.0, 4.0)	3.0 (2.0, 4.5)
Same or less AEDs	25	3.0/week (1.4, 4.0)	1.6/week (0.9, 2.9)	0.001	8 (32%)	2.0 (2.0, 3.0)	2.0 (2.0, 3.0)
Monthly							
All	17	2.4/month (1.2, 3.0)	1.5/month (0.9, 3.0)	0.36	3 (18%)	2.0 (2.0, 3.0)	3.0 (2.0, 3.0)
Altered AEDs	6	2.1/month (1.4, 2.4)	0.9/month (0.7, 1.3)	0.068	3 (50%)	2.0 (1.8, 3.3)	3.0 (2.8, 4.3)
Same or less AEDs	11	2.4/month (0.9, 3.6)	2.4/month (1.5, 3.6)	0.67	0	3.0 (2.0, 3.0)	3.0 (2.0, 3.0)

2 years follow-up

	No.	Seizure frequency Before Median (Q1, Q3)	Seizure frequency 2 years Median (Q1, Q3)	p*	≥50% effect N (%)	Number of AEDs Before Median (Q1, Q3)	Number of AEDs 2 years Median (Q1, Q3)
Daily							
All	60	2.5/day (1.0, 5.0)	1.5/day (0.6, 3.9)	<0.001	20 (35%)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)
Altered AEDs	28	2.8/day (1.1, 5.4)	2.5/day (1.0, 5.0)	0.162	8 (27%)	3.0 (3.0, 3.8)	4.0 (3.0, 4.0)
Same or less AEDs	27	1.5/day (1.0, 4.5)	1.0/day (0.5, 2.5)	<0.001	12 (43%)	3.0 (2.0, 4.0)	2.0 (2.0, 3.0)
Weekly							
All	33	2.5/week (1.3, 3.5)	1.5/week (0.5, 3.0)	0.018	13 (42%)	2.0 (2.0, 3.0)	3.0 (2.0, 3.0)
Altered AEDs	12	2.0/week (1.1, 3.5)	1.1/week (0.4, 2.8)	0.24	5 (42%)	2.0 (2.0, 4.0)	3.0 (2.0, 4.0)
Same or less AEDs	19	2.5/week (1.4, 4.0)	1.5/week (0.6, 3.0)	0.029	8 (42%)	2.0 (2.0, 3.0)	2.0 (2.0, 3.0)
Monthly							
All	15	2.4/month (1.5, 3.0)	1.5/month (0.9, 2.4)	0.167	7 (47%)	2.0 (2.0, 3.0)	3.0 (2.0, 3.0)
Altered AEDs	7	2.1/month (1.5, 2.4)	0.9/month (0.6, 1.5)	0.066	5 (71%)	2.0 (2.0, 3.0)	3.0 (3.0, 4.0)
Same or less AEDs	8	2.7/month (1.1, 3.5)	2.3/month (1.7, 3.0)	0.92	2 (25%)	2.5 (2.0, 3.0)	2.5 (2.0, 3.0)

5 years follow-up

	No.	Seizure frequency Before Median (Q1, Q3)	Seizure frequency 5 years Median (Q1, Q3)	p*	≥50% effect N (%)	Number of AEDs Before Median (Q1, Q3)	Number of AEDs 5 years Median (Q1, Q3)
Daily							
All	46	1.8/day (1.0, 3.9)	1.0/day (0.3, 2.5)	<0.001	21 (48%)	3.0 (2.0, 3.0)	3.0 (2.0, 4.0)
Altered AEDs	30	1.9/day (1.0, 5.0)	1.0/day (0.2-2.2)	0.004	14 (45%)	3.0 (2.0, 4.0)	4.0 (3.0, 4.0)
Same or less AEDs	14	2.0/day (1.0, 3.9)	0.9/day (0.3-3.4)	0.008	7 (54%)	2.0 (2.0, 3.3)	2.0 (2.0, 2.8)
Weekly							
All	24	3.0/week (1.5, 3.9)	1.9/week (0.6, 3.5)	0.41	8 (35%)	3.0 (2.0, 3.0)	3.0 (3.0, 4.0)
Altered AEDs	15	3.0/week (1.5, 4.7)	2.3/week (0.4, 3.0)	0.29	6 (40%)	2.0 (2.0, 3.5)	3.0 (3.0, 4.0)
Same or less AEDs	8	2.0/week (1.3, 3.9)	1.4/week (0.9, 3.9)	0.50	2 (25%)	3.0 (2.0, 3.0)	2.0 (2.0, 3.0)
Monthly							
All	10	2.4/month (0.9, 3.2)	1.5/month (0.2, 2.6)	0.038	6 (60%)	2.5 (2.0, 3.0)	3.0 (2.0, 3.3)
Altered AEDs	7	2.4/month (0.9, 3.0)	0.9/month (0.0, 3.0)	0.115	4 (57%)	2.0 (2.0, 3.0)	3.0 (2.0, 4.0)
Same or less AEDs	3	3.0/month (0.9, n.a.)	2.1/month (0.3, n.a.)	0.102	2 (67%)	3.0 (2.0, n.a.)	3.0 (2.0, n.a.)

*From paired Wilcoxon signed ranks test comparing seizures per day/week/month at VNS implantation and at actual follow-up time

AED = antiepileptic drug, Q = quartile

Table 6. Proportion of patients with specified number of AEDs before and after follow-ups

No. of AEDs	Before VNS (n=130)	After 1 year (n=123)	After 2 years (n=109)	After 5 years (n=79)
0	1 (0.8 %)	3 (2.4 %)	2 (1.8 %)	2 (2.5 %)
1	13 (10.0 %)	13 (10.6 %)	6 (5.5 %)	2 (2.5 %)
2	43 (33.1 %)	36 (29.3 %)	32 (29.4 %)	21 (26.6 %)
3	48 (36.9 %)	40 (32.5 %)	38 (34.9 %)	25 (31.6 %)
4	19 (14.6 %)	22 (17.9 %)	22 (20.2 %)	22 (27.9 %)
5	6 (4.6 %)	8 (6.5 %)	7 (6.4 %)	4 (5.1 %)
6	0	1 (0.8 %)	2 (1.8 %)	3 (3.8 %)

DISCUSSION

Neurogenesis (study I)

The positive effects on depression noticed in patients treated with VNS could be explained by an increased neurogenesis in the hippocampus. The results in **Study I** suggest that short-term VNS increases stem cell proliferation in the rat hippocampus. This supports the idea that hippocampal plasticity is involved in the treatment of depression and could be one of the mechanisms of action behind VNS. However, increased hippocampal neurogenesis can result from either increased progenitor cell proliferation, from enhanced progenitor survival with a decrease in the number of cells that normally succumb sometime after the cell division, or from a combination of both mechanisms[241]. Our results support the idea that progenitor cell proliferation and survival are regulated by different mechanisms, and that VNS can stimulate rapid cell proliferation in the hippocampus. This is believed to be facilitated through an increased noradrenergic release from the LC[99,105]. There is also evidence that the LC is involved in an increased indirect release of 5-HT in the hippocampus, since the LC has an excitatory influence on the serotoninergic dorsal raphe nucleus[99,100]. The increased 5-HT is, however, not as immediate as the NA release and could explain why antidepressant drugs induce progenitor cell proliferation in the hippocampus at a much slower pace, taking at least 2 weeks to induce cell proliferation[120,149,158]. The time delay in efficacy of antidepressants (in both humans and animal models) could also mirror the time required for newly proliferated neurons to become functional and hyperplastic[242]. Antidepressant drugs usually require 3–4 weeks before any sign of improvement in humans[243]. In clinical depression studies, VNS seems to induce improvement after 3 month but this improvement increases with time up to 2 years[134,244]. This is most likely a result of a long-term neuromodulatory effect. The role of VNS in the treatment of depression is, and will probably remain, as an adjunctive therapy for severe chronic cases, which may have necessitated repeated electroconvulsive therapies with short remission periods.

The up-regulation of progenitor cell proliferation appears to be dose dependent. The maximum effect was at an output current of 0.75 mA in **Study I**. This type of dose dependency has been reported in earlier experiments with long-term potentiation in the DG of the rat hippocampus[245]. The current 0.75 mA might be an optimal stimulation

parameter for progenitor cell proliferation in rats considering that there was a non-significant increase at 0.5 mA and 1.5 mA. On the other hand, the rat vagus nerve is approximately 25 times thinner than the human vagus nerve, and stimulation between 0.5 and 1.5 mA possibly actives the C-fibers as well. If extrapolating from animal data, stimulation with 0.125 mA would be sufficient to recruit C-fibers in the rat[246]. Hypothetically the C-fibers could be involved in the increase of progenitor cell proliferation in the rat hippocampus.

However, the question still remains if the progenitor cells mature into new neurons and integrate into existing hippocampal networks, in order to restore brain function in depressed patients. So far, VNS has been shown to be a potent stimulator of progenitor cell proliferation in experimental animal studies, but this have been difficult to correlate with behavioral improvements[157]. This suggests that there is probably more than just increased progenitor cell proliferation in the hippocampus needed to explain VNS induced efficacy in depression.

In animal studies the commonly used AEDs, carbamazepine, valproate, and lamotrigine have been found to cause an increase in hippocampal 5-HT[247-249]. These drugs seem to have mood stabilizing properties and are also used to treat psychiatric disorders, mainly bipolar affective disorder[250]. The anticonvulsant effect of carbamazepine can be blocked with 5-HT-depleting drugs in rats genetically prone to have seizures[251], suggesting that some antiepileptic drugs and antidepressants could have similar mechanisms of action. In experimental studies lamotrigine seems to increase the number of BrdU-positive cells in the rat hippocampus, whereas valproic acid does not[252]. Lamotrigine has been suggested as an augmented treatment in unipolar depression[253]. However, a recently published double-blinded placebo-controlled trial could not confirm its additive antidepressant effect[254]. Other antiepileptic drugs seem to have a negative effect on hippocampal neurogenesis, especially in the developing brain[255,256]. We hypothesize that the antidepressant mechanism of VNS through increased progenitor cell proliferation in the hippocampus is independent from its antiepileptic effects. However, further studies are needed to confirm this hypothesis.

In addition, there is a bidirectional relationship between epilepsy and depression. Patients with DRE have an increased risk of developing MDD, and patients with primary psychiatric disorders pose an increased risk of developing epilepsy. This comorbidity suggests that there could be shared underlying pathologies.[257] Since VNS has an impact on both epilepsy and depression it might be an optimal treatment in patients with DRE suffering from MDD.

Complications when implanting VNS in rat (Studies I and II)

In **Study I** approximately one third of the rats suffered from a left sided ptosis postoperatively. This was believed to be Horner's syndrome or oculosympathetic palsy. The phenomenon has been described as a complication of VNS implantation in rats[258]. In humans, one case of Horner's syndrome have been reported after VNS implantation[259]. In **Studies I and II** some of the rats developed an aseptic fluid collection around the stimulator after a few days to weeks. This was believed to occur because of device size-related irritation. In some cases the fluid was evacuated. Although these complications probably did not affect the results of **Studies I and II**, it is important to recognize that VNS implantation in rats can cause discomfort to the animals. This may be an ethical consideration in future studies.

VBLOC (Study II)

In order to accurately determine food intake, eating behavior, and metabolic parameters the state-of-the art CLAMS was used. CLAMS is particularly important when trying to analyze food consumption and weight reduction as accurately as possible in small rodents. Earlier experimental animal studies have reported a reduced body weight in response to VNS. However, these studies were mainly performed on the cervical vagus nerve[260,261]. In **Study II**, short-term (48 h) subdiaphragmatic blockage of the efferent vagus nerve did not alter eating behavior. Conversely, the long-term (6–8 weeks) VBLOC reduced food intake and body weight, particularly when being started with a high current. This is in line with a report that subdiaphragmatic VNS attenuated weight gain in obese minipigs during 14 weeks[262]. Data suggest that it is beneficial to start stimulation at a high current, rather than increasing it gradually, in order to achieve the body weight loss. On the other hand, one could speculate that starting with an initially high current without titration could cause aversive pain and thereby reduce food intake.

Theoretically VBLOC could activate the vagal signaling to the brainstem and hypothalamus and inhibit the signaling to the gut, leading to increased satiety, reduced food intake, and ultimately loss of body weight. Considering the newly approved VBLOC treatment for obesity, more studies are needed to further investigate the mechanism of action, safety, and long-term data.

Complications of VNS implantation (Study III)

Safety concerns must be seen in the long-term perspective since many patients receive their VNS at a young age with the expectancy to use it for many years. This includes a possible number of pulse generator replacement surgeries. To our knowledge **Study III** is the longest ever (up to 25 years, median 12 years) follow-up on surgical complications after VNS. With its longevity it covers not only immediate complications such as postoperative hematomas, wound infections, and vocal cord paralysis, but also more long-term complications such as lead malfunction and lead breaks which are probably more prone to occur over time. It is reasonable to believe that the complication frequency increases with a longer follow-up time considering the repeated surgeries needed due to depleted stimulators and hardware being worn out. The overall complication rate in **Study III** was 12.4 %, including both surgical and hardware complications. Most previous studies present frequencies ranging from 2.5 % to 12.5 %[24,38-42,218,263]. However, they rarely account for overall complication rates and seldom separate surgical and hardware complications. There are also different opinions about what is considered a complication, i.e. should a technical complication be considered as a complication to surgery or not? In the National Swedish Epilepsy Surgery Register (NSESR), compiling data from all Swedish epilepsy surgeries, complication is defined as "an unwanted, unexpected, and uncommon event after a diagnostic or therapeutic procedure"[264]. A great strength at our center is that the majority of patients are followed-up by the same physicians, and all primary implantations and stimulator replacements are performed at the same clinic by a limited number of experienced surgeons. If a suspected complication occurs, the implanting surgeon is immediately consulted. **In Study III** the most common complication was infection (2.6 %), and this is in the lower range compared to most previous complication studies (2–12.5 %)[24,25,32,34-42,265]. In the pediatric population the infection rate was almost twice as high (4.5 %) as in the adult. This could be a result of excessive wound manipulation, especially in children with intellectual disabilities. Extra postoperative vigilance could perhaps resolve this problem. The infection almost exclusively occurs at the stimulator implantation site below the collarbone and rarely at the neck. All but two patients with infection had to have their stimulator removed for successful treatment with antibiotics, and in all but two of these cases the electrode was simply cut at the stimulator and left in place. This indicates that it is most often safe to leave the leads in place. The number of extracted VNS devices due to infection varies in the literature (50–100 %)[34,41,43,266]. This is probably because there are both superficial and deep infections involved, and the superficial infections are easier to treat with antibiotics than the deep

infections. However, some centers advocate complete removal of both stimulator and lead if an infection occurs[266]. Infection rates after stimulator replacement in our study was only 1.1 % suggesting that this is a fairly low risk procedure compared to primary implantation. Lead malfunction and lead fractures have decreased significantly over the last 15 years of implantation at our center (2000–2014). This is probably a result of the development of better and more durable hardware. Lead fractures are not always visible on radiography. Increased impedance in combination with worsened seizure control can indicate a damaged lead leading to surgical revision or replacement of the lead. Lead failure in the absence of visible lead fractures have sometimes been described as "microlesions" in the cable[267]. Cardiac complications are rare and seem to occur mainly in the operating theatre when the leads are tested[26,30,39], resulting in bradycardia or short asystole. None of our patients have suffered from asystole during or after implantation, but atropine is always at hand during lead testing in the event of such an occurrence.

Complications should possibly be graded in different categories depending on time of occurrence and outcome. Kahlow et al suggests a grading into minor and major complication, where minor complications resolves within 3 months and a major complication is an event that hinders activity of daily living and lasts for more than 3 months, or any significant neurological deficit[24]. Perhaps an international registry database is the best solution for a more standardized and consistent follow-up regarding both complications and effectiveness.

In conclusion, in order to minimize surgical complications VNS implantations should be restricted to experienced surgeons with detailed familiarity with the procedure at hand and attention to detail.

VNS in drug refractory epilepsy (Studies III and IV)

In western Sweden VNS is offered exclusively to patients who have failed a multidisciplinary epilepsy surgery evaluation or when epilepsy surgery itself has been unsuccessful. Even though pre-surgical evaluation has become more and more developed in recent years, with new seizure monitoring techniques and better imaging modalities, many patients are poor surgical candidates because of multifocal seizure origins or involvement of eloquent areas[268]. VNS can be offered to these patients as an adjunctive palliative neuromodulatory treatment. Considering the fairly low surgical risks

involved, VNS has several advantages compared to pharmacological treatments. Contrary to drugs, compliance is not an issue, as VNS is continuous. Cognitive side effects and somnolence that is often seen with AED use have not been reported in VNS therapy[45]. In contrast, it is reported that many patients treated with VNS for epilepsy and depression can improve in cognition and alertness[226,269]. Furthermore, if stimulation related side effects occur, the parameters can be altered in order to alleviate them. Other important benefits that have been reported are decreased seizure severity and shortened duration of ictal and postictal events[270,271]. VNS does not interact with pharmacological treatments, and whereas the effect of AEDs seems to decrease over time due to tolerance[65], VNS tends to improve efficacy over time[56,223,272]. This improvement is reported in **Study IV** and was seen for up to 5 years post implantation. The largest increase was seen at 2 years. The magnet provides additional safety and control for the patient and caregiver[273]. In addition, VNS is reversible and can be turned off and removed if necessary. In **Study III** the most common cause for removal of the VNS was lack of efficacy in combination with discomfort from the stimulator.

Considering the fact that almost all patients implanted with VNS are treated with multiple AEDs, the question arises as to whether the reduction in seizure frequency is a result of VNS itself, the combination of VNS and AEDs, or merely a result of AED changes during VNS. There have only been two studies conducted so far without AED changes during follow-up, and the results were similar to most other studies but the follow-up time was limited (12–18 months)[182,214]. **In Study IV** we tried to account for AED changes and correlate them to the individual patient or group, and investigate if the actual response to VNS differed between patients who were on unchanged medication throughout the observation period compared to the patients with changes in AEDs. There were, however, no major differences in VNS efficacy between patients with altered medication compared to patients who remained on the same or withdrew AEDs during the follow-up period. The responder rate increased from 22.1 % to 43.8 % between the first and fifth year, with the largest increase between the first and second year (22.1 % to 38.1 %), in the whole cohort and regardless of AED changes. Conversely, there were also patients that initially were responders, with a seizure frequency reduction ≥ 50 %, who with time became non-responders. In our cohort the median number of AEDs were 3, but some of the patients had 5 or 6 different AEDs concomitantly. Some patients had up to 8 different medications during the follow-up period. The question whether there could be a synergistic effect between VNS and certain AEDs still remains unclear.

Seizure freedom is a rare phenomenon in patients treated with VNS, and this reflects the common impression that, in DRE, even if given a new drug or VNS, seizure freedom is seldom achieved[65]. Nevertheless, seizure freedom is the single best predictor of quality of life in patients with epilepsy[274,275]. In a recent meta-analysis of VNS, 8.2 % of the patients became seizure free after 24–48 months, and seizure freedom was predicted by age of epilepsy onset >12 years and predominantly generalized seizures[276]. Earlier reports have suggested seizure freedom in <5 %[277]. In **Study IV, 2** patients (1.5 %) became seizure free. This is not in line with the recent meta-analysis, but could reflect the fact that longer follow-up time is essential when determining seizure freedom and that relapses may occur after periods of remission.

In another recent meta-analysis the greatest benefit from VNS was seen in patients with posttraumatic epilepsy and with tuberous sclerosis, but patients with generalized epilepsy and children also benefited significantly from VNS[277]. Others have suggested that predictors of VNS response are low seizure frequency, temporal discharge on EEG-video, and the presence of an MRI lesion[213]. **Study IV** showed no difference in seizure frequency reduction between focal epilepsy and generalized epilepsy syndromes. Regarding etiology, the groups were too small for any statistical analyses to be made. It should be noted that in a recent meta-analysis, the number of patients that did not benefit from VNS at all was approximately 25 %[277]. **Study IV** presents a corresponding number (with no seizure frequency reduction) of 42.5 % after 5 years. However, the cut off between groups of patients with no benefit and patients with 1–49 % (McHugh class III) or 0–49 % seizure frequency reduction (Engel class IV) may in some cases be rather arbitrary and difficult to determine.

The recently published PuLSE (Open Prospective Randomized Long-term Effectiveness) trial assessed whether VNS as a treatment adjunct to best medical practice is superior to best medical practice alone, in improving health related quality of life in patients with pharmacoresistant focal seizures. The multicenter study demonstrated that adjunctive VNS therapy after 12-month follow-up was associated with significantly greater improvement in health related quality of life over best medical practice alone[278]. However, because of recruitment issues, the study was terminated prematurely and the enrolled number of patients did not meet the planned sample size. Sixty out of a 122 randomized patients completed the 1-year follow-up. When evaluating VNS effectiveness it is important not only to determine seizure reduction, but also other factors such as quality of life, pharmacological side-effects, adverse events, and seizure severity. By definition effectiveness is the

extent to which an intervention produces an outcome under ordinary day-to-day circumstances[279].

Many patients have intractable DRE and try multiple AEDs before they receive VNS. This procedure of trying multiple AEDs can require years of up-titrating and down-titrating different AEDs but to no avail. Since the efficacy of VNS is similar to adding a new AED, but possibly with fewer side effects, the question remains if VNS should be implanted at an earlier stage. In theory one can reason that this is a fair suggestion. Patients with DRE may respond to pharmacological therapies for shorter or longer periods but later relapse[280]. Seizure aggravation is also an important limitation of current AEDs[65]. Evaluation of the efficacy of adding just one new AED can, therefore, take over a year to complete. Patients with successful epilepsy surgery can also relapse after initial seizure freedom[281]. Among patients with DRE, 5 % per year enter seizure remission, but approximately 70 % of patients that experience a 12-month or greater seizure remission eventually relapse[280]. Thus, it is of great importance that all patients with DRE are referred to an epilepsy center for proper evaluation and follow-up.

VNS has a role in the treatment of refractory epilepsy but the regime on how to combine with AEDs or other treatments should be well thought through and monitored. Based on the results of **Study IV**, we suggest that VNS should be continuously and carefully evaluated for at least 2 years after implantation before deciding if it is helpful or not. Furthermore, since there seems to be increasing efficacy with time, a restrictive approach to AED changes should be applied if possible.

In summary, VNS is a well-tolerated, low risk procedure for the treatment of DRE and chronic depression. It has also been proposed for the treatment of several other diseases and was recently approved as a treatment for obesity. Over the past years multiple experimental studies have been conducted to better understand the mechanisms of action of VNS, and a number of clinical studies have also been performed in order to evaluate new possible treatment indications. Increasing evidence suggests that there are several pathways involved in the mechanism of action of VNS. As new research is published, further discoveries will hopefully unravel the full potential of VNS.

Strengths and weaknesses

Studies I and II
The main strengths of these studies are that the experiments were performed in a strictly methodological fashion and that the control animals were implanted with stimulators as well. In **Study I** the individuals performing the cell-counting procedures were blinded to whether the specimen was from a rat that had received VNS or not. Nevertheless, results from animal studies should always be interpreted with caution, especially if results are translated directly to humans. Although the laboratory environment is created not to stress the animals, the nature of the experiment itself could propose an increased level of stress. This could of course affect the results, especially since stress is a known inhibitor of hippocampal progenitor proliferation[142]. It cannot be excluded that some form of low-level stress was generated in the 1.5 mA group, which could affect the progenitor proliferation rate negatively. There is also a learning curve when it comes to animal surgery, and the anatomical variations between animals can result in more or less challenging procedures. The fact that some animals developed a left sided ptosis and a fluid collection around the implanted stimulator could reflect on this matter. However, both sham and stimulated rats were operated at all stages of the surgical learning curve and thereby, the effect of surgical experience should be equally distributed between groups. In **Studies I and II**, stimulation parameters were set in accordance with earlier similar studies, but there is no way of knowing if these were the ideal parameters for our experiments.

Studies III and IV
The strengths of the **Studies III and IV** are that they are population based, long-term studies with one of the longest registry follow-up periods in the world. Not only are primary implantations included, but also repeated surgeries such as battery and lead replacements. All surgeries were performed by a small number of surgeons, and between 1990 and 1999 one surgeon made all VNS implantations. On the other hand, analyzing registry data over a long time period can be difficult, especially since surgeries performed before 1997 were not registered automatically in the electronic charts at our hospital. Furthermore, different and rather small group sizes may have had an impact on the statistical analyses. Retrospective long-term studies also propose a challenge because of patients lost to follow-up and differences in the interpretation of VNS efficacy. It is not possible to conduct double-blinded randomized long-term studies in this patient population extending up to 25 years for ethical reasons and that it is simply not feasible. However, a prospective randomized study would have lifted the results up to a higher level of evidence. Differences in the extent and manner of documentation between physicians can also be a limiting factor when

analyzing retrospective data. Drug responsiveness of a patient's epilepsy should be regarded as a dynamic process rather than a fixed state. Instead of being constant, the course of epilepsy sometimes fluctuates[282]. Natural fluctuations in epilepsy can in part explain relapses in patients who otherwise are considered as responders.

CONCLUSIONS

The main findings of this thesis were that:

- VNS in the rat increases stem cell proliferation in the hippocampus, which supports the notion that hippocampal plasticity is involved in the antidepressant effect of VNS. (**Study I**)

- VNS did not effect progenitor cell survival in the rat hippocampus. (**Study I**)

- The mechanism of action of VBLOC as a treatment for obesity could be regulated by inducing satiety through vagal signaling, leading to reduced food intake and loss of body weight. The treatment was well tolerated in rats. (**Study II**)

- The most common complications to VNS implantation, with an occurrence rate of about 2 %, were infection, postoperative hematoma, and vocal cord paralysis. (**Study III**)

- Hardware quality has improved since the introduction of VNS, and significantly less lead associated complications occurred during 2000–2014 compared to 1990–1999. (**Study III**)

- VNS efficacy did not differ between patients that had altered or remained on the same AEDs throughout the study period. (**Study IV**)

- VNS efficacy increases with time with improvements seen up to 5 years after VNS implantation. (**Study IV**)

FUTURE PERSPECTIVES

Recently two non-invasive devices for external VNS have been introduced on the European market. The NEMOS (Cerbomed, Erlangen, Germany) is an external device that provides transcutaneous VNS (tVNS) by using an intra auricular electrode, which stimulates the auricular branch of the vagus nerve. The device is approved for epilepsy. The gammaCore (electroCore LLC, Basking Ridge, NJ USA) is a handheld non-invasive VNS device (nVNS) that is held against the neck and provides a single 180-second burst of VNS. The nVNS is approved for the treatment of primary headache, bronchoconstriction, epilepsy, gastric motility disorders, anxiety, and depression. So far, only very small, mainly feasibility and proof-of-concept studies have been conducted on epilepsy for the NEMOS[283], and none for the gammaCore[284]. More studies will hopefully cast a light on whether these treatments can become an alternative to invasive VNS. One can speculate that instead of implanting a VNS device immediately, a test trial with an external device could be performed to investigate if the patient is likely to be a responder to VNS. Hopefully this could reduce the number of implanted patients not responding to VNS. It could also give the patient a premonition of the stimulation related adverse events, such as voice alterations and hoarseness.

In other neuromodulatory treatments such as spinal cord stimulation (SCS) and deep brain stimulation (DBS), there are several rechargeable alternatives on the market. With rechargeable VNS devices, repeated surgeries due to depleted batteries could be avoided and lower the complication risk.

With **Studies III and IV** comprising the adverse events for up to 25 years and the efficacy for up to five years, as well as multiple other long-term studies, further retrospective cohort studies are no longer needed. There is today evidence for the effectiveness of VNS in epilepsy. Future studies could, however, be prospective randomized controlled trials focusing on VNS in combination with specific pharmacological treatments or on special patient groups who might benefit more than others from VNS therapy.

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