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Hippocampal deep brain stimulation for drug resistant epilepsy in a rodent model

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List of abbreviations

ACh	acetylcholine
AD	afterdischarge
ADT	afterdischarge threshold
AED	anti-epileptic drug
ANT	anterior nuclei of the Thalamus
AP	aminopyridine
BDNF	brain-derived neurotrophic factor
BOLD	blood-oxygen-level dependent
CA	cornu ammonis
CE	conformité européenne
CoRaStiR	controlled randomized stimulation versus resection
DBS	deep brain stimulation
DC	direct current
DCX	doublecortin
DG	dentate gyrus
EEG	electro-encephalogram
FDA	food and drug administration
GABA	gamma-aminobutyric acid
GAERS	genetic abscence rats from Strasbourg
HFS	high frequency stimulation
HMPAO-Tc99^{m}	hexamethylpropyleneamine oxime Technecium
HS	hippocampal sclerosis
ILAE	international league against epilepsy
ip	intraperitoneal
KA	kainic acid
LFP	local field potential
LFS	low frequency stimulation
LTD	long-term depression
LTLE	lateral temporal lobe epilepsy
LTP	long-term potentiation

MCAO	middle cerebral artery occlusion
METTLE	multicenter study of hippocampal electrical stimulation in mesial temporal lobe epilepsy
MFS	Mossy fiber sprouting
MOA	mechanism of action
MRI	magnetic resonance imaging
MTLE	mesial temporal lobe epilepsy
PBS	phosphate buffered saline
PDS	Poisson distributed stimulation
PED	periodic epileptiform discharge
PET	positron emission tomography
PTZ	pentylenetetrazol
rCBF	regional cerebral blood flow
RNS	responsive neurostimulation
rTMS	repetitive transcranial magnetic stimulation
SANTE	stimulation of the anterior nuclei of Thalamus for epilepsy
SE	status epilepticus
SRS	spontaneous recurrent seizures
STN	subthalamic nucleus
SWD	spike-wave discharge
TBI	traumatic brain injury
tDCS	transcranial direct-current stimulation
TLE	temporal lobe epilepsy
TNS	trigeminal nerve stimulation
t-VNS	transcutaneous Vagus nerve stimulation
VHC	ventral hippocampal commissure
VNS	vagus nerve stimulation
(μ)CT	(micro) computed tomography
(μ)SPECT	(micro) single photon emission computed tomography

Outline of the thesis

Chapter 1: "Rationale and aims of the thesis"

Chapter 2: "Introduction on temporal lobe epilepsy" is a general introduction on epilepsy, with an emphasis on temporal lobe epilepsy

Chapter 3: "Deep brain stimulation in epilepsy" gives an introduction into the use of deep brain stimulation as a treatment for epilepsy

Chapter 4: "Animal models for translational epilepsy research" provides a short overview of the different animal models used in translational epilepsy research and an in depth characterization of the systemic kainic acid rat model for temporal lobe epilepsy used in this thesis

Chapter 5: "Optimization of hippocampal deep brain stimulation parameters for the treatment of drug resistant epilepsy" describes a study that compares the seizure suppressive effect of unilateral and bilateral hippocampal stimulation in a rat model for temporal lobe epilepsy.

Chapter 6: "**Long-term hippocampal deep brain stimulation in a rat model for temporal lobe epilepsy contradicts seizures beget seizures hypothesis"**. In this study the effect of long term continuous hippocampal deep brain stimulation on seizure rate is investigated in a rat model for temporal lobe epilepsy.

Chapter 7: "Hippocampal deep brain stimulation: mechanism of action" describes a study where the mechanism of action of hippocampal deep brain stimulation is investigated by monitoring changes in regional brain perfusion with μSPECT in healthy rats subjected to hippocampal deep brain stimulation

Chapter 8: "General discussion and conclusions"

Chapter 9: "Future perspectives"

Chapter 1

Rationale and aims of the thesis

Rationale

Epilepsy is a chronic neurological condition, characterized by spontaneous recurrent seizures. These seizures are the consequence of excessive and hypersynchronous electrical activity in the brain. Epilepsy is the second most common neurological disorders, with 50 to 70 new cases per 100000 individuals each year (Banerjee PN 2008; Forsgren et al. 2005; Hauser et al. 1996). Temporal lobe epilepsy (TLE) is the most common form of epilepsy (Panayiotopoulos 2007). Current treatment of epilepsy patients is typically based on administration of anti-epileptic drugs (AEDs) in order to restore the imbalance between excitation and inhibition in the brain. In about 75% of TLE patients seizures are poorly controlled with currently available AED treatment (Spencer 2002). Systemic administration of AEDs is associated with side-effects such as sleepiness, dizziness, memory – and mood disorders, etc. Information processing in the brain is regulated by means of excitatory and inhibitory postsynaptic potentials, and this information is coded as sequences of action potentials. In this system, the neurotransmitters on which most AEDs act are only the messengers, not the message. The development of new treatments that are based on affecting innate neurophysiological mechanisms of the brain may herald superior efficacy. Furthermore, AEDs act on a timescale of minutes to hours, and cannot replicate the precise timing of electrical information processing in the brain (Montgomery 2010).

A promising, but currently experimental treatment for drug resistant epilepsy is hippocampal deep brain stimulation (DBS). Hippocampal DBS is a neurostimulation strategy, where electrical pulses are delivered to hippocampal tissue through a subclavicularly or paraumbilical implanted pulse generator connected to subcutaneous wires and a stereotactically implanted electrode in the hippocampus. Treatment with hippocampal DBS has demonstrated promising results for drug resistant epilepsy patients. About 70% of all drug resistant epilepsy patients treated with hippocampal DBS experience a >50% seizure reduction during stimulation (Boex et al. 2011; Cukiert et al. 2014; McLachlan et al. 2010; Tellez-Zenteno et al. 2006; Velasco et al. 2007; Vonck et al. 2013). Although the results of these experimental open label and small randomized controlled trials should be interpreted with caution, the therapeutic potential of hippocampal DBS may increase when the mechanism of action and the most optimal stimulation parameters are further investigated. In parallel with pharmacokinetics and – dynamics studies for drug therapy, the different variables of hippocampal DBS need to be better understood to design an efficient stimulation strategy which can be safely used in patients.

Aim

The aim of this thesis is to increase the applicability of hippocampal DBS by investigating

1) the mechanism of action of hippocampal DBS

2) strategies for optimal stimulation parameters for hippocampal DBS

3) the neuromodulatory properties of hippocampal DBS on disease progression in TLE.

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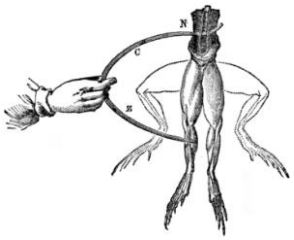
Chapter 2

Introduction on temporal lobe epilepsy

The history of epilepsy and epilepsy research

Epilepsy is a neurological disorder that has been covered in a veil of mystique and superstition since the beginning of mankind. The first historical record of a secondarily generalized seizure dates back to ancient Mesopotamia (current Iraq) about 3000 years ago. In this first record, the seizure was attributed to the god of the moon. Advances in medical treatment and scientific discoveries have gradually withdrawn epilepsy from the mystique to a more enlightened view on this complex neurological disorder. A pioneering manuscript titled *"On the sacred disease*" by the hand of Hippocrates (Hippocrates 460 - 370 BC) played an important role in this process. In this manuscript, Hippocrates refutes the common belief that seizures are caused by divine intervention. Instead of divine intervention, Hippocrates states that epilepsy was a brain disease caused by a superfluity of phlegm that had led to abnormal brain consistency. He was the first to suggest treatment with drugs and dietary measures. In the middle Ages, Hippocrates' writings became discarded and epilepsy was again covered in superstition and magic. Anatomic drawings of anatomic dissections from enlightened minds like da Vinci and Vesalius provided more insight and increased the interest in neuropathology. In the 18th century this led to the theory that seizures were caused by structural abnormalities like hardening or abscesses in the brain. Further understanding of the pathophysiology of epilepsy was driven by the discovery of animal electricity, reported in *"De viribus electricitatis in muto musculare commentarius* "nby Luigi Galvani (Galvani 1791). He discovered the existence of intrinsic electrical activity in animals by showing that electrical stimulation of the crural nerve resulted in a signal that was transmitted to and through the spinal cord and nerves, causing distal leg muscles to contract (figure 1).



Legend figure 1: *drawing of Galvani's experiments. Touching the nerves of a dissected frog with an electrode causes movement of the legs.*

In 1875, Richard Caton was the first person ever to observe the continuous, spontaneous electrical activity of the brain by placing two electrodes on the external surface of the skull (Caton 1875). In 1914, Cybulsky and Jelenska-Macieszyna were the first to publish photographs of abnormal cortical EEG activity during experiment focal seizures caused by electrical stimulation of the cortex in a dog (Cybulsky N 1914). During the twentieth century discoveries occurred and are still occurring at an unusually rapid rate thanks to close collaboration of clinicians and experimental scientists (Goldensohn et al. 1997).

Definition, epidemiology and diagnosis

Between 5 to 10% of the population experiences one single seizure during his or her lifetime. After a single unprovoked seizure, the risk for a second seizure is 40–52% (Berg and Shinnar 1991). With two unprovoked non-febrile seizures, the chance of having another seizure within 4 years is 73%, with a 95% confidence interval (CI) of 59–87% (Hauser et al. 1998). Patients are only diagnosed with epilepsy when at least 2 unprovoked seizures have occurred. The "two unprovoked seizures" definition of epilepsy has served us well, but it is inadequate in some clinical circumstances. Therefore a task force of the International League Against Epilepsy (ILAE) recently proposed the following practical definition for special circumstances that do not meet the two unprovoked seizures criteria (Fisher et al. 2014). Epilepsy is a disease of the brain defined by any of the following conditions:

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

Epilepsy is a neurological disorder with a high prevalence affecting 0.5-1% of the general population and is the second most common chronic neurological disease following cerebrovascular disorders (Banerjee PN 2008; Forsgren et al. 2005; Hauser et al. 1996). Epilepsy affects males and females equally and the incidence rate is age related. The highest incidence rate is observed in the first year of life, with a peak in the first week. Incidence is lowest during adult years and increases in the elderly as a result of the higher prevalence of cerebrovascular disorders (Hauser et al. 1996).

An epileptic seizure is the transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al. 2005). Depending on the location of onset in the brain, patterns of propagation, maturity of the brain, confounding disease processes, sleep-wake cycle, medications, and a variety of other factors, seizures can affect sensory, motor, and autonomic functions, consciousness, emotional state, memory, cognition and behavior. Not all seizures affect all of these factors, but all influence at least one (Fisher et al. 2005).

Pathophysiology

Epilepsy is a disease originating from abnormal excessive and synchronous neuronal activity in the brain (Fisher et al. 2005). Epilepsy is attributed to an imbalance between excitation and inhibition in the brain. Under normal physiological conditions there is a constant equilibrium between excitation and inhibition in the brain. Epileptic seizures arise as a result of an imbalance between this equilibrium, leading to the occurrence of spontaneous seizures. The underlying pathophysiological process is called epileptogenesis, and is characterized by a dynamic cascade of molecular and cellular changes progressively altering neuronal excitability and hereby transforming a normal brain into an epileptic brain. This dynamic process includes neurodegeneration, neurogenesis, gliosis, blood brain barrier damage, axonal sprouting, dendritic plasticity and inflammatory processes (Pitkanen A. 2011). These transformations from a healthy brain into an epileptic brain are currently not fully understood. With the advances of genetics in clinical practice and research, more and more genetic defects are identified to be involved in the development of epilepsy. Many of these identified genetic defects are defects in voltage- or ligand-gated ion channels. Changes in the expression of these ion channels disturb the excitation-inhibition equilibrium and result in an increased excitability of the brain (Berkovic et al. 2006).

Classification

The ILAE subdivides epileptic seizures in generalized epileptic seizures and focal epileptic seizures based on the onset zone and propagation of abnormal hypersynchronous firing neurons in the brain. Generalized epileptic seizures are conceptualized as originating at some point within, and rapidly engaging, bilaterally distributed networks in the brain. Focal or partial epileptic seizures are conceptualized as originating within networks limited to one hemisphere (Berg et al. 2010). Partial seizures can be subclassified based on the effect of the seizure on consciousness. Simple partial seizures are partial seizures with retention of consciousness, whereas complex partial seizures are partial seizures associated with loss of consciousness. The clinical manifestations of partial seizures depend on the involved brain region in seizure onset and seizure propagation (Panayiotopoulos 2007). Partial seizures may gradually develop into secondary generalized seizures when epileptic activity spreads to the contralateral hemisphere.

Generalized seizures can be subdivided according to the clinical manifestations of the seizure into tonic-clonic seizures, clonic seizures, tonic seizures, absence seizures, atonic and myoclonic seizures. Tonic-clonic seizures are characterized by an initial tonic and a subsequent clonic phase and are

accompanied with loss of consciousness from onset to the late phase of recovery. A typical tonic-clonic seizure is often preceded by an aura, and starts with a tonic phase during which there is a contraction of virtually the complete body musculature. This tonic phase then evolves into the clonic phase, characterized by the clonic jerking of the extremities. Postictal stupor, confusion, automatic semi-purposeful behavior and sleep may occur (Zifkin and Dravet 2008). Tonic or clonic generalized seizures manifest with only the tonic or clonic phase of a generalized tonic-clonic seizure. Absence seizures are characterized by brief paroxysmal loss of consciousness associated with typical signature spike-wave discharges on the EEG (Panayiotopoulos 2007). Myoclonic seizures manifest as involuntary contractions of muscles while consciousness remains intact (Engel 2006). Next to these seizures characterized by increased muscle tone, atonic seizures can also occur. These manifest as a sudden loss of muscle tone, often resulting in patients falling to the ground (Panayiotopoulos 2007).

Epilepsy syndromes are subdivided according to their etiology into three categories: *idiopathic* (primary, without a known or suggested genetic origin), *symptomatic* (secondary, resulting from known origins e.g. tumors, lesions, infections, vascular cause) or *cryptogenic* (presumably symptomatic but currently of unknown specific etiology). These categories are still used, but the most recent ILAE report on the categorization of epilepsy syndromes proposes to discard the terms symptomatic, idiopathic and cryptogenic. Epilepsy syndromes should be subdivided into genetic (in case of a known or presumed genetic defect associated with seizures), structural/metabolic (known structural or metabolic condition associated with increased risk for developing epilepsy) and of unknown cause (the nature of the underlying cause is as yet unknown)
(Berg et al. 2010)

Drug resistant epilepsy & treatment options

Patients diagnosed with epilepsy are treated with antiepileptic drugs (AED). In patients with newly diagnosed epilepsy, 47% become seizure free under treatment with a first AED. Treatment with a second AED renders another 13% seizure free, and treatment with a third AED results in an additional 3% of the patients becoming seizure free (Kwan and Brodie 2000). This leaves around 30% of all epilepsy patients who do not respond to conventional AED treatment. These patients are drug resistant epilepsy patients. This patient population shows excess mortality, particularly due to sudden unexpected death (Nilsson et al. 1999). Furthermore, prolonged seizures and high seizure frequency in these patients can lead to cognitive decline (Devinsky 1999). An extended period of imperfect seizure control can produce disturbed psychosocial integration resulting, for example, in poor academic achievement, diminished self-esteem, dependent behavior, and a restricted lifestyle (Sillanpaa et al. 1998). These factors highlight the need to develop new treatment strategies such as the development of new AEDs with novel mechanisms of action, epilepsy surgery, gamma knife surgery, dietary measures, and neurostimulation techniques such as vagus nerve stimulation, transcranial magnetic stimulation, transcranial direct current stimulation, trigeminal nerve stimulation and deep brain stimulation.

Anti-epileptic drugs (AED)

Adjunctive treatment with novel AEDs provides seizure freedom in 6% of the treated patients and a 50% seizure reduction in 21% of the treated patients compared to adjunctive placebo treatment. This placebo-corrected efficacy of adjunctive treatment with modern AEDs is disappointingly small and suggests that other strategies for drug development are required for drug resistant epilepsy (Beyenburg et al. 2010). During the last decades, considerable advances have been made in the understanding of the mechanism of action (MOA) of AEDs. The main mechanisms by which AEDs suppress epileptic seizures are decreasing neuronal excitation (glutamatergic system), increasing neuronal inhibition (GABAergic system) or by modulation of ion channel activity (modification of cellular excitability) (Bradford 1995).

Epilepsy surgery

Surgical treatment may be considered in cases where the patient does not achieve adequate seizure control after failure of two well-tolerated treatment regimens (Panayiotopoulos 2007). Surgery is aimed at removing or disconnecting the ictal onset zone believed to be responsible for seizure occurrence from the surrounding brain tissue (Spencer 2002). The most successful procedure in epilepsy surgery is the focal resective procedure in which the seizure generating zone is completely

removed. Depending on the localization of the seizure focus, this procedures leads to seizure freedom in 60-80% of the patients (Wiebe 2004). Other procedures in epilepsy surgery aimed at transecting the routes through which seizure activity spreads in the brain include corpus callosotomy and multiple subpial transections. These procedures are indicated only in a well-defined subpopulation of patients with drug resistant epilepsy. The risk-benefit-analysis for surgery must be individualized using a presurgical evaluation protocol and eventually a substantial number of patients have to be rejected (Boon et al. 1999). Although many patients with medically resistant epilepsy have a good response to established surgical management, it has been demonstrated that as many as 30% of medical drug resistant epilepsy patients are not candidates for surgery (Cohen-Gadol et al. 2006). For example, patients with multifocal epilepsy or patients where the seizure onset zone co-localizes with language function are considered unsuitable candidates for resective surgery (Kwan and Brodie 2000).

Gamma knife surgery

Gamma knife surgery is a type of radiotherapy based on the convergence of 200 tiny gamma radiation beams on a well-defined small volume of brain tissue rendering it less prone to seizures (Romanelli and Anschel 2006). The mechanism through which gamma knife surgery exerts its therapeutic effect on seizures has not been elucidated, but it is clear that seizure control does not entirely correlate with radiation-induced necrosis. A neuromodulatory rather than ablative effect is suggested by the fact that the dose clinically applied is below what is known to induce necrosis as well as by post-treatment neuroimaging and pathology studies (Regis et al. 1996; Romanelli and Anschel 2006; Srikijvilaikul et al. 2004). While the short-term mortality seems lower compared to open resections, long-term side effects are insufficiently known (Quigg and Barbaro 2008; Romanelli and Anschel 2006).

Dietary measures

The ketogenic diet allows high consumptions of fat (80%) and restricts the amount of daily consumed carbohydrates to 5% of the total consumed nutrients, while allowing sufficient protein (15%) intake. The high-fat, adequate protein, low carbohydrate diet mimics the biochemical changes associated with starvation. Due to the lack of carbohydrates in the diet and the fact that the brain cannot use fatty acids directly as an energy source, the body is forced to metabolize stored body fat in the liver. The end product of this metabolic process are ketones which are used as an alternative energy source (Huffman and Kossoff 2006). Long-term prospective studies show a reduction of seizure frequency of more than 90% in one third of the patients who are able to continue the diet (Freeman et al. 1998; Freeman et al. 2006). Despite the high efficacy of the diet, the ketogenic diet is primarily

used in catastrophic childhood epilepsy, such as epileptic encephalopathies because of the high chance of side effects and restrictiveness of the diet (Dhamija et al. 2013). Because of this high restrictiveness, an alternative for the ketogenic diet is the modified Atkins diet. The modified Atkins diet induces ketosis as does the ketogenic diet, without restrictions on protein, calories and fluids intake. These measures make the diet more tolerable for the patient, while keeping most of its seizure suppressive capacities. Under the modified Atkins diet around 35% of the patients have a more than 90% reduction in seizure rate and 65% has a more than 50% reduction in seizure rate (Kossoff et al. 2006).

Neurostimulation

Neurostimulation techniques for the treatment of drug resistant epilepsy imply the application of electric or magnetic fields in the vicinity of nerves or neuronal tissue to suppress seizure activity. Electrical pulses are administered directly to or in the vicinity of nervous tissue in order to prevent or suppress seizure occurrence. Neurostimulation strategies can either involve the intracranial implantation of a stimulation electrode, extracranial implantation of a stimulation electrode, or it can be performed by applying a non-invasive extracranial stimulation device to specific regions in the body (Boon et al. 2009; DeGiorgio and Krahl 2013).

Vagus nerve stimulation (VNS)

Vagus nerve stimulation (VNS) is an extracranial form of neurostimulation developed in the 1980s, where the left vagus nerve is stimulated in the neck area by means of an implanted helical stimulation electrode connected to a subclavicularly implanted pulse generator (Ben-Menachem 2002). Following the implantation, the stimulation intensity is slowly increased from 0.25 mA to 2.0 – 3.0 mA. Other stimulation parameters used in clinical setting are a stimulation frequency of 20 – 30 Hz, 250 – 500 µs pulse width and a duty cycle with a 30s ON and 3 – 5 min OFF time. Since the approval of this therapy for the treatment of drug resistant epilepsy patients in 1997, more than 70,000 patients worldwide have been implanted with a VNS-device (Magdaleno-Madrigal et al. 2014). Despite 20 years of experience with VNS treatment, the optimal stimulation parameters and precise mechanism of action remain largely unknown. The current consensus on efficacy of VNS for the treatment of drug resistant epilepsy patients is that about 1/3 of patients experience a reduction in seizure rate of at least 50%, while another 1/3 experiences a reduction in seizure rate of 30 – 50%, and in the remaining 1/3 of patients there is little to no effect of treatment. Better comprehension of the antiepileptic mechanism of action of VNS could lead to the identification of certain seizure types and epilepsy syndromes that respond best to VNS and to the identification of responder characteristics. In addition, it may guide the search for a more appropriate choice of stimulation

parameters. Despite these gaps in the understanding of VNS, it has been shown that efficacy improves with longer treatment duration (De Herdt et al. 2007; Vonck et al. 2009). VNS is usually well tolerated by patients. The main side-effects are mild to moderate stimulation related side-effects like hoarseness, coughing and voice alternations. These can be reduced by adjusting the stimulation parameters of stimulation (De Herdt et al. 2007).

Transcutaneous vagus nerve stimulation (t-VNS)

Transcutaneous vagus nerve stimulation (t-VNS) is a recently developed non-invasive alternative for VNS. The neurostimulation treatment consists of stimulating the auricular branch of the vagus nerve by means of an external electrode and generator. t-VNS is presumed to activate the auricular branch of the vagus nerve, which further propagates along the vagus nerve to the nucleus tractus solitarius (NTS). t-VNS for the treatment of drug resistant epilepsy patients has been shown to be safe, effective, well-tolerated and practicable for long-term treatment (Stefan et al. 2012). Based on these first promising clinical data concerning feasibility and safety, the t-VNS device received CE approval in 2012. Further experiments are needed to evaluate the efficacy and replacement potential for implanted VNS of this treatment.

Trigeminal nerve stimulation (TNS)

Trigeminal nerve stimulation (TNS) is a novel form of non-invasive neurostimulation. With TNS the superficial branches of the trigeminal nerve are stimulated with an external stimulation electrode. The presumed antiepileptic mechanism of action involves the desynchronization of thalamic and cortical activity and a generalized arousal by activating the reticular-activating system in the brainstem (Fanselow et al. 2000). TNS has been shown to be a safe and effective treatment for drug resistant epilepsy both in human and animal experiments (DeGiorgio et al. 2009; DeGiorgio et al. 2011; Fanselow et al. 2000)

Repetitive transcranial magnetic stimulation (rTMS)

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neurostimulation technique in which magnetic pulses are created in a stimulation coil and are applied to the patients' scalp. When a magnetic field is created close to conductive media, electrical currents are created perpendicular to this magnetic field. The neuromodulatory effect of rTMS is dependent on the created magnetic and electrical pulses. High-frequency stimulation (> 3 Hz) results in synaptic facilitation, while low-frequency rTMS (≤ 1 Hz) induces a reduction of synaptic efficiency (Fitzgerald et al. 2006; Gersner et al. 2011). Therefore low frequency stimulation is explored for the treatment of drug resistant epilepsy patients. The therapeutic effect of rTMS outlasts the stimulation duration with at least 2-4 weeks (Hsu et al. 2011) by increasing or decreasing the excitability of neuronal networks.

Randomized, double blind, sham-controlled trials demonstrate that the effect of rTMS for the treatment of drug resistant epilepsy patients varies from a trend in reduction of seizures (Theodore et al. 2002) to no change in seizure rate (Cantello et al. 2007).

Transcranial direct current stimulation (tDCS)

Transcranial direct current stimulation (tDCS) is a non-invasive neurostimulation strategy where direct electrical currents of 1 – 2 mA are delivered via anodal and cathodal electrodes through the scalp to the brain. Anodal tDCS is presumed to increase excitability, while cathodal tDCS is presumed to reduce excitability. It has been shown that tDCS can affect motor, somatosensory, visual, affective and cognitive functions (Been et al. 2007). Safety of the treatment and some seizure suppressive effects have been reported in a small open label trial (Auvichayapat et al. 2013) but the efficacy of tDCS to reduce seizure activity in drug resistant epilepsy patients still needs to be confirmed in additional experimental trials.

Deep brain stimulation (DBS)

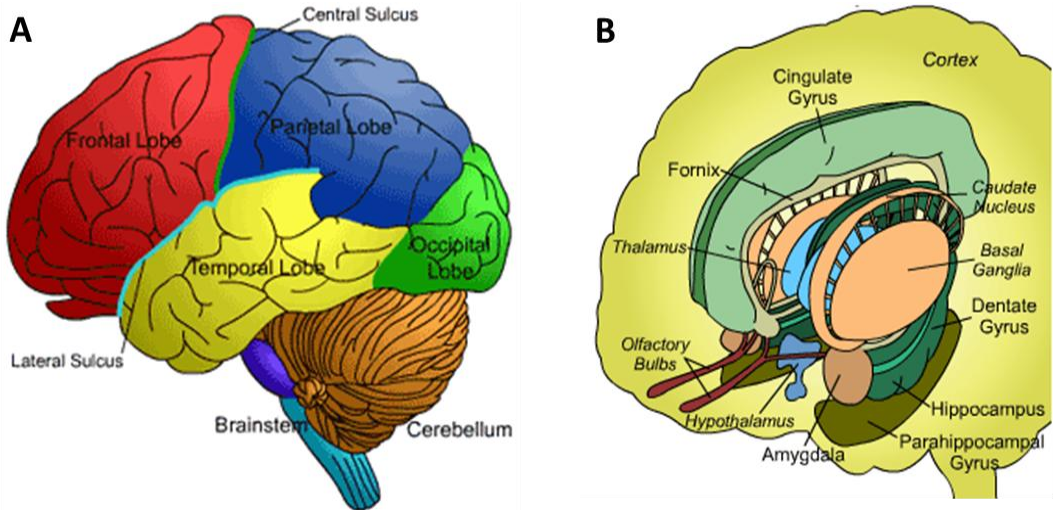
Deep brain stimulation (DBS) is an intracranial neurostimulation strategy in which intracerebral structures are targeted. Via a subclavicular or paraumbilical implanted pulse generator and a subcutaneous lead, electrical pulses are directed to intracranial electrodes implanted in specific parts of the brain to interfere with the neural activity of the target site. Various brain structures have been targeted with DBS in preclinical (Wyckhuys et al. 2009) and clinical trials (Sprengers et al. 2014) resulting in variable results. Several mechanisms of action have been proposed. Continuous delivery of electrical pulses to the targeted brain structures may cause a functional inhibition of the targeted region. The effect of the stimulation consequently is associated with the targeted brain structure. The two main strategies to suppress seizures in drug resistant epilepsy patients with DBS are either stimulation of the epileptic onset zone causing a local inhibition of the hyperexcitable region or stimulation of structures responsible for seizure propagation which may result in suppression of seizure spread (Boon et al. 2007). The precise mechanism of action of DBS still needs to be elucidated. Seizure suppressive effects of DBS have been observed with both strategies in two large multicenter randomized controlled trials. The SANTE (Stimulation of the Anterior Nuclei of Thalamus for Epilepsy) trial showed that high frequency (145Hz) stimulation in a structure responsible for seizure propagation (i.e. the anterior nuclei of the thalamus (ANT)) in patients with focal epilepsy resulted in a 29% greater reduction in seizure rate compared to non-stimulated controls 3 months after start of the study. By 2 years, more than half of the patients had a >50% reduction in seizure rate (Fisher et al. 2010). This SANTE trial showing safety and efficacy of ANT-DBS led to premarket FDA approval for ANT-DBS in 2009, and CE marking in 2010. The efficacy of the strategy of

stimulating the epileptic onset zone has been shown in a randomized controlled trial, where a responsive neurostimulation (RNS) was used to stimulate the onset zone whenever a seizure is detected by the system (NeuroPace®RNS®). In this study a 20% greater reduction in seizure rate compared to non-stimulated controls after a 3 month blinded period has been reported. By 2 years, the median seizure reduction was 53% (Heck et al. 2014). The Neuropace study led to premarket FDA approval of the NeuroPace®RNS® system for the treatment of adults with partial onset seizures who have not been controlled with two or more antiepileptic drugs in 2013. Despite these positive results with responsive focal neurostimulation, it is not known whether responsive stimulation heralds superior or inferior effects over scheduled stimulation. A systematic review analyzing all small randomized controlled trials conducted on intracranial DBS showed that next to ANT-DBS and responsive focal DBS, hippocampal DBS reduces seizure frequency in drug resistant epilepsy patients (Sprengers et al. 2014). Both at Ghent University Hospital (Boon et al. 2007; Vonck et al. 2002; Vonck et al. 2013) as in other clinical research centers (Boex et al. 2011; Cukiert et al. 2014; McLachlan et al. 2010; Tellez-Zenteno et al. 2006; Velasco et al. 2000; Velasco et al. 2007) promising seizure suppressive effects of hippocampal DBS have been observed. Both in the open label phase of the SANTE and NeuroPace trial, as in observations after long-term treatment with hippocampal DBS, an improvement in efficacy with longer treatment duration has been observed (Fisher et al. 2010; Heck et al. 2014; Vonck et al. 2013)

Temporal lobe epilepsy

Temporal lobe epilepsy (TLE) is the most prevalent type of epilepsy in adults (Panayiotopoulos 2007). Studies have shown that up to 53% of the TLE cases had febrile seizures as a child, suggesting this as an important risk factor (Arzimanoglou et al. 2002). In about 75% of the TLE patients seizures are poorly controlled, resulting in drug resistant epilepsy making TLE the most drug resistant type of epilepsy (Spencer 2002). Two main types can be distinguished: mesial TLE (MTLE) with a seizure focus in medial temporal lobe structures and the lateral TLE (LTLE) with a seizure focus in the lateral temporal lobe. Unlike patients with MTLE, there are no reported large series of patients with well-documented lateral temporal lobe seizure origin. This partly explains why LTLE is not well described. Therefore, in this dissertation, we refer to MTLE when we describe TLE. TLE is characterized by complex-partial seizures, with or without secondary generalization.

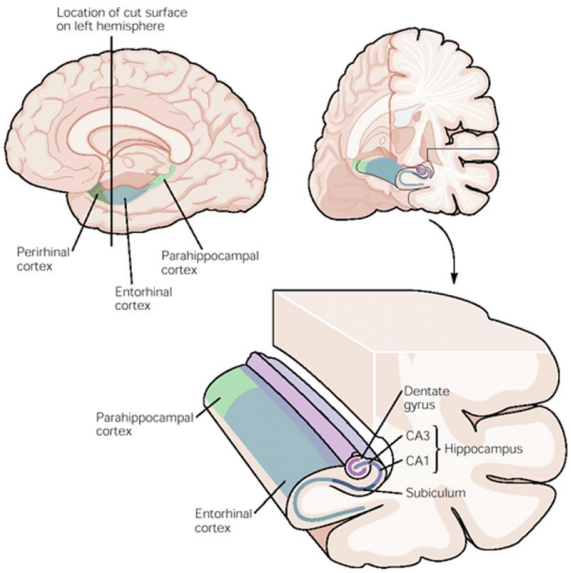
The hypothesis on TLE development is that these recurrent seizures originate from the medial temporal lobe and the limbic system (figure 2) after an acute brain insult followed by a latent period, during which cellular and molecular changes occur. This period can last for months to years before it eventually leads to a condition with spontaneously occurring recurrent seizures. It has been postulated that a second hit may be necessary to trigger chronic epilepsy. This second hit could be an environmental factor or time-dependent gene expression (Scharfman 2007)



Legend fig. 2: *(A) Schematic overview of the anatomical location of the temporal lobe in the human brain and (B) overview of the limbic system. Adapted from the HOPES Brain tutorial.*

Due to the high % of drug resistant TLE patients, understanding the mechanisms that lead to the development of chronic TLE is crucial to improve the existing treatment. The underlying cause of TLE remains to be elucidated but electroencephalography and neuroimaging studies suggest that the limbic system plays an important role in the process of developing TLE (Mathern et al. 1996). Since this dissertation is focused on hippocampal DBS, a more detailed overview of the normal anatomy and physiology of the hippocampus and the changes the brain structure undergoes in TLE is provided.

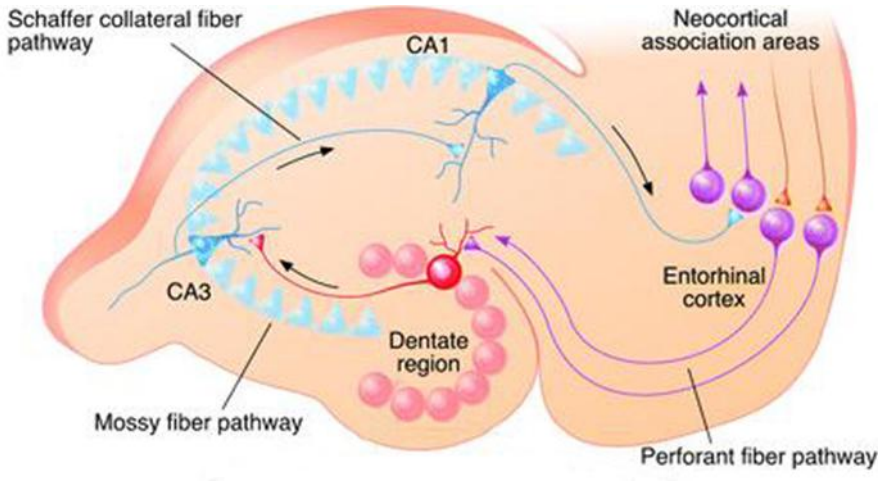
In the last decades, the (patho)physiology of the limbic system and the hippocampus in particular has been extensively studied. The hippocampus can easily be identified by its characteristic shape and unique macroscopic and microscopic structure. In essence, the hippocampus is a cortical wrapping of the archaeocortex. It evolved from a simple cortical plate in amphibians to a complex structure in mammals, tightly connected to the neocortical regions (El-Falougy and Benuska 2006). The hippocampus itself is divided into two U-shaped, interlocking sectors (figure 3), defining the "hippocampal formation".



Legend fig. 3: *Location of the hippocampus within the temporal lobe and a detailed view of the hippocampus showing the 2 U-shaped interlocking sectors composed of the dentate gyrus and CA4→CA1 regions (Kandel 2000)*

The hippocampal formation consists of 4 structures, each with different histological and functional properties (figure 4). These 4 structures are:

- the dentate gyrus (DG)
- the hippocampus proper (subdivided in regions CA1 → CA4)
- the subiculum
- the entorhinal cortex



Legend fig. 4: *Schematic representation of the different regions of the rodent hippocampal formation and information flow. Neurons of the dentate gyrus receive input from the entorhinal cortex via the perforant pathway, and extent projections into the CA3 via the mossy fiber pathway. Subsequent information flows to CA1 from which extra-hippocampal areas are targeted. Adapted from (Lie et al. 2004)*

Within the hippocampus there are well established unidirectional connections. They form closed loops that originate mainly in the entorhinal cortex. From the entorhinal cortex, the main input to the hippocampus is the perforant path. These connections project to either the granule cells of the DG, or to pyramidal cells of the CA1 region. Axons of granule cells in the DG, the so-called mossy fibers, project mainly to CA3 pyramidal cells. More than one granule cell can synapse onto a single CA3 pyramidal cell. Axons from the CA3 pyramidal cells project to the ipsi – and contralateral CA1 region pyramidal cells. These projections are called Schaffer collaterals.

The hippocampus plays an important role in the regulation of emotional behavior and a brain structure primarily involved in memory and learning (El-Falougy and Benuska 2006). The hippocampus plays a crucial role in formation of new memories. This is reflected by the fact that people with damage to the hippocampus are usually able to recall memories prior to the incident, but they have difficulties creating new memories. The DG is one of two locations where adult neurogenesis takes place (Li and Pleasure 2005). Adult neurogenesis has been shown to play an important role in learning and memory (Deng et al. 2010).

The hippocampus seems to be highly vulnerable to seizure-induced neuronal damage. Histological evaluation of human epileptic hippocampal tissue shows that the typical histopathological characteristics of TLE are neuronal cell loss (Lewis 2005) and granule cell dispersion (Fahrner et al. 2007), gliosis (Blumcke et al. 2002), synaptic plasticity under the form of mossy fiber sprouting (Parent et al. 1999), neurogenesis (Parent 2002), inflammation (Vezzani et al. 2002), and molecular reorganization in cellular membranes and extracellular matrix (Avanzini and Franceschetti 2003) in

the limbic system. Despite a large body of research and many theories, up to now it remains incompletely understood how these pathophysiological changes in the hippocampus contribute to, or are the consequence of, the ongoing epileptogenic process.

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Chapter 3

Deep brain stimulation in epilepsy

The history of deep brain stimulation in epilepsy

Early developments in the field of neurostimulation

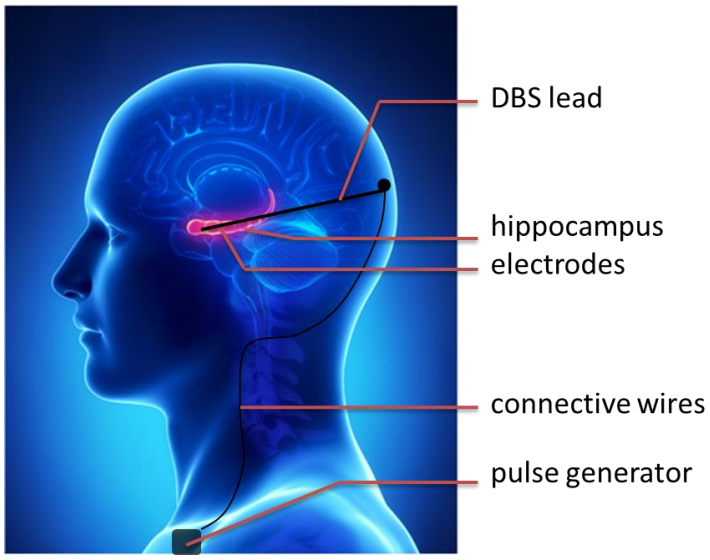
Deep brain stimulation may be perceived as a rather new and emerging treatment for various neurological and psychiatric disorders. Electrical stimulation however has been used to modulate the nervous system and to treat neurological disorders since ancient times (Rossi 2003). Scribonius Largo, the physician of the roman emperor Claudius already suggested to apply the electric ray fish (*Torpedotorpedo* and *Torpedo nobiliana)* to the cranium as a treatment for headache. These fish are known for their capability to produce electrical discharges (Debru 2006). Electric fish were later used for the treatment of seizures, depression and pain until the 18th century (Schwalb and Hamani 2008). The later development of neurostimulation evolves together with the development in the understanding of epilepsy as discussed in chapter 1. The discovery of animal electricity in 1791 by Luigi Galvani (Galvani 1791) inspired his nephew Giovanni Aldini (1762–1834) to further unravel Galvani's discoveries by performing electrical stimulations on the exposed human cerebral cortex of recently decapitated prisoners. In 1804, Aldini reported that cortical stimulation evoked horrible facial grimaces. This finding led him to conclude that the cortical surface could be stimulated electrically (Aldini 1804). This finding is the origin of the development of using brain stimulation for neurophysiological investigations to understand the functioning of the brain and the use of brain stimulation to treat neurological and psychiatric disorders. After various animal experiments Robert Bartholow (1831-1904) was the first to perform electrical stimulation of the cerebral cortex in awake humans (Bartholow 1874) demonstrating the electrical excitability of the cortex. the contribution of this research in determining the motor topography of the human brain nonetheless remained poorly exploited except to confirm the electrical excitability of the cortex. For more precise and systematic observations on the topography of the brain we should wait for until 1950 – when fundamental studies of the neurosurgeon Wilder Penfield (1891–1976) were published – before the brain stimulation of the human cortex could give a real accurate representation of the human brain functions, including motor and somatosensory areas(Penfield 1950).

The development of deep brain stimulation as a treatment for epilepsy

The first modern therapeutic intervention with brain stimulation is the use of electroshock for the treatment of severe psychosis (Kalinowsky 1986). The application of an electrical current on the skull evoked an epileptic seizure that "roughly" remodelled the neural connections, providing a clinical improvement to the patients. In the 1950s we see the first reports on brain stimulation for pain

control with good effects through temporarily implanted electrodes in the brain (Rezai 2002). In 1952, José M. Delgado is the first to describe the technique of implantation of intracranial electrodes in humans, indicating the importance of this method for diagnosis and its possible therapeutic role in patients with mental disorders (Delgado et al. 1952). The first reports on chronically implanted DBS systems in the thalamus for the treatment of pain emerge in the early 1970s (Hosobuchi et al. 1973). The extensive experience of Irving S. Cooper (1922-1985) in placing electrodes over the cerebellum and into deep thalamic nuclei for the treatment of cerebral palsy, spasticity and epilepsy marked a new step in the scientific approach to neurostimulation techniques (Cooper 1973). Due to the diagnostic use of neurostimulation prior to coagulation for the treatment of movement disorders to asure correct localisation, the positive effect of thalamic stimulation on tremor was well known (Gildenberg 2005). The first reports on chronically implanted DBS systems in the thalamus for the treatment of pain emerge in the early 1970s (Hosobuchi et al. 1973). The extensive experience of Irving S. Cooper (1922-1985) in placing electrodes over the cerebellum and into deep thalamic nuclei for the treatment of cerebral palsy, spasticity and epilepsy marked a new step in the scientific approach to neurostimulation techniques (Cooper 1973). Due to the diagnostic use of neurostimulation prior to coagulation for the treatment of movement disorders to asure correct localisation, the positive effect of thalamic stimulation on tremor was well known (Gildenberg 2005). The early reports of Prof. Benabid on stimulation of the ventral intermediate nucleus of the thalamus further empowered this notion (Benabid et al. 1987). In 1991 Benabid shows the safety and efficacy of long-term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus (Benabid et al. 1991). Based on Benabid's findings, the interest in using neurostimulation to treat Parkinson's disease led to an enormous increase in experimental studies in the field of neurostimulation. Exploring new stimulation targets and improving stimulation parameters eventually resulted in the approval by the Food and Drug Administration (FDA) of DBS for the treatment of essential tremor in 1997, for Parkinson's disease in 2002, and for dystonia in 2003. Because of the success of DBS in the treatment of movement disorders, the early observations of Cooper with cerebellar stimulation for the treatment of epilepsy (Cooper 1973) and the observation that epileptiform activity during functional mapping can be aborted by brief pulses of cortical stimulation (Penfield 1950). DBS regained interest with epileptologists in the 1990s. Various brain structures have been targeted with DBS in preclinical (Wyckhuys et al. 2009) and clinical trials (Sprengers et al. 2014) resulting in variable results. Two large multicenter trials - the SANTE (scheduled Stimulation of the Anterior Nuclei of Thalamus for Epilepsy) trial & the RNS® system (responsive focal neurostimulation) pivotal randomized controlled trial - showing safety and efficacy of both stimulation strategies for the treatment of drug resistant epilepsy patients (Fisher et al. 2010; Heck et al. 2014) led CE approval for ANT-DBS in 2009 and FDA approval of the NeuroPace®RNS®

system in 2013. Next to responsive focal and ANT-DBS, various reports have shown that hippocampal DBS is a promising treatment option for drug resistant epilepsy patients (Sprengers et al. 2014). Despite the various positive reports on the efficacy of hippocampal DBS as treatment for drug resistant epilepsy in open label studies(Boex et al. 2011; Cukiert et al. 2014; Velasco et al. 2007; Vonck et al. 2013) and very small RCTs (McLachlan et al. 2010; Tellez-Zenteno et al. 2006), more research is required to unravel the mechanisms underlying the therapeutic effect of hippocampal DBS and optimize the stimulation parameters to improve clinical outcome and to further explore the potential of this neurostimulation technique to modulate neuronal network activity.



Legend fig. 5: *schematic overview of an implanted hippocampal DBS system. A subclavicularly implanted pulse generator is connected with subcutaneous wires to an implanted electrode in the hippocampus.*

Clinical efficacy of hippocampal DBS in epilepsy

Several centers have explored the efficacy of scheduled hippocampal DBS as a treatment for drug resistant epilepsy patients.

Velasco and colleagues were the first to systematically study hippocampal DBS in 10 drug resistant epilepsy patients prior to temporal lobectomy (Velasco et al. 2000b). Hippocampal stimulation with 1-min trains of square wave pulses at 130Hz, a pulse duration of 450µs and an amplitude of 300µA followed by 4-min stimulation free intervals resulted in seizure freedom in 4/9 (44%) patients and a reduction in seizure rate of 50-70% in 5/9 (56%) at the 18 month follow-up (Velasco et al. 2007). In this study 5 patients receiving no stimulation during the first month of the double-blind period experienced no change in seizure rate compared to baseline, whereas the 4 patients in the treatment

arm during the first month of the double blind period had immediate reductions in seizure rate during this first blinded month.

At Ghent University, so far 13 drug resistant TLE patients have been implanted with DBS electrodes. Results of treatment in 12 drug resistant TLE patients with hippocampal DBS showed that after a mean follow-up of 31 months (range, 12–52 months), 1 of the 12 stimulated patients was seizure free (>1 year), 1 had a >90% reduction in seizure rate, 5 had a >50% reduction in seizure rate, and 2 had a seizure rate reduction of 30% to 49%, 1 patient was a non-responder and the remaining 2 patients eventually underwent underwent selective amygdalohippocampectomy (Boon et al. 2007). All the patients in this study were stimulated unilaterally except for one patient with a bilateral onset who received bilateral hippocampal stimulation. Stimulation intensity was set for each individual patient to 0.1-0.2V subthreshold of the stimulation intensity generating a stimulation artifact on the hippocampal electrode (mean output voltage of 2.3-3V). Long-term follow-up of these chronically stimulated patients and 1 additional patient implanted at a later time point showed that switching from unilateral to bilateral stimulation in 5 patients with an initial <90% seizure rate reduction improved seizure control in 3/5 patients. After a mean follow-up of 8.5 years, 6/11 (55%) have a >90% seizure rate reduction, 3/11 (27%) have a 50-90% reduction in seizure rate, and 2/11 (18%) experience <30% reduction in seizure rate (Vonck et al. 2013).

Tellez-Zenteno and colleagues report on 4 patients with drug resistant left TLE whose risk of postoperative memory deficits prevented resective surgery (Tellez-Zenteno et al. 2006). Continuous left hippocampal stimulation with a frequency of 190Hz, a pulse width of 90µs and stimulation intensity subthreshold of patients' conscious threshold (1.8 – 4.5V) delivered in a double-blind, randomized, crossover design resulted in a median seizure rate reduction of 15%, with 2/4 patients experiencing a reduction in seizure rate between 30 – 50%, and the remaining 2 patients experienced no change in seizure rate or a slight increase in seizure rate during stimulation.

The same research group reports in 2010 on 2 additional patients with independent bilateral mesial temporal lobe seizure onset (McLachlan et al. 2010). Continuous bilateral hippocampal stimulation with a frequency of 185Hz, a pulse width of 90µs and a stimulation intensity subthreshold of patients' conscious threshold delivered in a double-blind, randomized, cross-over design resulted in a median reduction in seizure rate of 33% compared to when stimulation was switched off in these patients.

Boëx and colleagues showed that treatment with unilateral hippocampal DBS with 130Hz, 450µs pulse width and a stimulation intensity of 0.5-2V at the side of most frequent seizure onset in 8 TLE patients resulted in seizure freedom in 2/8 of the patients. Four out of eight patients had a 50-90% reduction in seizure rate, and 2/8 patients were non-responders and showed no change in seizure rate during stimulation (Boex et al. 2011).

Recently, Cukiert et al. reported on the effect of hippocampal DBS in 9 patients with drug resistant TLE where patients with unilateral HS were implanted unilaterally at the affected side with hippocampal DBS electrodes and patients with a normal MRI or bilateral HS were implanted bilaterally. Two out of 9 patients were subjected to bilateral hippocampal DBS and 7/9 patients received unilateral hippocampal DBS. The decision to stimulate either bilateral or at the right or left side was based on the preceding non-invasive video-monitoring. Stimulation was started from the side where the majority of seizures came from. If this unilateral stimulation resulted in poor seizure rate improvement, the other side was stimulated (personal communication). They showed that continuous high frequency stimulation (130Hz, 300µs pulse width, 1-3.5 V) resulted in 2/9 seizure free patients, 5/9 patients with a >50% seizure rate reduction, and 2/9 patients with no reduction in seizure rate.

When combining these small open label and small randomized controlled trials, a total of 43 patients have been treated with hippocampal DBS for drug resistant epilepsy. Thirty out of these 43 (70%) patients experience a >50% reduction in seizure rate (table 1). This high responder rate should be interpreted with caution, as in the open label studies stimulation parameters were tailored to achieve optimal seizure control in the individual patient during daily clinical practice.

		seizure rate reduction			
study	# TLE patients	>90%	50-90%	30-50%	<30%
Velasco et al.,2000 **Velasco et al.,2007**	**9**	4	5		
Vonck et al.,2002 Boon et al.,2007 **Vonck et al.,2013**	**11***	6	2	1	2
Tellez-Zenteno et al., 2006	**4**			2	2
McLachlan et al.,2010	**2**			2	
Boëx et al., 2011	**8**	2	4		2
Cukiert et al., 2014	**9**	2	5		2
total	**43**	14	16	5	8

Legend table 1: *summary of all published open label and small randomized controlled trials on hippocampal DBS as treatment option for drug resistant epilepsy patients. Numbers are based on the most recent report on the patient group in bold. * In the Ghent university series, 13 patients were implanted with hippocampal DBS electrodes of which 2 eventually underwent selective amygdalohippocampectomy.*

Due to the small series including mainly open-label data, the value of hippocampal DBS remains difficult to assess. A recent Cochrane analysis on all small randomized controlled trials conducted on intracranial DBS showed that next to ANT-DBS and responsive focal DBS, hippocampal DBS seems to be a valid option to reduce seizure frequency in drug resistant epilepsy patients (Sprengers et al. 2014). Two independent randomized trials on hippocampal DBS have been initiated of which both the METTLE trial (multicenter study of hippocampal electrical stimulation in mesial temporal lobe epilepsy) and the CoRaStiR study (Controlled Randomized Stimulation versus Resection) have been discontinued due to insufficient enrolment.

Hippocampal DBS parameters for the treatment of epilepsy

Neurostimulation is defined by 2 main parameters; the frequency of stimulation and the charge density of an individual stimulation pulse. In this next section, we will briefly discuss the parameters that have been explored in hippocampal DBS for the treatment of drug resistant epilepsy. Continuous DBS pulses are delivered with square wave pulses according to a charge balanced design to avoid accumulation of electrical charges and to avoid tissue damage. It has been shown that biphasic stimuli are more efficient than pseudo-monophasic pulses in suppressing interictal activity with hippocampal DBS in drug resistant epilepsy patients (Tyrand et al. 2012).

Stimulation frequency

It has been suggested that low frequency stimulation (LFS) induces EEG synchronization, whereas high frequency stimulation (HFS) is associated with desynchronization of the EEG, which might have a therapeutic effect in epilepsy (Boon et al. 2007). Various animal studies have explored both LFS and HFS parameters, and both have shown positive effects on reducing seizure activity (Cuellar-Herrera et al. 2006; Urino et al. 2010; Weiss et al. 1995; Wyckhuys et al. 2010b). Animal studies directly comparing the efficacy of LFS with HFS are sparse. Wyckhuys et al. showed that HFS (130Hz) is more efficient compared to LFS (5Hz) in reducing evoked seizure activity in the kindling rat model (Wyckhuys et al. 2010a). A later study by Shigeto et al. showed that stimulation frequencies between 50Hz and 100Hz had the highest epileptiform activity evoking potential and there was no difference between irregular and regular stimulation (Shigeto et al. 2013). Contrary to this it has been shown that HFS of 130Hz delivered with a more irregular interpulse interval (Poisson distributed) was more efficient in suppressing seizures compared to HFS delivered with fixed interpulse intervals (Wyckhuys

et al. 2010b). Further animal experiments are needed to determine the most optimal stimulation frequency.

Because of the experience with HFS in the treatment of Parkinson patients and the observed safety of HFS hereby, only HFS has been used in patient studies exploring hippocampal DBS. As discussed in the previous section on efficacy of hippocampal DBS, HFS has promising seizure suppressive potential. Boëx et al. showed that HFS (130Hz) for 3-6h resulted in a decrease in interictal spiking during stimulation, while this effect was absent when using LFS (5Hz), suggesting that HFS should be preferred over LFS for the treatment of drug resistant epilepsy patients (Boex et al. 2007).

Charge density

The charge density is the product of the stimulation intensity and the pulse width. In animal studies various stimulation intensities and pulse durations have been used. There are no studies available that systematically evaluate the effect of different charge densities on seizures. In the clinical hippocampal DBS trials stimulation output was often determined by gradually increasing voltage until a stimulation artifact was observed on the hippocampal electrode (Boon et al. 2007) or until the patient experienced conscious appreciation of the stimulation (McLachlan et al. 2010; Tellez-Zenteno et al. 2006) and then the output voltage was decreased by 0.1–0.2 V to eliminate the stimulation artefact and determined to be subthreshold for conscious appreciation. The gradual increase in voltage is thought to affect a larger volume of epileptic tissue, which could result in better seizure control. Pulse widths were kept unchanged in all clinical hippocampal DBS trials and were set at either 90µs, 300µs or 450µs.

It must be emphasized that the basic assumptions with regard to stimulation parameters remain speculative and, as only limited data from animal and human studies are available. Current setting of stimulation parameters are mainly based on safety concern rather than based on clinical and experimental data suggesting efficient seizure control.

Safety & side-effects of hippocampal DBS

Complications associated with the implantation procedure of hippocampal DBS electrodes and generator that have been reported are skin erosion, local infection and asymptomatic haemorrhages around the electrode track (Boon et al. 2007; Cukiert et al. 2014; Velasco et al. 2007). The design of the Velasco study to perform hippocampal DBS in patients prior to temporal lobectomy allowed histopathological analysis of resected tissue subjected to hippocampal stimulation. The stimulated

hippocampal tissue showed histopathological abnormalities attributable to the depth-electrode penetration damage. However, no evident histopathological differences were found between the stimulated and non-stimulated hippocampal tissue (Velasco et al. 2000b). In none of the open label and small randomized controlled trials on hippocampal DBS had the stimulation pronounced side effects (Boex et al. 2011; Boon et al. 2007; Cukiert et al. 2014; McLachlan et al. 2010; Tellez-Zenteno et al. 2006; Velasco et al. 2007). Since the hippocampus is primarily involved in the process of memory and learning (El-Falougy and Benuska 2006), it should be very carefully monitored whether hippocampal DBS affects learning and memory. The available clinical data suggest that hippocampal DBS has no major effects on memory and learning (Velasco et al. 2007), but there seems to be an overall improvement in emotional well-being of the treated patients (Miatton et al. 2011). The issue of possible side-effects of hippocampal DBS on memory is more and more studied in preclinical animal experiments. Recent research has shown that rather than deteriorating or disturbing memory, hippocampal DBS might enhance short-term memory (Luna-Munguia et al. 2012). Overall, the procedure appears to be safe and does not appear to carry significant neuropsychological risks. However, additional studies with more included patients and long-term follow-up are needed to identify complications that might not be obvious in pilot-trials with limited sample sizes.

Mechanism of action of hippocampal DBS in epilepsy

Since the subject of this thesis is HFS for the treatment of drug resistant epilepsy, and HFS is being explored in human clinical trials as described above, we will limit this section to the proposed mechanisms of action of HFS. The mechanism by which high frequency DBS reduces seizure activity still needs to be elucidated. Several mechanisms of action have been proposed.

Continuous delivery of electrical pulses to the targeted brain structures may cause a reversible functional inhibition of the targeted region. The effect of the stimulation is then directly related to the targeted brain structure. The two main strategies to suppress seizures in drug resistant epilepsy patients with DBS are either stimulation of the epileptic onset zone causing a local inhibition of the hyperexcitable region or stimulation of structures responsible for seizure propagation which may result in suppression of seizure spread (Boon et al. 2007).

Some studies have suggested that the effect of hippocampal DBS is due to a microlesioning effect of the implantation of the electrodes and is independent from the applied electrical stimuli (Vonck et al. 2005). This theory is supported by the observation of prolonged seizure control in patients who underwent invasive recording with conventional electrodes (Katariwala et al. 2001). Studies showing a significant difference between stimulation ‘ON’ and stimulation ‘OFF’ conditions both in animal

studies and in clinical trials are in contrast with the microlesioning hypothesis (Velasco et al. 2007; Wyckhuys et al. 2010b)

Apart from the local functional inhibition, the mechanism of action of DBS may be related to the effect on projections leaving from the stimulation area to other structures and thereby suppressing neuronal excitability. The hypothesis of affecting the network excitability is supported by the fact that continuous high frequency stimulation of the hippocampus suppressed acute cortical epileptic activity effectively in the penicillin – induced epilepsy model (Akman et al. 2011). As the hippocampus is not only the presumed ictal focus in TLE but is also a structure involved in networks regulating neuronal excitability, it is plausible that focal targeting may subsequently affect the epileptogenic network (Boon et al. 2007). Animal studies have shown that decreased neuronal excitability by HFS can be induced by suppression of axonal conduction (Jensen and Durand 2009), induced potassium efflux and membrane hyperpolarization (Bikson et al. 2001; Lian et al. 2003). The decrease in neuronal excitability caused by HFS is supported by increasing threshold and decreasing duration of the hippocampus-induced afterdischarges during hippocampal HFS (Velasco et al. 2000b) and HFS induced hypoperfusion in a positron emission tomography (PET) study in the hippocampal region(Velasco et al. 2000a). This inhibitory effect of hippocampal DBS has also been shown to be supported by the involvement of the inhibitory neurotransmitter system gamma-aminobutyric acid (GABA). Increases in GABA levels in stimulated brain tissue both in animal studies as in patients have been shown (Cuellar-Herrera et al. 2004; Luna-Munguia et al. 2012).

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Chapter 4

Animal models in translational epilepsy research

Animal models in translational epilepsy research

The use of animal models plays a critical role in all modern biomedical research, because they allow us to study disease mechanisms in ways that would be unethical in patients. Developing models that reproduce critical features of clinical syndromes and phenotypes are therefore indispensable.

The International League against Epilepsy (ILAE) defined epileptic seizures and epilepsy as follows: *"An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain." "Epilepsy is a chronic disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychosocial, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure" (Fisher et al. 2005).* Therefore, when modeling human epileptic seizures and epilepsy it is essential to distinguish between (1) models of acute epileptic seizures that do not necessarily indicate the presence of an epileptic condition, and (2) models of epilepsy that are associated with permanent "epileptogenic" disturbances (Engel J 2006). It is debatable whether animal models exist or can be created in which the complex features of a seizure or syndrome are faithfully reproduced. Therefore animal modeling uses the strategy to focus on key components of seizures and syndromes that will help define and address the critical questions associated with the varied and complex seizure and syndrome phenotypes that have been clinically described (Engel 1998). Therefore several animal models are available for epilepsy research and different models are appropriate for studying different issues (Engel J 2006). In epilepsy research, 4 categories of animal models can be distinguished: in vitro models, genetic models, acute seizure models, and chronic models.

This section is not intended to give a complete overview and discussion on all available animal models used in epilepsy research. The goal of this section is to provide the reader with some background understanding on the advantages and disadvantages of different animal models in translational epilepsy research and to identify the animal model with the best fit to achieve the aims of this thesis to optimize hippocampal deep brain stimulation parameters to achieve better seizure control, and to explore the neuromodulatory properties of hippocampal DBS on disease progression in TLE.

In vitro models

To investigate the basic physiology of neurons and neuronal circuits in epilepsy, several in vitro preparations can be used ranging from single nerve cells acutely dissociated from animal and human brains, over cell culture models and organotypic brain slices to study in vitro isolated whole brains

from experimental animals (Pitkanen 2006). These models are useful to study basic ionic and electrophysiological mechanisms, but extracted preparations from its normal tissue surrounding produce distorted views of the intact in vivo situation (Stewart M. 2006). The use of in vitro models in epilepsy research is out of the scope of this thesis and will therefore not be further discussed.

Genetic animal models

Several transgenic mouse models exist with several epilepsy related phenotypes depending on the affected genes. The disadvantage of these models is that although epilepsy related phenotypes are mimicked and/or epilepsy related genes are targeted, it is unclear to what extent this mimicked situation represents human epilepsy (Burgess 2006). Idiopathic epilepsy syndromes are modeled by means of strains of rats who are predisposed to develop epilepsy. Most other genetic models are models of "reflex epilepsy" and display no spontaneous seizures. Animals with reflex epilepsy are sensitive to sensory stimulation like audiogenic or visual stimuli. The major drawback of these models is that this type of epilepsy is very rare in human epilepsy patients (Loscher 1997). The WAG/Rij and genetic absence epilepsy rats from Strasbourg (GAERS) are two genetic validated models for spontaneous absence seizures in humans. The hallmark of these absence seizure models is the appearance of bilateral generalized spike-wave discharges (SWDs) on the EEG, which are of higher frequency compared to human absence SWDs (7-12 Hz versus 3 Hz, respectively). In the GAERS rats the absences are characterized by behavioral arrest and are sometimes accompanied by rhythmic twitching of the vibrissae. The main difference between GAERS and the WAG/Rij strain is that the latter has two types of SWDs on the EEG. The type 2 SWD is not accompanied by behavioral signs and seems to be a more localized phenomenon (Midzianovskaia et al. 2001). Since this thesis addresses TLE, an interesting genetic model that should be mentioned is the Ihara epileptic rat that has a genetic predisposition to develop TLE (idiopathic) and displays neuronal microdysgenesis and gliosis in the hippocampus (Arai et al. 2003).

Acute animal models

Acute seizure models are mostly used as an initial screening model for the antiepileptic potential of new treatments. In these models, seizures are evoked by chemical or electrical stimuli applied in non-epileptic animals. The advantage of these models is the "on demand" availability of seizure activity and the reproducibility of these phenomena. A major drawback is that these models do not represent the human epileptic feature of recurring spontaneous seizures.

Systemic administration of convulsant agents is the most widely used route to create acute seizure models. The procedure is convenient, straightforward , and simple (Velisek 2006). Many convulsants have been used and can by roughly subdivided in GABA-related substances, excitatory amino acid-

related substances and acetylcholine (Ach) – related substances. The most used animal acute chemically induced seizure model is the pentylenetetrazol (PTZ) model in which the GABA-A receptor antagonist PTZ blocks GABA-A receptors. PTZ-induced clonic seizures represent a routine test for screening anticonvulsants (Swinyard EA. 1989).

Electrically induced seizures can be subdivided according to the mode of stimulation into two main types: those elicited by stimulation of the whole brain (electroshock seizures) and those induced by local stimulation of a defined brain structure (epileptic afterdischarges) (Mares 2006). There are two commonly used types of electroshock models: (1) stimulation with an alternating current of 50-60Hz and low frequency stimulation (6Hz). According to the stimulation intensity two types of seizures can be induced. Either *minimal* clonic seizures involving muscles of head and forelimbs without affecting the righting ability of the rat, and *maximal* (i.e. generalized tonic-clonic) seizures with a loss of the righting reflexes (Mares 2006). Both stimulation frequencies (50-60Hz or 6Hz) may be used, however the low-frequency stimulation (6Hz) results in less intensive seizures (Walker MC and Fisher A 2004). *Minimal* electroshock seizures represent a model of myoclonic seizures and are generated in the forebrain (Loscher and Schmidt 1988). *Maximal* electroshock seizures represent a model of generalized tonic-clonic seizure and are generated in the hindbrain (Loscher 2002). Local single stimulation of specific brain structures or repeated stimulations with the same intensity with short intervals not resulting in kindling can be used to evaluate electroencephalographic afterdischarges (ADs). ADs have specific electrographic patterns and behavioral correlates according to the stimulated structure. Epileptic ADs represent a model of complex partial seizures when stimulating limbic structures or myoclonic seizures when stimulating sensorimotor cortex (Mares 2006).

Chronic animal models

Chronic epilepsy models more closely resemble the epileptic state in which spontaneous and recurrent seizures occur (Loscher 2002). Several methods are used to induce spontaneous seizures in normal rodents and the description of methods in this section is not exhaustive. The most used methods to induce spontaneous seizures in normal rodents are brain infarction (Kelly et al. 2006; Srejic et al. 2013), traumatic brain damage(Bolkvadze and Pitkanen 2012; Kharatishvili et al. 2006), kindling(De Smedt et al. 2007; Michalakis et al. 1998) or SE (Raedt et al. 2009; Williams et al. 2009).

To model brain infarction, the standard technique is occlusion of the middle cerebral artery (MCAO). The MCAO procedure is intended to model post-stroke neocortical epilepsy characterized by focal seizures with elementary clonic or inhibitory motor signs, with or without secondary generalization. Despite the observation of electrographic seizures up to 3 days after lesioning (Hartings et al. 2003),

a drawback of this model is that long-term monitoring did not provide evidence of seizure activity up to 1 year after lesioning (Karhunen et al. 2003).

Traumatic brain injury (TBI) results in a complex assembly of acute and delayed molecular, cellular and network alternations, some of which are directly caused by trauma, whereas others are delayed and secondary to the initial physical impact (Laurer and McIntosh 1999). The most used model of traumatic brain injury is the lateral fluid percussion injury model. It produces several focal and diffuse characteristics of moderate to severe closed head injury in humans, including focal contusion, blood-brain barrier disruption, altered cerebral metabolism, altered cerebral blood flow, subdural hematoma, intraparenchymal and subarachnoidal hemorrhage, local and remote axonal injury, progressive neuronal loss, acute seizures and acute and behavioral abnormalities (Thompson et al. 2005). A drawback of this model are the relative high mortality of 30% to 40% after lateral fluid percussion injury and only about 50% of the surviving rats develop spontaneous seizures (Kharatishvili et al. 2006).

The kindling phenomenon is based on the repetitive induction of a focal seizure discharge, which eventually produces a progressive, highly reliable and permanent increase in the epileptic response to the inducing agent. The inducing agent can be chemical or electrical stimulation, with the latter being most widely used. The progression begins on the first day with a brief, low frequency electrographic AD, which is associated with little to no behavioral response. This response evolves over days, resulting in the triggering of long, high frequency ADs associated with strong convulsive responses (Goddard et al. 1969). Electrical stimulation can be focused to different brain structures such as the amygdala, the hippocampus or the pyriform cortex by using implanted electrodes. The behavioral responses to the repeated stimuli is scored with the so-called Racine's scale (Racine 1972). An animal is termed "fully kindled" when electrical stimulation reproducibly evokes generalized tonic-clonic seizures (stage 5 seizures according to the Racine's scale). Once an animal is fully kindled it remains displaying stage 5 seizures in response to stimulation for months or years (Goddard et al. 1969). When kindling is continued in these fully kindled animals they eventually develop spontaneous seizures, albeit rarely (Michalakis et al. 1998). This almost complete absence of spontaneous seizures is the main drawback of the model to study the effect of treatments on spontaneous seizures. Several stimulation protocols exist to kindle rats, ranging from delivering 1 kindling pulse per day during 15 days, over alternate day kindling with 48 kindling stimuli spread over 4 alternating days (Lothman and Williamson 1994), to rapid kindling protocols where rats receive 40 kindling stimuli in one day (Smith et al. 2005). Despite the clear development of seizure during the kindling process, a drawback of this model is that even after extensive kindling, gross morphological damage, as observed in patients with HS, are only moderately evident(Michalakis et al. 1998).

SE can be induced in animals by a number of different stimuli. In rodents, it is typically induced chemically by systemic or intracerebral kainic acid (a glutamate agonist) (Raedt et al. 2009; Williams et al. 2009) or pilocarpine (a cholinergic muscarinic agonist) (Cavalheiro et al. 1991; Glien et al. 2001) administration, or by sustained electrical stimulation of the amygdala (Brandt et al. 2003; Nissinen et al. 2000), perforant path (Gorter et al. 2001; Mazarati et al. 2002) or hippocampus (Bertram and Cornett 1994). This induced SE is the initial precipitating insult causing the process of epileptogenesis which eventually results in the occurrence of spontaneous seizures. Next to the occurrence of spontaneous seizures, SE models also display typical histopathological changes in different temporal lobe regions, which closely resemble the pathology seen in human TLE (Babb et al. 1995; King et al. 1995; Mello et al. 1993; Parent et al. 1997). A major advantage of the chemically induced post SE models is the high proportion of animals that develop spontaneous seizures after SE compared to the stimulation post SE models. While a disadvantage of the chemically induced post SE models is the high mortality during the SE, this mortality is very low in the stimulation post SE models. To reduce the high SE associated mortality, a repeated low-dose KA injection protocol has been developed by Hellier et al.(Hellier et al. 1998). This repeated low dose KA post SE model for TLE seemed to be the ideal rat model to achieve the goals of this thesis to optimize hippocampal deep brain stimulation parameters to achieve better seizure control, and to explore the neuromodulatory properties of hippocampal DBS on disease progression in TLE. Therefore in the next section of this thesis we show the results of a detailed characterization of this TLE model.

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The systemic kainic acid rat model of temporal lobe epilepsy: long-term EEG monitoring and histopathologic features

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BASED ON SUBMISSION IN BRAIN RESEARCH

ABSTRACT

Animal models that closely resemble the characteristics of human epilepsy are essential for the elucidation of the pathophysiology and in the search for novel treatment strategies. In this study we describe the natural evolution of epilepsy in the systemic kainic acid post status epilepticus rat model for temporal lobe epilepsy and evaluate the presence of histopathological features of human TLE. Rats were implanted with depth EEG recording electrodes in the left and right hippocampus. Rats received repeated low-dose KA injections (5mg/kg) until induction of a self-sustained SE. Continuous EEG monitoring was started from the moment of KA injection and was continued during 30 weeks. Mortality due to KA injections was limited to 7%, while 100% of the surviving KA injected rats developed spontaneous recurrent seizures (SRS). The median duration of the electrographic SE was 10.5 to 11.5 hours depending on the analysis method. The latency to the first spontaneous electrographic seizure was 7.4 ± 0.7 days after SE. Mean seizure rate progression of all rats in the KA rat model followed a sigmoid curve from 1 ± 0.2 seizures per day during the second week after SE to 24.4 ± 6.4 seizures per day during week 30 after SE. Seizure rate progressed to a plateau phase 122 ± 9 days after SE in 8 out of 9 rats. Although the development of a high stable seizure rate according to a sigmoid function was similar for these rats, the individual seizure rate during the final plateau phase was quite variable with a minimum of 14.5 seizures per day and a maximum of 48.6 seizures per day. A circadian rhythm in seizure occurrence was observed in all rats. Histological features typically found in human TLE such as astrogliosis and aberrant mossy fiber sprouting were also observed in the post SE KA model. Correlation analysis of SE and seizure rate progression parameters shows that rats with a fast increase in seizure rate during the exponential growth phase have higher seizure rates when reaching the plateau phase, compared to rats with a slower exponential increase in seizure rate. This long-term EEG monitoring study shows that the post SE model obtained with repeated low-doses of KA injections has a low mortality and results into the occurrence of SRS in all surviving rats after a silent period of about 1 week. All rats experienced an exponential increase in seizure rate during the 30 week long EEG monitoring period and reached a plateau in 8 out 9 rats after 122 ± 9 days. All rats displayed extensive astrogliosis and mossy fiber sprouting 30 weeks after SE, which are typical histopathological features of human TLE.

KEYWORDS

temporal lobe epilepsy, animal model, kainic acid, long-term EEG monitoring, seizures

INTRODUCTION

Over 50 million people world-wide are currently suffering from epilepsy (Chang and Lowenstein 2003; Engel 2008). The most common type of epilepsy is temporal lobe epilepsy (TLE). Patients with TLE typically have complex partial seizures, with or without secondary generalization. TLE is a condition often caused by an initial precipitating event such as febrile seizures, encephalitis, or status epilepticus (SE) leading to a cascade of molecular and cellular events, known as epileptogenesis, and eventually giving rise to spontaneous recurrent seizures (SRS).

There are no biomarkers to identify who will develop epilepsy after an initial event, and the period between the initial event and the occurrence of the first spontaneous seizure varies from patient to patient, ranging from months up to years (Annegers et al. 1998; French et al. 1993). Therefore, studying the natural history of epileptogenesis and the potential effects of disease modifying anti-epileptic treatments in patients is unfeasible. The development of new anti-epileptic drugs (AEDs) often relies on the use of preclinical animal models for acutely induced seizures. These treatments often prove to be ineffective in patients with TLE (Loscher and Schmidt 2011). In order to study epileptogenesis and to develop successful disease modifying anti-epileptic treatments, disease models that more closely reflect the characteristic features of human TLE such as the gradual occurrence of spontaneous recurrent seizures (SRS) and the typical histopathological changes found in TLE are required. Although no experimental model currently reproduces all the features of human TLE, several chronic epilepsy models allow to study the epileptogenic process and the effect of anti-epileptic treatments on SRS.

The most widely used animal models in this field are the post-status epilepticus (SE) models, where SE is induced chemically, either by intracerebral or systemic injection of pilocarpine or kainic acid (KA), or electrically by stimulation of the perforant path, the ventral hippocampus or the amygdala (Bertram and Cornett 1994; Brandt et al. 2003; Gorter et al. 2001; Lothman et al. 1989; Mazarati et al. 2002; Nissinen et al. 2000). This study focuses on the systemic KA model. Kainic acid (KA) [2-carboxy-4 (1-methylethenyl)- 3-pirrolidiacetic acid] is a cyclic analog of L-glutamate and an agonist of ionotropic, non-NMDA glutamate AMPA and KA receptors. KA is isolated from the seaweed *Digenea simplex.* The disadvantage of post SE epilepsy models obtained with intracerebral injections of chemoconvulsants is the required equipment and investigator skills. Systemic administration of chemoconvulsants requires no special training nor advanced equipment. Another advantage of the systemic KA model is the low mortality rate (15%) when using multiple injections of KA according to the protocol of Hellier et al. (Hellier et al. 1998) compared to the systemic pilocarpine model (30-50%) (Curia et al. 2008). In the systemic KA model a high proportion of rats develops SRS (97%)

(Hellier et al. 1998) compared to the electrically induced self-sustained SE model (54-80%) (Brandt et al. 2003).

Previous studies exploring the systemic KA model have either focused on histopathological changes (Sharma et al. 2008) or on the analysis of seizure rate progression during epileptogenesis (Hellier et al. 1998; Williams et al. 2009). One study performed long-term but not continuous EEG recordings throughout the disease progression in the KA model (Williams et al. 2009). In this study we combined long-term, continuous EEG monitoring to evaluate spontaneous seizure occurrence and progression with a histopathological analysis of hippocampal regions in systemically treated KA rats. An analysis of the EEG during SE was performed to investigate possible correlations between SE parameters (i.e electrographic SE duration and electrographic SE severity), seizure progression parameters and the histopathological findings.

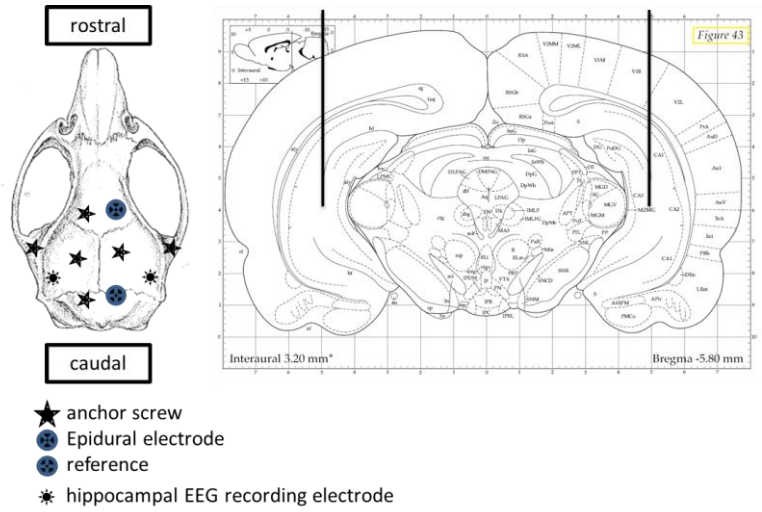
MATERIALS & METHODS

Animals

Male Sprague-Dawley rats (Harlan, the Netherlands) weighing 200–275 g, were treated according to guidelines approved by the European Ethics Committee (decree 86 / 609 / EEC). The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University Hospital (ECP 09 / 16). All animals were kept under environmentally controlled conditions (12 h normal light / dark cycles, 20–23°C and 50% relative humidity) with food and water intake *ad libitum*. The rats described in this paper served as a control group for an experiment that investigated the effect of long-term hippocampal DBS (unpublished data). The unique situation of continuously monitoring EEG in 9 control KA-injected rats during SE and for the following 30 consecutive weeks offered the opportunity to do an in depth analysis of the disease evolution in these rats in order to gain insight in the characteristic progression of the post SE KA rat model.

Surgery

Thirty rats were anesthetized with a mixture of isoflurane (5% for induction, 2% during implantation) and medical oxygen. At the start of the surgery, rats received subcutaneous Temgesic (0.03mg/kg) to reduce discomfort during recovery. After exposure of the skull, 10 small burr holes were drilled; six for the positioning of anchor screws (1.57 mm diameter; Bilaney), one for an epidural screw electrode above right frontal cortex, one for the reference/ground electrode over right cerebellum, one for a left-sided hipppocampal EEG recording electrode and one for right-sided hippocampal EEG recording electrode. The hippocampal EEG electrodes were made up from two polyimide coated stainless steel wires (bare diameter of 70 µm – CFW, CA, USA) with a distance of 500µm between the recording tips. These EEG recording electrodes were implanted at -5.6 mm AP, -5.9 DV, and ±5.1 ML relative to Bregma (Paxinos C 1998) (fig 1). The electrode leads ended in a common connector that was fixed to the skull with screws and acrylic dental cement. Immediately after surgery rats were injected subcutaneously with an NSAID, Metacam (1mg/kg) to provide analgesia and reduce the inflammatory reaction. Lidocaine and Neobacitracine pomade were applied to the wound, to lower discomfort during recovery and to avoid bacterial infections of the wound respectively. After surgery, rats were allowed 8 to 18 days of recovery before initiation of the experiment. One rat died during post-surgical recovery.



gend fig. 1: *schematic overview of the configuration of electrode implantation: hippocampal EEG recording electrode in the right and left hippocampus (AP -5.6, DV -5.9, ML ±5.1 relative to Bregma (Paxinos C 1998)) 1 epidural electrode above the right frontal hemisphere, 1 reference electrode just posterior to the sutura lambdoidea above the right hemisphere, and 6 anchor screws.*

Kainic acid injection & status epilepticus

In order to induce status epilepticus (SE), 29 rats (267 +/- 4 g) received kainic acid (KA) (5 mg/kg; Tocris Bioscience, USA) by intraperitoneal injections according to the protocol of Hellier et al. (Hellier et al. 1998). Seizure activity of all rats was continuously monitored visually and electrographically. The KA treatment was repeated hourly until the animals displayed a stable self-sustained SE for ≥3 hours (i.e., > 10 behavioral seizures per hour). Animals that exhibited excessive motor or excessive lethargic behavior were further given reduced dosages (2.5mg/kg) of KA to avoid exaggerated toxicity and mortality (Williams et al. 2009). Of the 29 rats undergoing the KA injection protocol, two rats died during SE.

EEG recording

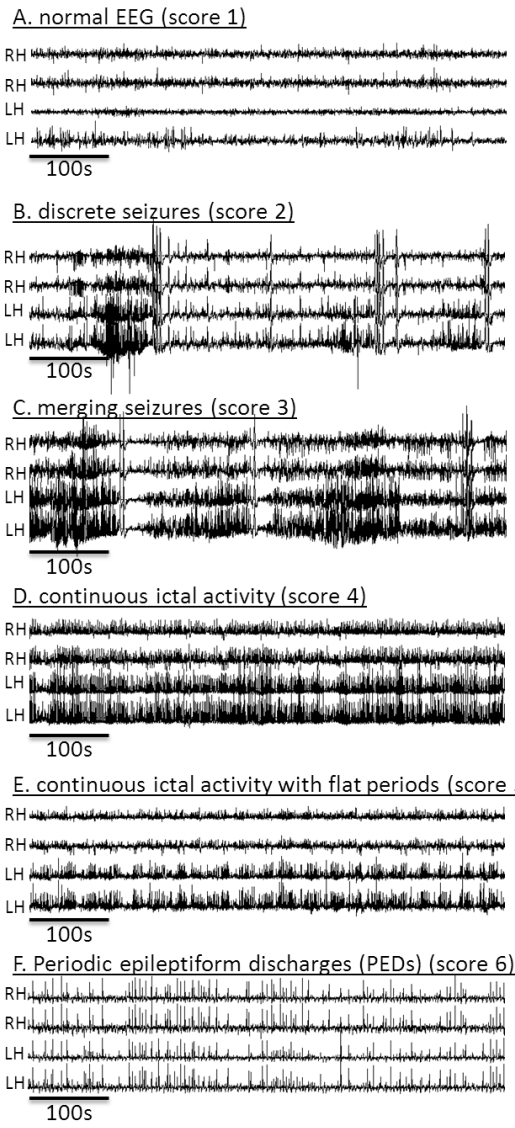
EEG signals were recorded with the hippocampal EEG recording electrodes via a head stage, carrying unity gain preamplifiers, and a commutator connected to custom-built amplifiers (gain: 510 x; band-width: 0.13Hz – 5.8kHz). A data acquisition card (NI-USB-6259, National Instruments, Belgium) digitized the EEG signals which were then stored onto a hard-drive for later offline analysis. The EEG sampling rate was set at 2 kHz. EEG recording was started in all rats before the first injection of KA and continued for 30 weeks. The EEG recording sessions were interrupted weekly for 30 minutes

from 9h00 until 9h30 to clean the cages and refresh food and water. Other than this, EEG was monitored continuously for 30 weeks without interruption.

Analysis of status epilepticus EEG

The EEG recordings during the first 24h of SE allowed us to analyze the electro-encephalographic SE characteristics. Continuous EEG monitoring allowed us to determine mean latency to the first electrographic seizure after KA injection. The EEG of 9 rats was scored during the first 24h after KA injection in 30' epochs. Two different methods of analysis were used to evaluate SE characteristics; a categorical scoring method and an automated spike detection method.

The so-called 'categorical six-point scoring' method consists of scoring EEG based on the occurrence of electrographic patterns such as discrete seizures, merging seizures, continuous ictal activity, continuous ictal activity with flat periods and periodic epileptiform discharges (PEDs) (fig. 2) (Lehmkuhle et al. 2009; Treiman et al. 1990; Walton and Treiman 1988). The SE duration was defined as the time from the appearance of the first discrete electrographic seizure (score 2) up to the epoch where no more continuous ictal activity was observed and only periodic epileptiform discharges (PEDs) were visible on the EEG (score 6) (fig. 2). The total SE severity score was calculated by adding all severity scores for the 30' epochs until a score 6 epoch appears (i.e. occurrence of PEDs superposed on normal EEG).



six-point method		
1	normal or slow isolated EEG spikes	
2	discrete seizures	SE
3	merging seizures	
4	continuous ictal activity	
5	continuous ictal activity with flat periods	
6	periodic epileptic discharges (PEDS)	

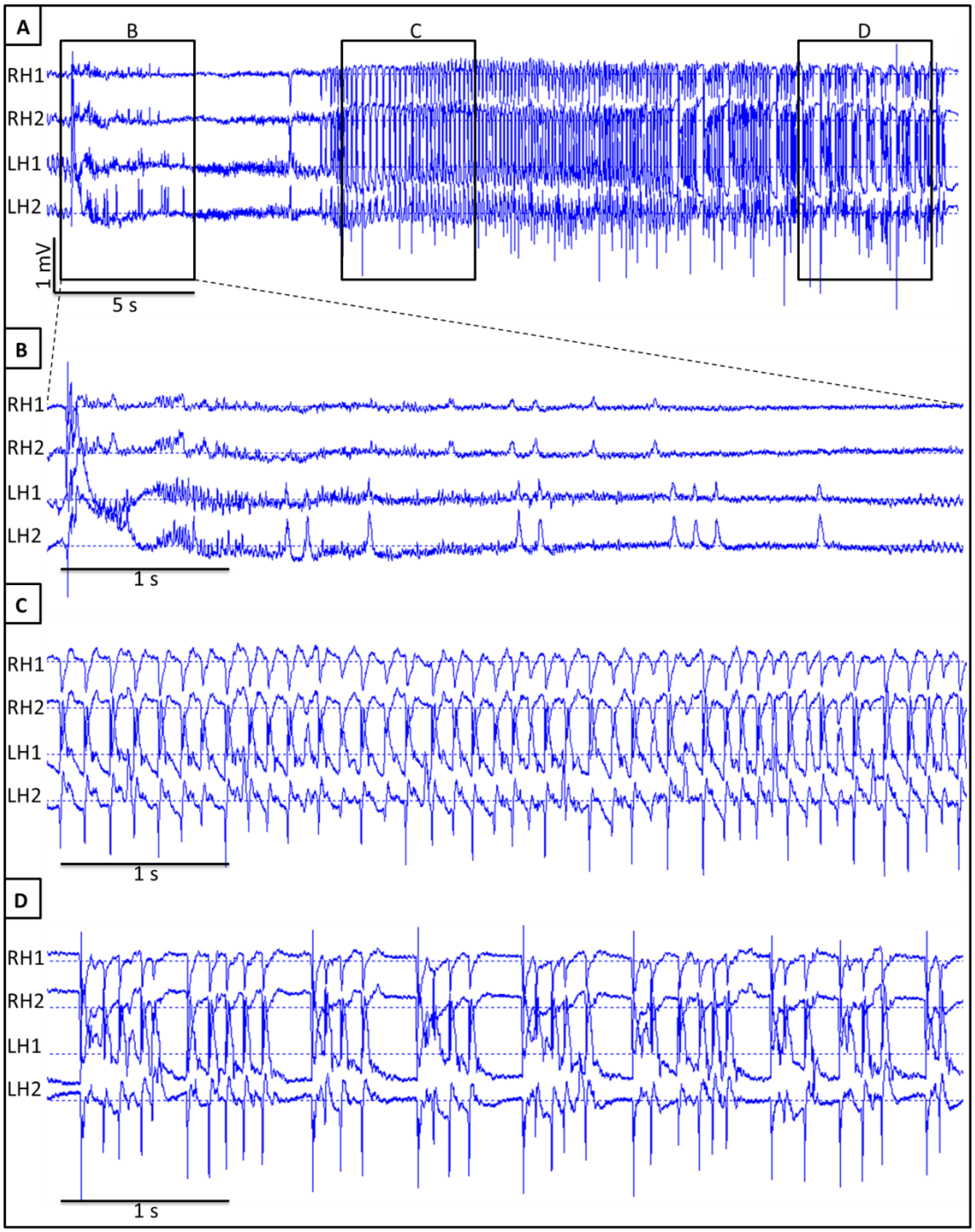
Legend fig. 2: *extracts of a typical sequence of electrographic patterns during status epileptics; RH-right hippocampus, LH-left hippocampus* ***A:*** *normal EEG before KA injection.* ***B:*** *SE starts with a period of discrete seizures.* ***C:*** *period of merging seizures.* ***D:*** continuous ictal activity*.* ***E:*** *continuous ictal activity with flat periods.* ***F:*** periodic epileptiform discharges. six-point scoring method: rats are defined as "in status" when score 2-5 epochs are registered.

The total number of epileptiform spikes during SE and the SE duration was determined with an 'in house developed' Matlab software application. Before running the spike detection software, the EEG traces were digitally filtered with a band pass filter (1-50Hz) to reduce low-frequency movement artifacts and high frequency noise. The spike number was determined by setting thresholds 3 standard deviations (SD) above the mean amplitude of a baseline period of 3 minutes taken before KA injection. Each upward event with a minimum width of 10ms and a maximum width of 100ms was counted to determine the total number of spikes. SE severity was assessed by counting the total

number of spikes during 24h after SE onset (Pitkanen et al. 2005). SE duration was calculated by multiplying the number of analyzed epochs during which the spiking frequency was higher than 1 Hz with the duration of the epoch (ie. 30 minutes).

EEG analysis of spontaneous seizures

Seizures were defined as episodes of rhythmic spiking activity with a high amplitude (>3 x baseline) and a high frequency (>5Hz) during at least 10 seconds. These seizures often started with a large positive or negative potential followed by a decrease in amplitude, that progressed into the described high frequency, large amplitude rhythmic spiking activity, (White et al. 2006; Williams et al. 2009; Wyckhuys et al. 2010). The end of a seizure was determined as the last spike of this large amplitude rhythmic spiking activity. Based on these annotated seizures, latency to the first electrographic seizure, mean daily seizure rate and seizure duration were determined during the entire 30 week long EEG monitoring period. Determining seizure rate per hour allowed us to check for possible day – night differences in seizure occurrence.



Legend fig.3: *A typical electrographic seizure following a latent period after KA-induced SE; RH-right hippocampus, LH-left hippocampus* ***A:*** *electrographic seizure that lasts for 34s.* ***B:*** *Seizure initiation characterized by a large positive or negative potential followed by a decrease in amplitude.* ***C:*** *Progression of the spikes into rhythmic, high frequency, high-amplitude EEG spiking.* ***D:*** *Lingering epileptic activity near the end of the seizure.*

Histology

At the end of the experiment, all rats were deeply anesthetized with pentobarbital (100mg/kg, i.p.) and transcardially perfused with a 0.37% Na_2S solution followed by 4% paraformaldehyde. The brains were removed and post-fixed in a 4% paraformaldehyde solution for at least 24 hours. Then, brains were transferred to a 10%, 20%, and 30% sucrose solution (at least one day in each solution), frozen in ice-cold isopentane and stored in liquid nitrogen until sectioning. Coronal sections (70µm) were cut at the level of the hippocampus. Sections were collected in 30% ethylene glycol and 25% glycerol in 50 mM phosphate buffered saline (PBS) and stored at -20°C until they were processed after rinsing in PBS. Starting at a point 2.3mm posterior from Bregma (Paxinos C 1998), a 1-in-6 series of coronal sections of each rat brain was processed for Nissl staining to confirm electrode location.

A second 1-in-6 series of coronal sections from 6 out of 9 rats was processed for Timm's staining to evaluate the presence of mossy fiber sprouting. Sections were mounted, dried, dehydrated and developed for 60 minutes in developer solution made by mixing 120ml of 50% gum Arabic, 20ml of 2M citrate buffer, 60ml of 0.5M hydroquinone and 1 ml of 17% silver nitrate solution. After rinsing, sections were again dehydrated and coverslipped with Entellan. Brain slices processed for Timm staining were scored independently by 3 investigators with the Timm scoring method of Cavazos et al. (Cavazos et al. 1991). In brief, a score of 0 represents no Timm granules in the supragranular layer. Score 1 represents very light, sparse granules in the supragranular layer. Score 2 represents a very light distribution of granules over a continuous region in the supragranular layer. Score 3 represents a continuous pattern of granules in a band over the supragranular region. Score 4 represents a confluent dense laminar band of granules, not entirely filled. Score 5 represents an entire band of granules in the supragranular layer, extending into the inner molecular layer. To evaluate inter-rater reliability, a split-half reliability analysis was performed to compare the ratings of injury by the 3 investigators (BVN, RR, MS). Scores of the 3 investigators were averaged; reported values represent the mean and SEM of the averaged scores of the 3 investigators.

Immunohistochemical analysis was performed on a third 1-in-6 series of coronal sections of all 9 rats to evaluate the presence of reactive astrocytes (Vimentine) using a protocol adapted from Raedt et al. (Raedt et al. 2009). Sections were rinsed with PBS and subjected to 30 minutes of antigen retrieval in NaCitrate buffer (10 mM, pH 8.5). Thereafter sections were treated with 0.6% H2O2 in PBS for 30 min to block endogenous peroxidase activity. The sections were then incubated for 30 minutes with a blocking solution containing PBS, 0.25% Triton X-100 and 3% donkey serum. Primary mouse anti-Vimentin antibody (DAKO, Heverlee, Belgium) was applied overnight at 4°C using a 1:500 dilution. The next day sections were washed and incubated for 2h with biotinylated donkey anti-mouse IgG antibody (1:1000; JacksonImmunoResearch, Suffolk, England), before avidin-biotin-

peroxidase complex solution (Vectastain Elite ABC-kit Standard, Vector Laboratories, Burlingame, CA,USA) was applied for 1h prior to peroxidase detection (0.25 mg/ml 3,3-diaminobenzidine, 0.01% H2O2 and 0.04% NiCl for 5 min). These sections were mounted, dehydrated and coverslipped with Entellan.

Statistics

All data was tested for normality before statistical analysis. In case conditions for parametric testing were not fulfilled, non-parametric testing was used and values were expressed as median and interquartile range (IQR). Data fulfilling conditions for parametric testing were expressed as mean and standard error of the mean (sem). Differences between the 6 point status scoring method and the spiking detection method were tested using the non-parametric Wilcoxon signed rank test. Differences in mean daily seizure duration over the 30 week EEG monitoring period were tested using a one-way repeated measures ANOVA. Difference in seizure rate during lights on periods and lights off periods were tested using a paired t-test. Correlation analysis for SE, seizure rate progression parameters and histopathological quantifications were performed with the non-parametric Spearman Rank test. Parameters of SE for correlation analysis were the total KA dose, SE duration as determined with the spike detection and the six point analysis method and total number of spikes during SE. Parameters for seizure rate progression were latency to the first spontaneous seizure, maximum seizure rate, time after SE to reach the half maximum seizure rate, the slope of the exponential increase in seizure rate, and days after SE to reach the final stable plateau phase in seizure rate progression. Parameters for histopathological quantification were the Timm score of ventral and dorsal hippocampus. Significance is assumed for p-values < 0.05.

RESULTS

Status epilepticus in the KA model

KA injections according to the protocol of Hellier et al. (Hellier et al. 1998) resulted in a self-sustained SE in all rats. Two out of 29 rats died during SE. The rats described in this paper served as a control group for an experiment that investigated the effect of long-term hippocampal DBS (unpublished data). Of the 27 surviving rats, 9 rats were randomly selected for long term EEG monitoring and are described in this paper. The semiology of the SE was characterized by continuous seizure activity ie. stereotyped sniffing, head nodding, wet dog shakes, behavioral arrest, repetitive chewing and excessive salivation, repeatedly interrupted by generalized convulsive seizures. The EEG traces of all rats displayed the typical sequential electrographic patterns of SE that have been described in status epilepticus animal models: discrete seizures with interictal slowing; merging seizures with waxing and waning amplitude and frequency of EEG rhythm; continuous ictal activity; continuous ictal activity interrupted by low voltage flat periods; and periodic epileptiform discharges (Treiman et al. 1990) (fig 2).

The mean latency between the first KA injection and the appearance of the first discrete seizure on the hippocampal EEG was 48 ± 11 minutes. For quantitative analysis of the electrographic SE 2 methods were used as described in the methods section. With the human categorical six-point method we found a median total SE severity score of 81 (IQR: 80 – 90) and a median SE duration of 10.5 (IQR: 10.0 – 11.1) hours (fig.4).

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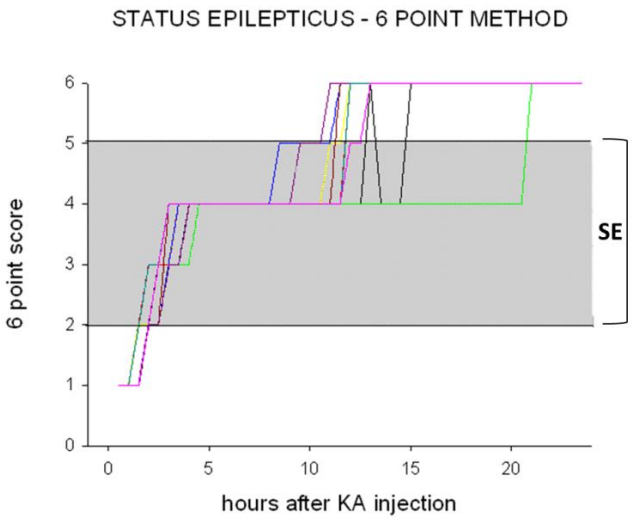
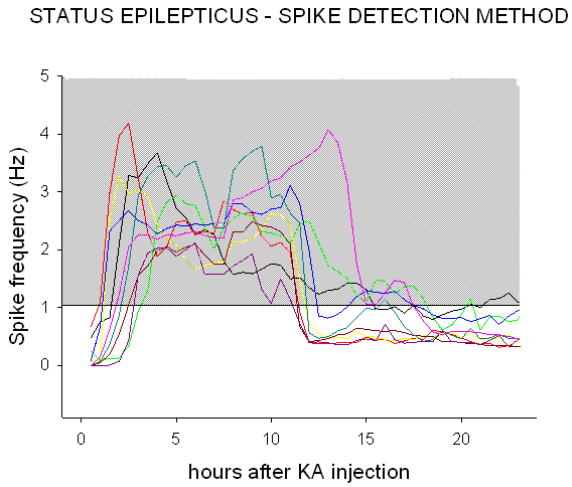


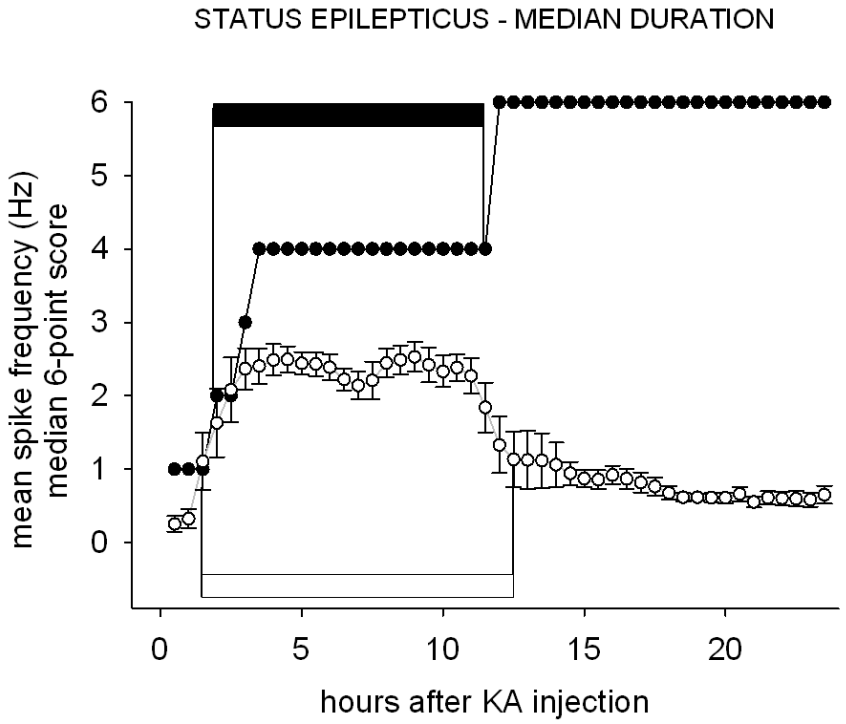
Legend fig 4: SE progression of all rats according to the six-point scoring method. Rats are defined in status once discrete seizures appear (score 2) until relative flat EEG superposed with periodic epileptiform discharges (PEDs) is observed (score 6).

In all rats, the automated spike detection method described in the methods section revealed a similar progression in spike frequency over time during SE. The appearance of discrete electrographic seizures evolving into merging seizure activity could be observed as a steep rise in spiking frequency, after which spiking frequency remained at a relatively stable level corresponding to continuous electrographic ictal activity. A sudden decrease in spiking frequency is observed at the time of the transition from continuous electrographic ictal activity to EEG traces superposed with periodic epileptiform discharges (PEDs), (fig. 5).



Legend fig 5: *SE progression of all rats according to the automated spike detection method. Status is defined as the period where spiking frequency is higher than 1Hz.*

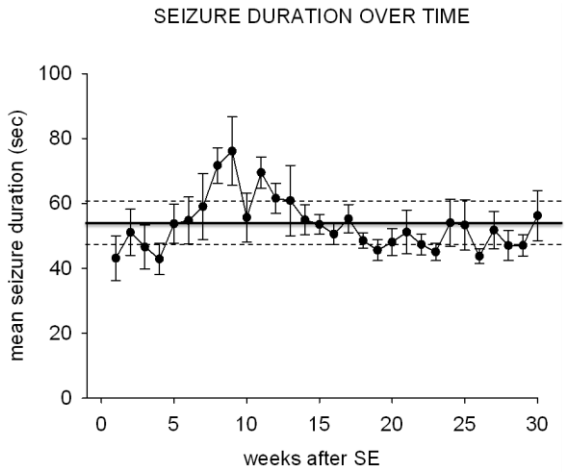
As determined by the automated spike detection method, SE duration was 11.5 (IQR: 10.9 – 16.6) hours. During the first 24h after KA injection 129,665 (IQR: 108,544 – 134,199) spikes were counted. There was a good concordance between SE progression as determined with the six-point method and as calculated with the automated spike detection method (fig.6).



Legend fig 6: *median SE progression for all rats according to the six-point method. Status is defined as the time spanning from the appearance of discrete seizures (score 2) until relative flat EEG superposed with periodic epileptiform discharges (PEDs) is observed (score 6) (black dots). Black bar represents SE duration according to the six-point method and according to the spike detection method. Status is defined as the period when spike frequency is higher than 1Hz.(white dots).White bar represents SE duration according to the spiking detection method*

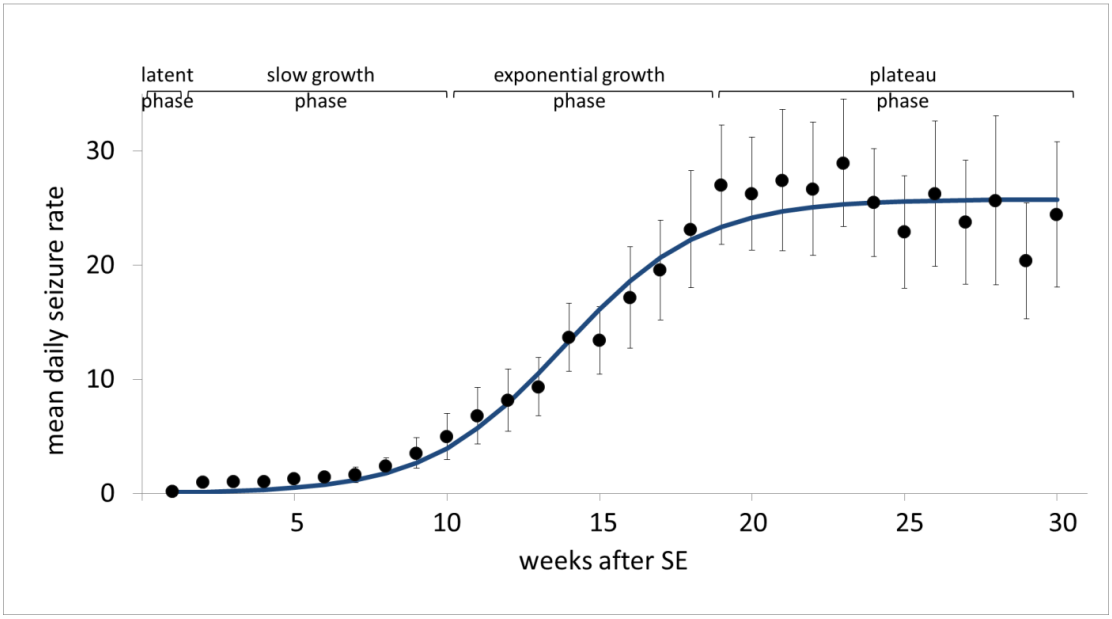
Spontaneous seizures in the KA model

EEG was continuously monitored for 30 weeks in 6 out of 9 rats. Due to the unexpected loss of the head cap (1/9) or due to unexpected death (2/9) the EEG monitoring period was shorter in 3 rats (24, 18 and 15 weeks respectively). Continuous EEG recording allowed us to determine the latency to the first spontaneous seizure after SE, and the evolution over time in seizure rate and seizure duration. The mean latency to the first seizure was 7 ± 1 days with a maximum latency of 10 days and a minimum latency of 4 days after the onset of SE. The mean duration of SRS in the KA model was 56 ± 5 seconds. The mean duration of seizures remained unchanged throughout the entire 30 week follow-up period (fig. 7).



Legend fig.7: *mean seizure duration over 30 weeks of continuous EEG monitoring. The solid line represents the mean seizure duration for all rats over the entire 30 weeks period. Dashed lines are the sem of the mean seizure duration for all rats over the entire 30 weeks*

The mean increase in seizure rate of all rats evolved from a mean seizure rate of 1.0 ± 0.2 seizures per day (n=9) during the second week after KA injection to a mean seizure rate of 24.4 ± 6.4 seizure per day (n=6) during week 30 after KA injection. A Boltzmann sigmoid function can be fitted on the mean seizure rate progression during the entire EEG monitoring period starting immediately after KA injection (n=9) (fig. 8).



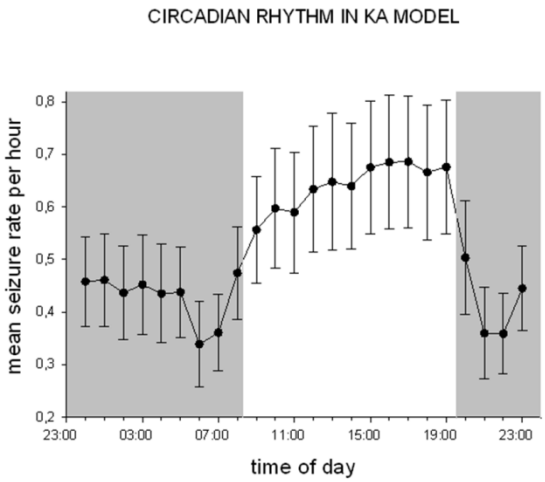
Legend fig. 8: *Progression in seizure frequency (dots) as a function of time after KA induced status epilepticus. The blue line is a sigmoid Boltzmann function fitted to the data. Four stages in seizure rate progression could be distinguished. Stage 1 the latent period for electrographic seizures. Stage 2 is the period of a slow increase in seizure rate (the slow growth phase), which was followed by the exponential growth phase in seizure rate and eventually going into the plateau phase.*

The expected plateau phase (Williams et al. 2009) was found in 8 out of the 9 rats. In these 8 rats a Boltzmann sigmoid curve could be fitted to the time dependent increase in seizure rate after KA injection. The function describing the fitted curves has the following form:

$$y(t) = y_{in} \pm \frac{y_{max}}{(1+e^{\frac{\tau - t}{k}})}$$

Where y(t) is the seizure rate at time point t in weeks after KA injection; y_{in} is the initial seizure rate, y_{max} is the maximum seizure rate; τ is the time in weeks after KA injections the increase in seizure rate reaches its half maximum value; k is the slope factor describing the steepness of the Boltzmann sigmoid curve. These parameters were calculated for 8 out of 9 rats describing the progression in seizure rate. Mean maximum seizure rate was 27.7 ± 3.1 seizure per day. The half maximum of this seizure rate was reached after 13.5 ± 1.2 weeks. Mean slope factor was 1.5 ± 0.2. For comparison purpose with the paper of Williams et al., the same seizure rate progression data could be subdivided in 4 periods (Williams et al. 2009). First, a latent period during which no seizures occurred with a mean duration of 7.4 ± 0.7 days. Second, a slow growth phase characterized by slow steadily increase in seizure rate until the mean daily seizure rate was >5 seizures per day. The mean number of days after SE to obtain this seizure rate was 67.5 ± 6.3 days. The third phase of seizure rate progression, called the exponential growth phase, is characterized by a very fast increase in seizure rate. The end of this stage was mathematically defined as the time in which the fitted sigmoid curve attained 95% of its maximal value. The mean time to the end of the exponential growth phase was 122.3 ± 9.0 days. The last phase in seizure rate progression, known as the plateau phase is characterized by stable high seizure rates. Despite this uniformity in seizure rate progression among rats, the seizure rate at the final stage was quite variable among rats, with a minimum of 14.5 seizure per day up to a mean daily seizure rate of 48.6 seizures in the rat with the highest seizure rate.

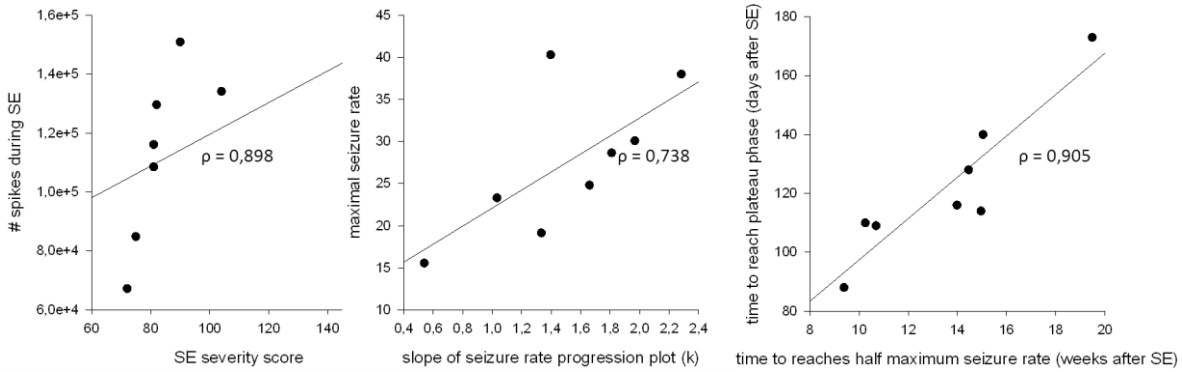
A clear circadian rhythm was observed in all rats. Over the entire 30 week period, mean seizure rate during the light phase was 0.63 ± 0.12 seizures per hour. Whereas mean seizure rate during the dark phase was significantly lower with 0.42 ± 0.09 seizures per hour. Both the increase and decrease in seizure rate related to the light phase is immediately apparent from the first hour the light is either switched on or off (fig 9).



Legend fig.9: *Circadian rhythm of SRS. Based on mean hourly seizure rate for all rats over the entire 30 week long period. Wilcoxon signed rank test showed that seizure rate was significantly higher during the lights-on phase (08:00-20:00) (0.63 ± 0.12 seizures) per hour compared to the lights-off phase (20:00-08:00 (marked in grey) (0.42 ± 0.09))*

Correlation analysis SE & seizure rate progression parameters

A positive correlation was found between SE score and the number of spikes during SE (ρ = 0.898). When looking at seizure progression parameters, a positive correlation was found between the maximum seizure rate (y_{max}) and the slope factor (k) of seizure rate progression (ρ = 0.738). This positive correlation was also observed between the time in weeks after KA injections the increase in seizure rate reaches its half maximum value (τ) and the time after KA injection seizure rate progression reaches the plateau phase (i.e. seizure rate > 95% of y_{max}) (ρ = 0.905) (fig.10).

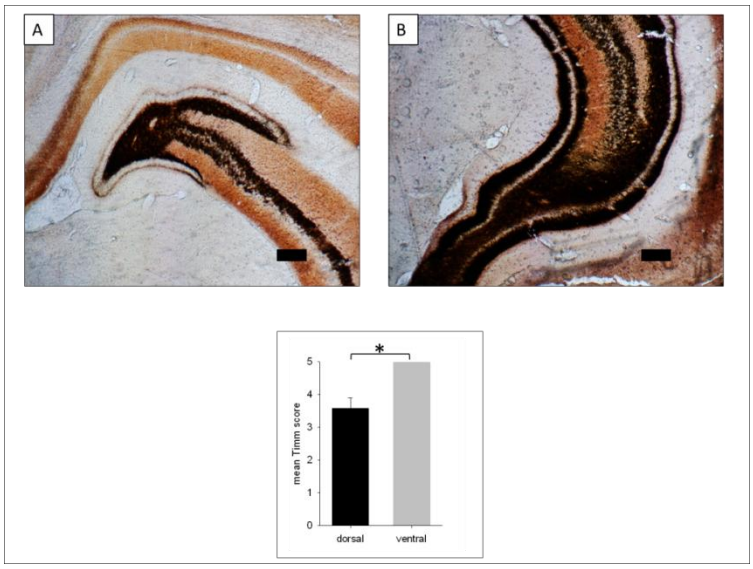


Legend fig.10: *statistically significant correlations between all analyzed SE parameters and seizure rate progression parameters (p < 0.05); ρ, correlation coefficient Spearman rank test*

Histopathological characteristics of the KA model

Mossy fiber sprouting

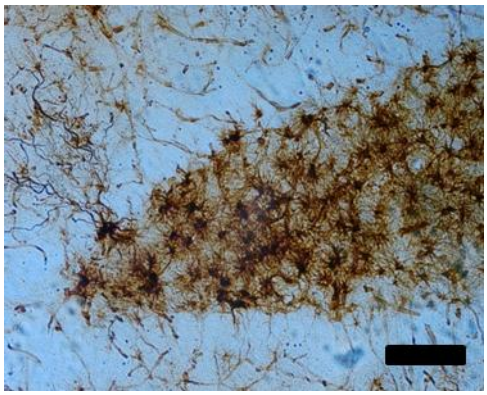
Brains of 6 out of 9 rats were processed for Timm staining to asses mossy fiber sprouting (MFS) in the intraperitoneal KA model 30 weeks after SE. Timm staining showed extensive mossy fiber sprouting throughout the entire hippocampus in all rats. Inter-rater correlation of the scoring of the 3 investigators was 0.915, and did not differ significantly. Scoring of the amount of mossy fiber sprouting in the ventral and dorsal hippocampus, revealed a significant difference. Mossy fiber sprouting was more extensive in the ventral part of the hippocampus for all rats (mean Timm score of 5 ± 0) compared to the Timm score in the dorsal part of the hippocampus (3.6 ± 0.3) (fig 11).



Legend fig. 11: *Mossy fibre sprouting. Representative examples of sections (70 µm) of the dorsal part of the HC (A), and ventral part of the HC (B) stained by the Timm staining protocol for evaluation of mossy fibre sprouting. MFS was present in all rats. MFS was more intense in the ventral HC compared to the dorsal HC. Scale bar is 200 µm.*

Astrogliosis

Immunohistochemical analysis revealed the presence of reactive astrocytes (vimentine positive) throughout the entire hippocampus of all 9 rats, indicating extensive astrogliosis in the hippocampi of ip. treated KA rats 30 weeks after SE (fig 12). Astrogliosis was most prominent in the hilus.



Legend fig. 12: Presence of reactive astrocytes in the hippocampus. Representative example *of section (70 µm) of the hilus stained for Vimentine (Vim). Vim-expressing astrocytes were found throughout the entire hippocampus and were most prominent in the hilus. A) Scale bar 100 µm.*

DISCUSSION

In our study the mortality rate of repetitive low-dose KA injections was only 7% (2/29). Single dose injections of KA induced much higher mortality (up to ± 50%) and often did not consistently induce chronic epilepsy (Cronin and Dudek 1988; Meier and Dudek 1996). In comparison to the repeated low dose KA-injection protocol, systemic pilocarpine induced SE has a higher mortality rate of 30-50% (Curia et al. 2008), and mortality rate in the stimulation SE models is comparable with our observations. All rats we injected with KA developed a self-sustained SE consistent with reports on the intrahippocampal KA model (Raedt et al. 2009), while in the ip. post SE pilocarpine model only 83% of the rats develop SE (Mello et al. 1993), in the continuous hippocampal and perforant path stimulation model only 70% (Bertram and Cornett 1994; Gorter et al. 2001) and in the amygdala stimulation model 87% of the rats develop SE (Nissinen et al. 2000). In all rats, the typical sequential electrographic patterns of status epilepticus that have been described in patients with generalized convulsive status epilepticus and various rat models of SE such as the single dose systemic KA SE model, the cobalt/homocysteine SE model, and the lithium/pilocarpine SE model could be observed: discrete seizures with interictal slowing; merging seizures with waxing and waning amplitude and frequency of EEG rhythm; continuous ictal activity; continuous ictal activity interrupted with low voltage flat periods; and periodic epileptiform discharges (Treiman et al. 1990). All the rats (100%) in our study developed SRS after SE. This corresponds to observations in the systemic pilocarpine model, where more than 90% of the surviving animals became epileptic (Cavalheiro et al. 1991; Glien et al. 2001). In the amygdala stimulation SE model 88% of the rats develop SRS, but only 31% of these rats progress into a state with frequent SRS (Nissinen et al. 2000). This high amount of rats developing SRS, but with only a small fraction of the rats (27%) having frequent seizures was also observed in the intrahippocampal KA rat model (Raedt et al. 2009). In the perforant path stimulation SE model only about 54-80% of the rats develop SRS (Brandt et al. 2003). Therefore, the ip. KA model described in this report was as effective as the systemic pilocarpine model in inducing a chronic epileptic state, but with a lower mortality. In humans, the risk of SRS after SE is 37% within one year and 56% within three years (Hauser et al. 1990). This lower risk may be a reflection of the attempts to interrupt SE in patients and subsequent chronic treatment SE patients with anti-epileptic drugs (AEDs). In this study repeated low-dose KA injection resulted in a median SE duration of 10.5 – 11.5 hours depending on the method used to determine SE duration, which was in accordance to the observations in the intrahippocampal KA model (Raedt et al. 2009). With a SE duration of 8-12 hours in the pilocarpine model (Cavalheiro et al. 1991; Cavalheiro et al. 1994), around 12h in the amygdala stimulation SE model (Nissinen et al. 2000) and 8 hours in the perforant path stimulation SE model (Brandt et al. 2003), SE duration in other post SE models are quite similar to our observation in the

post SE KA model. The short latency of 7.4 days after KA before the first spontaneous seizure occurs is in accordance with the observation of Williams et al., who observed a latency of 7-9 day after KA was observed before the first spontaneous seizure occurred with nearly continuous EEG monitoring (Williams et al. 2009). This latent period is slightly shorter than the latent period of around 14 days that has been reported in the pilocarpine model (Cavalheiro et al. 1991; Curia et al. 2008), and much less variable compared to latent periods in the amygdala stimulation SE model varying between 6 to 85 days (Nissinen et al. 2000) or in the perforant path stimulation SE model with a latent period of 23-99 days (Brandt et al. 2003). This narrow variability range in the systemic repeated low-dose KA model makes it an appropriate epilepsy model to test experimental treatments aimed at postponing or suppressing the occurrence of SRS after an initial event like SE. Data on seizure rate progression confirm the findings of Williams et al., that a simple step function is inadequate to describe acquired epileptogenesis (i.e. development of SRS) and that the latent period between SE and the first spontaneous seizure may be the first of many long interseizure intervals and a poor measure of the time frame of epileptogenesis (Williams et al. 2009). Our 30 week long EEG monitoring period showed that the transition from the latent period to the final plateau phase is best modeled as a sigmoid Boltzmann function with a clear phase characterized by exponential increase in seizure rate. Gorter et al. (Gorter et al. 2001) emphasized the presence of rats with a progressive increase in seizure rate after an electrically induced SE and rats in which this progressive increase was not observed during a 12 week long monitoring period. In this study, all rats displayed a phase of exponential increase in seizure rate during the 30 weeks of EEG monitoring. However, if monitoring would have been ended after 12 weeks as in the study by Gorter et al., 6 out of 9 rats could have been defined as a non-progressive rat. This emphasizes the need for extended long term EEG monitoring in epilepsy model characterization studies. Eight out of 9 rats reached a plateau in seizure rate after 122 ± 9 days. Although seizure rate progression is similar among different rats, a substantial variation in seizure rate at the end stage of seizure rate progression is present in this model with a minimum of 14.5 seizure per day and a maximum of 48.6 seizures per day. To evaluate experimental treatments at the end stage of seizure progression in KA treated rats with stable seizure rates, this variation in seizure rate among rats could obscure any positive or negative effects of the intervention. Therefore rats should thus serve as their own controls to evaluate experimental treatments in KA treated rats with stable seizure rates. Seizure duration remained very consistent around 56 seconds throughout the entire 30 week long EEG monitoring period, which corresponds well with observations in the pilocarpine model and the post stimulation SE models, where seizure duration varied between 30 s and 60 s (Brandt et al. 2003; Cavalheiro et al. 1991; Glien et al. 2001; Nissinen et al. 2000). In TLE patients, secondary generalized seizures usually have a duration of about 60 seconds(Theodore et al. 1994), which shows the similarity between patients and animal models

concerning seizure duration. The seizure rate in our study followed a clear circadian rhythm with 0.63 ± 0.12 seizures per hour during the time lights were switched on and 0.42 ± 0.09 seizures per hour when the lights were switched off in the rat room. This circadian rhythm has previously been described in the KA post SE model (Wyckhuys et al. 2010), in the amygdala stimulation model (Nissinen et al. 2000) and in the intrahippocampal KA model (Raedt et al. 2009). It has been shown that seizures occur more often in the KA model during periods of inactivity (Hellier and Dudek 1999). In TLE patients, seizures occur more often between 13h30 and 16h30 (Quigg et al. 1998). Rats are more active during night time – which can be confirmed by video-monitoring of the rats – whereas humans are usually more active during the day. This discrepancy between the human situation and the rat models indicate that light on/off and the associated hormonal shifts may be responsible for the circadian rhythm in seizure rate rather than activity level. However, this discrepancy might be a reflection of essential differences between humans and rats.

We observed a positive correlations between the number of spikes during SE and the SE severity score. Apart from the positive correlation between the time to reach the half maximum seizure rate and the time to reach the plateau phase, an interesting positive correlation between the slope factor and the maximum seizure progression rate was found. This shows that rats with a fast increase in seizure rate during the exponential growth phase will have higher seizure rates when reaching the plateau phase, compared to rats with a slower exponential increase in seizure rate. Histopathologic analysis revealed the presence of mossy fiber sprouting and extensive astrogliosis in all rats at 30 weeks after SE. Mossy fiber sprouting was present in all cases, which is also typical for patients with chronic symptomatic TLE (Babb et al. 1991; Houser et al. 1990). Mossy fiber sprouting was more extensive in the ventral part of the hippocampus than in the dorsal part of the hippocampus. This is in accordance with previous research in the pilocarpine model where It was postulated that the dorsal hippocampus seems to be more resistant to seizure and KA-induced injury than the ventral hippocampus (Williams et al. 2009). In the amygdala stimulation SE model there was a gradient in sprouting: the density of Timm granules was heavier in the ventral portion of the dentate gyrus than in the dorsal part (Nissinen et al. 2000). This indicates that higher vulnerability of the ventral hippocampus compared to the dorsal hippocampus seems quite similar among the different experimental models. Besides MFS, extensive astrogliosis was observed in all rats, corresponding with observations in the intrahippocampal KA model, the systemic pilocarpine model and the electrical stimulation models of TLE (Babb et al. 1995; Bertram and Cornett 1994; Nissinen et al. 2000; Raedt et al. 2009) as well as in TLE patients (King et al. 1995).

Despite the similarities between the characteristics of the systemic KA post SE model and the situation in human TLE patients, it should be noted that a very important difference can be found in the generalization of seizure activity. In our study, all visually observed electrographic seizure onsets

recorded with the hippocampal depth EEG electrodes originated from both hippocampi simultaneously. Due to the limited electrographic coverage of our EEG (local field potential – LFP) recordings (only hippocampal recordings) and the rapid spread of seizure activity in this rat model differentiation between left or right-sided onset cannot be performed. In theory, seizures may have initiated either in the left hemisphere, in the right hemisphere or bilaterally. When translating an epileptic situation from the rat brain to the human situation, the anatomic differences between rats and humans should be kept in mind. In rats there are strong connections between both hippocampi along the full length of their axis (Laurberg 1979; Swanson 1977), whereas these pathways are vestigial in primates and humans. Because of these strong interactions between both hippocampi, seizures may spread more easily over both hemispheres in rats compared to the situation in humans. However, the hippocampal involvement of seizure onset has been shown previously with depth EEG recording electrodes implanted bilaterally in the dorsal and ventral hippocampus, amygdala, enthorhinal cortex, striatum and cortical surfaces electrodes placed over the parietal and frontal cortex (Lothman and Collins 1981). Lothman and Collins suggested that KA induced seizures are preferentially initiated in the hippocampus and rapidly spread to other limbic structures and cortical regions (Lothman and Collins 1981). Our recordings are in accordance with this observation that seizures rapidly spread over the brain in rodents, whereas secondary generalization of seizures in TLE patients is rare.

CONCLUSION

The systemic KA post SE model replicates key features of human TLE and may prove to be a highly useful tool for mechanistic studies of the development of seizures in this disease. Long-term continuous EEG monitoring of the systemic KA post SE rat model for TLE allows to model seizure rate progression without interference of treatment and may be useful for future antiepileptogenesis or disease modifying studies. The repetitive low dose KA induced post SE model is a highly efficient and useful animal model to model all stages of human TLE, but as for all animal models differences between the human and animal situation should be kept in mind when translating data obtained in animal models to the human situation.

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Chapter 5

Optimalization of hippocampal deep brain stimulation parameters for the treatment of drug resistant epilepsy

In search of optimal DBS paradigms to treat epilepsy: bilateral versus unilateral hippocampal stimulation in a rat model for temporal lobe epilepsy

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Brain Stimulation 2014

ABSTRACT

Background: In about 20% of patients with temporal lobe epilepsy (TLE), both hippocampi are seizure onset zones. These patients are unsuitable candidates for resective or disconnective epilepsy surgery but may be amenable to hippocampal deep brain stimulation (DBS). The optimal DBS parameters for these patients are unknown. Recent observations also suggest that even in patients with a unilateral affected hippocampus switching from unilateral hippocampal DBS to bilateral hippocampal DBS could improve seizure control.

Objective: To compare the effect of unilateral with bilateral hippocampal DBS on seizures in a rat model for TLE.

Methods: In the post status epilepticus (SE) intraperitoneal kainic acid rat model for TLE continuous EEG monitoring was performed for 50 days during which rats were subjected to 10 days of unilateral and 10 days of bilateral Poisson distributed high frequency hippocampal DBS in a cross-over trial where unilateral and bilateral DBS were separated by stimulation free periods of 10 days. When bilateral DBS was delivered, each hippocampus was stimulated with a separate stimulator and its own generated Poisson distribution with a mean and variance of 1/130s.

Results: Electrographic seizure rate was 23% lower during bilateral hippocampal DBS compared to electrographic seizure rate during unilateral hippocampal DBS ($p<0.05$). No effect of unilateral nor bilateral hippocampal DBS was observed on electrographic seizure duration. When bilateral hippocampal DBS was applied, lower stimulation intensities were required to evoke afterdischarges ($p<0.05$), reflecting a higher potency of bilateral hippocampal DBS compared to unilateral hippocampal DBS to affect hippocampal networks.

Conclusion(s): Superior outcome in seizure control with bilateral hippocampal DBS compared to unilateral hippocampal DBS indicates that targeting larger regions of the hippocampal formation with more than one stimulation electrode may be more successful in suppressing seizures in TLE.

KEYWORDS

Deep brain stimulation, epilepsy, hippocampus, temporal lobe epilepsy

INTRODUCTION

Electrical stimulation of deep brain structures (Deep Brain Stimulation, DBS) is a promising treatment option for refractory epilepsy patients (Fisher and Velasco 2014; Sprengers et al. 2014). Several brain structures involved in the epileptic network have been targeted and the hippocampus appears to be an effective target for the treatment of temporal lobe epilepsy (TLE) (Akman et al. 2011; Cuellar-Herrera et al. 2006; Urino et al. 2010; Wyckhuys et al. 2007; Wyckhuys et al. 2010). The hippocampus is often involved in seizure onset and is a brain region with a low seizure threshold (McIntyre and Gilby 2008; Parrent and Almeida 2006; Theodore and Fisher 2004). Some patients with TLE have bilateral seizure onset zones. These patients are unsuitable candidates for resective or disconnective epilepsy surgery but may be amenable to hippocampal deep brain stimulation (DBS). Clinical outcome of DBS in the hippocampal formation using various stimulation parameters has been reported both in patient trials (Boex et al. 2011; Boon et al. 2007; Cukiert et al. 2014; McLachlan et al. 2010; Osorio et al. 2005; Tellez-Zenteno et al. 2006; Velasco et al. 2000a; Velasco et al. 2000b; Velasco et al. 2007; Vonck et al. 2002) and in pre-clinical experiments (Bragin et al. 2002; Carrington et al. 2007; D'Arcangelo et al. 2005; Goodman et al. 2005; Kile et al. 2010; Lopez-Meraz et al. 2004; Rashid et al. 2012; Sun et al. 2010; Velisek et al. 2002; Weiss et al. 1995; Zhang et al. 2009) with variable results. Short-term randomized trials report moderate effects of 15% seizure rate reduction (Tellez-Zenteno et al. 2006), while long-term trials demonstrate further improvement with time with efficacy ranging from 50% seizure reduction up to seizure freedom (Sprengers et al. 2014; Velasco et al. 2007). Further improvement in seizure control by means of hippocampal DBS is dependent on the search and the identification of optimal stimulation parameters and stimulation strategies to be applied in this region. Pre-clinical trials investigating the effect of hippocampal DBS on spontaneous seizures showed that short trains of either low (1Hz), medium (50Hz), or high (200Hz) frequency stimulation had no effect on seizure rate (Bragin et al. 2002), whereas long-term (10 days) continuous hippocampal high frequency (130Hz) stimulation in a rat model for TLE resulted in a significant reduction in seizure rate in 54% of the rats (Wyckhuys et al. 2010). A recent study showed that stimulation at 2 separate locations in the perforant path using different stimulation frequencies is more potent in terminating seizures compared to stimulation with identical stimulation frequencies (Cymerblit-Sabba et al. 2013). The aim of this study was to investigate whether there is a difference in seizure control induced by bilateral high frequency DBS compared to unilateral DBS in a randomized cross-over trial in the KA rat model for TLE.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, the Netherlands) weighing 150–200 g, were treated according to guidelines approved by the European Ethics Committee (decree 86 / 609 / EEC). The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University Hospital (ECP 09 / 16). All animals were kept under environmentally controlled conditions (12 h normal light / dark cycles, 20–23°C and 50% relative humidity) with food and water intake *ad libitum*.

Status epilepticus

Eighty rats (177 +/- 8.96 g) received kainic acid (KA) (5 mg/kg; Tocris Bioscience, USA) by intraperitoneal injections according to the protocol of Hellier et al. to induce status epilepticus (SE) (Hellier et al. 1998). Seizure activity of all rats was continuously monitored visually. The KA treatment was repeated hourly until the animals displayed a stable self-sustained SE for ≥3 hours (i.e., > 10 seizures per hour). Animals that exhibited excessive motor or excessive lethargic behavior were further given reduced dosages (2.5mg/kg) of KA to avoid exorbitant toxicity and mortality (Williams et al. 2009). Eleven rats died during or after SE. Between 107 & 120 days after the KA injection, the 69 surviving rats were observed during 3 consecutive days for 8h/day (9am-18pm) and spontaneous behavioral seizures were counted. Rats with 2 or more convulsive seizures (ie. Stage 3,4 or 5 seizure according to Racine's scale) during one of the three 8 hour observation periods were selected for further use in this study (n=34).

Surgery

Out of these 34 rats fulfilling the selection criterion, 28 rats were used for surgery. Rats were anesthetized with a mixture of isoflurane (5% for induction, 2% during implantation) and medical oxygen. After exposure of the skull, 10 small burr holes were drilled; six for the positioning of anchor screws (1.57 mm diameter; Bilaney), one for the epidural electrode, one for the reference electrode, and two for recording/DBS depth electrodes. A custom-made epidural electrode was screwed in the right side of the skull at the height of the frontal cortex. A similarly constructed reference electrode was placed on the right side posterior to the sutura lambdoidea. The quadripolar recording/DBS electrodes were custom-made by twisting four polyimide coated stainless steel wires. The 2 outer electrode contacts of the quadripolar electrode have a diameter of 125 µm (Bilaney, Germany) with a

distance of 3 mm between the exposed tips and were used for stimulation. The two inner electrode contacts have a diameter of 70 µm (CFW, CA, U.S.A.) with a distance of 500 µm between the exposed tips and were used for recording. Each of the wires is straight-cut, which results in a quadripolar electrode: the innermost two contact points were used for EEG recording, the outermost two contact points were used to deliver DBS pulses. The quadripiolar recording/DBS depth electrodes were implanted in the left and right hippocampus respectively ((AP -5.6, DV -7.4, ML +/- 5.1 relative to Bregma) (Paxinos C 1998) (Fig 1). The electrode leads were coupled to a connector that is fixed to the skull with screws and acrylic dental cement. After surgery, rats were allowed 2 weeks of recovery before initiating the experiment. Four rats died during post-surgical recovery.

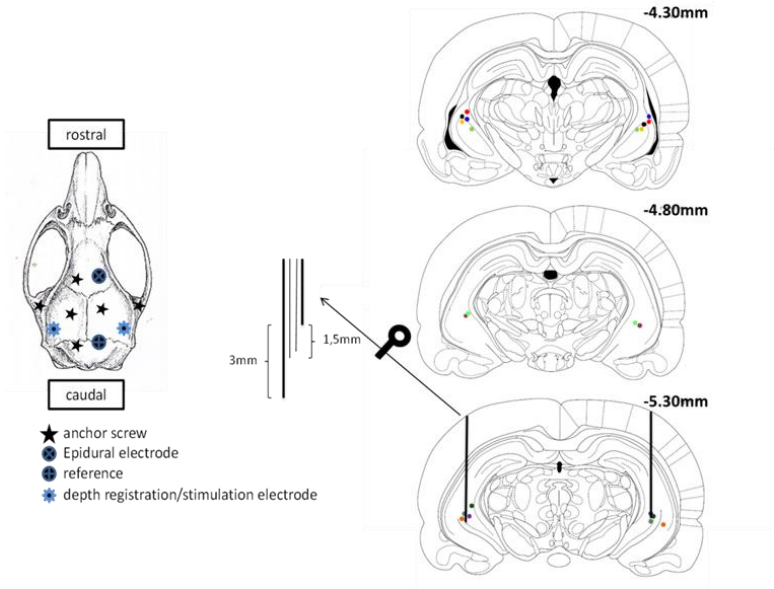

Figure 1: *schematic overview of the configuration of electrode implantation: a quadripolar recording/DBS depth electrode in left & right hippocampus ((AP -5.6, DV -7.4, ML -5.1 and 5.1 relative to Bregma [39]), 1 epidural electrode above the right frontal hemisphere, 1 reference electrode posteriorly of the sutura lambdoidea above the right hemisphere, and 6 anchor screws. Colored dots represent the position of the deepest electrode tip of the stimulation electrodes of all rats.*

EEG recording and stimulation set up

EEG signals were recorded continuously for 24 hours per day during the entire experiment via a head stage, carrying unity gain preamplifiers, and a commutator connected to custom-built amplifiers (gain: 510 x; band-width: 0.13Hz – 5.8kHz). A data acquisition card (NI-USB-6259, National Instruments, Belgium) digitized the EEG signals which were then stored onto a hard drive. The intracranial signals are composed of synchronized postsynaptic potentials (local field potentials), recorded directly from neuronal tissue surrounding the registration electrodes. The EEG sampling rate was set at 2 kHz which is sufficient to detect the stimulus artifacts, and remove them by interpolation. A trained investigator (BVN) visually quantified electrographic seizures on these continuous EEG recordings. Electrographic seizures were defined as episodes characterized on the EEG by ongoing rhythmic spiking activity with a high amplitude (>3 x baseline) and frequency >5Hz during at least 10 seconds. These electrographic seizures were often initiated by a large positive or negative potential followed by a decrease in amplitude, which progressed into the described rhythmic, high frequency, large amplitude EEG spiking (White et al. 2006; Williams et al. 2009; Wyckhuys et al. 2010) (Fig. 2).

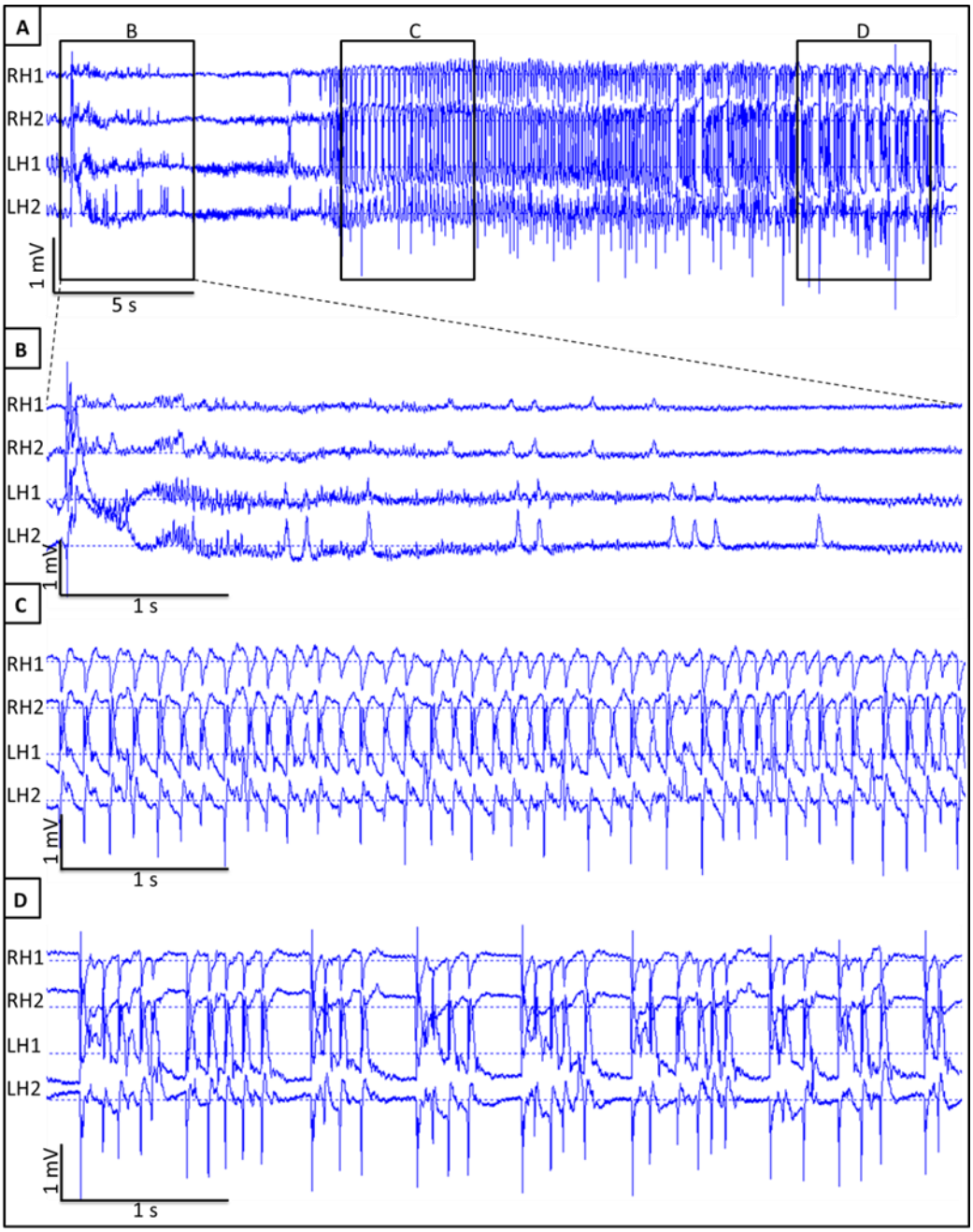

Figure 2: *A typical electrographic electrographic seizure following a latent period after KA-induced SE; RH-right hippocampus, LH-left hippocampus* ***A:*** *electrographic seizure that lasts for 34s.* ***B:*** *Seizure initiation characterized by a large positive or negative potential followed by a decrease in amplitude.* ***C:*** *Progression of the spikes into rhythmic, high frequency, large-amplitude EEG spiking.* ***D:*** *Lingering epileptic activity near the end of the seizure. Notice that seizure activity starts simultaneously in the left and right hippocampus.*

Stimulation pulses were delivered by connecting each bipolar stimulation electrode to a computer controlled custom-built constant current stimulator. The stimulation paradigm consisted of a continuous train of bipolar, biphasic, charge-balanced square-wave pulses with a pulse width of 100 µs. The interpulse intervals were drawn from a Poisson distribution with a mean and variance of 1/130 s (130Hz-PD). In the unilateral stimulation protocol, the stimulation pulses were delivered through the stimulation electrode implanted in the right hippocampus. In the bilateral stimulation protocol, the stimulation pulses were delivered through both stimulation electrodes. When bilateral DBS was delivered, each hippocampus was stimulated with a separate stimulator and its own generated Poisson distribution. Stimulation intensity was set at 60% of the afterdischarge threshold (ADT), i.e. the minimum stimulus intensity giving rise to seizure activity on the EEG. The ADT was measured using 10s pulse trains with parameters identical to those applied for the continuous therapeutic stimulation – i.e. 130Hz-PD frequency, 100µs pulse width. There was a 1 minute interval in between pulse trains during which the EEG was evaluated for epileptic discharges (ADT). For each pulse train, stimulation intensity was increased starting from 25 µA in steps of 25 µA until the first sign of an AD was detected on the EEG or 500 µA was reached. This ADT was determined in each rat for each stimulation paradigm (unilateral right hippocampal DBS, and bilateral hippocampal DBS) on 3 consecutive days before baseline video-EEG monitoring was started. One rat was left out for further analysis because no typical AD activity could be evoked. After the last ADT determination rats were allowed one day of rest before baseline EEG monitoring was started. During the stimulation periods, electrode impedance was measured daily (IMP-2 (1kHz sine wave testing stimuli), Bak electronics, Sanford Florida, USA). Five rats were left out of the analysis because of unintended loss of the head cap during the experiment.

Experimental protocol

To determine baseline electrographic seizure rate, rats underwent video-EEG monitoring during 10 days. Rats that were not in a stable plateau phase with regards to daily electrographic seizure rate (Williams et al. 2009) during the baseline period were left out for further analysis (n=4). This baseline period was followed by two stimulation periods of 10 days each. During these stimulation periods, stimulation was delivered continuously (24 h/24 h) at a fixed intensity (60% of ADT). Half of the rats were treated with unilateral hippocampal DBS during the first stimulation period and with bilateral hippocampal DBS during the second stimulation period. In the other rats this treatment order was reversed. This treatment order was chosen at random, and the investigators were blinded during analysis for the assigned treatment. Between each stimulation period and after the last stimulation

period, there were 10 day long periods during which no stimulation was given (baseline periods) in order to check for possible outlasting effects and to rule out carry-over effects (Fig. 3).

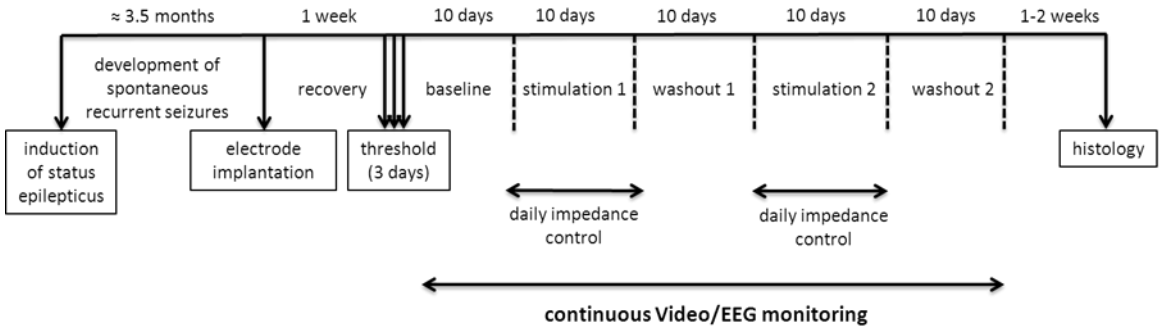

Figure 3: *Schematic timeline of the experimental design. After induction of SE by repeated ip. KA injections, selected rats with the highest seizure rates were implanted with recording/DBS depth electrodes in the right and left hippocampus. Before the start of the 50 days continuous EEG monitoring period, the seizure thresholds for the three stimulation paradigms were determined on 3 consecutive days. Half of the rats received unilateral hippocampal DBS during stimulation period 1 and bilateral hippocampal DBS during stimulation period 2, and vice versa for the other half of the rats. At the end of the experiment the rats were euthanized to verify correct positioning of the electrodes in the hippocampus.*

Histology

At the end of the experiment, all rats were deeply anesthetized with pentobarbital (100mg/kg, i.p.) and transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were removed and post-fixed in a 4% paraformaldehyde solution for at least 24 hours. Then, brains were transferred to a 10%, 20%, and 30% sucrose solution (at least one day in each solution), frozen in ice-cold isopentane and stored at -80°C until sectioning. Coronal sections (70µm) were cut at the level of the hippocampus, and mounted onto glass slides. Nissl staining was performed to allow for electrode location determination using light microscopy. Rats with aberrant electrode position either in the left or right hippocampus (n=1) or signs of intracranial hemorrhage or lesion (n=2) were not included for the further analysis.

Statistics

All data is tested for normality before statistical analysis was performed. Non-normally distributed continuous variables are expressed as median and interquartile range (IQR). Normally distributed continuous variables are expressed as mean and standard error of the mean (SEM). No significant difference between baseline and washout periods could be observed, indicating no outlasting effects of either unilateral or bilateral hippocampal DBS. This allowed us to combine both treatment arms when comparing either unilateral hippocampal DBS or bilateral hippocampal DBS to its respective baseline EEG monitoring period. A repeated measures ANOVA with post hoc Bonferroni correction was used to compare outcome parameters during DBS-free and DBS periods. The individual response to treatment was tested with a Mann-Whitney-U test comparing electrographic seizure rate for the individual rat during the 10 days stimulation free period with the 10 stimulation period. Electrographic seizure rate during unilateral hippocampal DBS was compared with electrographic seizure rate during bilateral hippocampal DBS using a paired t-test. For the comparison of the reduction in electrographic seizure rate during unilateral versus bilateral hippocampal DBS and to compare ADTs and stimulation intensities during unilateral and bilateral hippocampal DBS a non-parametric Wilcoxon signed rank test was used. $P < 0.05$ are assumed to reflect significant differences.

RESULTS

Threshold, impedance & stimulation intensity

Impedance of the stimulation electrodes remained stable at 91 ± 5 kΩ throughout all stimulation periods. Afterdischarge (AD) evoking stimulation intensity thresholds were determined daily, during three consecutive days prior to baseline EEG monitoring. The median threshold to evoke an AD with bilateral hippocampal stimulation (median:75 µA IQR:56 – 231µA) was lower compared to the median threshold to evoke an AD with unilateral hippocampal stimulation (median:100µA IQR:75 – 250µA) (p=0.03). As our protocol was to therapeutically deliver DBS at 60% of AD evoking threshold, stimulation intensity was lower during bilateral DBS (median:45µA IQR:34 – 134µA than during unilateral DBS (median:60µA IQR:45 – 150µA) (p=0.03). The stimulation intensities based on the initial AD evoking thresholds were used during the entire experiment.

The effect of unilateral hippocampal DBS on electrographic seizure rate

Unilateral hippocampal DBS (n=11) did not significantly affect mean daily electrographic seizure rate. The mean electrographic seizure rate during baseline was 36.8 ± 5.6 electrographic seizures per day and was 29.1 ± 5.6 electrographic seizures per day during unilateral hippocampal DBS (p=0.2). During the 10 day long stimulation-free period following unilateral hippocampal DBS, mean daily electrographic seizure rate was 37.6 ± 6.5 electrographic seizures per day (fig.4A). Four out of 11 rats (36%) experienced a significant reduction in seizure rate during unilateral hippocampal DBS compared to the baseline EEG monitoring period (Mann-Whitney-U-tests) . In this group of rats (n=4) electrographic seizure rate showed a nearly significant decrease of 60% from 36.8 ± 10.6 electrographic seizures per day during baseline to 14.5 ± 5.4 electrographic seizures per day during unilateral hippocampal DBS (p=0.08, Repeated Measures ANOVA). After stopping DBS electrographic seizure rate returned to 36.2 ± 12.7 electrographic seizures per day in these rats (fig.4B).

The effect of bilateral hippocampal DBS on electrographic seizure rate

Bilateral hippocampal DBS (n=11) significantly reduced mean daily electrographic seizure rate from 35.5 ± 6.4 electrographic seizures per day during baseline to 22.5 ± 6.0 electrographic seizures per day during bilateral hippocampal DBS (p=0.01, Repeated Measures ANOVA). Electrographic seizure rate returned to baseline values (32.1 ± 6.2 Sz/day) when stimulation was stopped (fig.4C). Seven out of 11 rats (64%) experienced a significant reduction in seizure rate during bilateral hippocampal DBS

compared to the baseline EEG monitoring period (Mann-Whitney-U-test). Two of these rats with a significant reduction in seizure rate became seizure-free during the 10 day period of bilateral hippocampal DBS from the first day of bilateral hippocampal DBS on. Mean daily electrographic seizure rate of these rats with a significant reduction in seizure rate (n=7) dropped by 60% from 34.7 ± 10 electrographic seizures per day during baseline to 14.1 ± 7.3 electrographic seizures per day during bilateral hippocampal DBS (p=0.005, Repeated Measures ANOVA). Immediately after stopping stimulation, mean daily electrographic seizure rate returned to 37.5 ± 9.5 electrographic seizures per day (fig.4D).

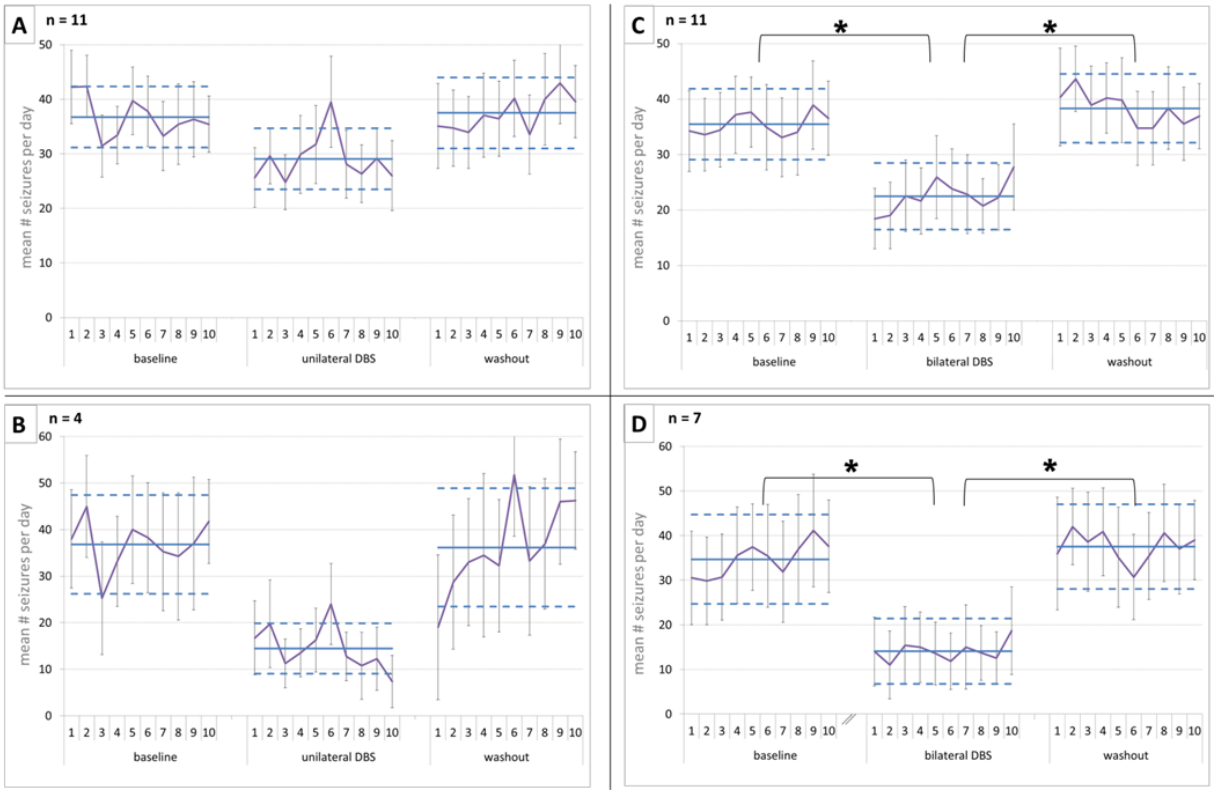

Figure 4: *Graph showing the effect on mean daily electrographic seizure rate of;* ***A)*** *unilateral DBS in all rats (n=11),* ***B)*** *rats with a significant reduction in seizure rate during unilateral DBS (n=4),* ***C)*** *bilateral DBS in all rats (n=11),* ***D)*** *rats with a significant reduction in seizure rate during bilateral DBS (n=7). X-axis represents consecutive days in the corresponding monitoring period (baseline, unilateral or bilateral DBS, washout). Purple bars represent the mean and SEM of the number of electrographic seizures for each day of all rats. Horizontal blue lines indicate the mean (full lines) and SEM (dotted lines) of the daily electrographic seizure rate of all rats for the 10 day long period. Repeated measures ANOVA with post hoc Bonferroni correction reveals significant differences (*) between the condition without DBS and bilateral DBS at group level (n=11) (p=0.003) and in the bilateral DBS responder group (n=7) (p=0.001).*

Unilateral hippocampal DBS versus bilateral hippocampal DBS

All changes in electrographic seizure rate caused by switching stimulation on or off are instantaneous given the time resolution of one day both during unilateral and bilateral hippocampal DBS. Once stimulation is switched on, electrographic seizure rate is lower from the first day of stimulation on. Once stimulation is stopped, electrographic seizure rate returns to baseline values already at the first day of the post stimulation EEG monitoring period. The mean decrease in electrographic seizure rate compared to baseline during bilateral hippocampal DBS (45 ± 12%) was significantly (p=0.03, Wilcoxon signed rank test) higher compared to during unilateral hippocampal DBS (14% ± 13%), while electrographic seizure rates during both baseline periods preceding unilateral and bilateral DBS were similar (table 1). The electrographic seizure rate during bilateral hippocampal DBS (22.5 ± 6.0 electrographic seizures per day) was significantly lower compared to electrographic seizure rate during unilateral hippocampal DBS (29.1 ± 5.6 electrographic seizures per day) (p=0.03). During bilateral hippocampal DBS was 7/11 (64%) rats experienced a significant reduction in seizure rate, and during unilateral hippocampal DBS 4/11 (36%) rats experienced a significant reduction in seizure rate (table 1). In these rats, the mean electrographic seizure rate reduction was 60% during unilateral (n=4) and during bilateral (n=7) DBS (fig 4B&C). Bilateral hippocampal DBS led to seizure freedom from day 1 during a 10 day long stimulation period in 2/11 (18%) rats. Seizure freedom was not achieved in rats undergoing unilateral hippocampal DBS.

	unilateral DBS		bilateral DBS	
	mean daily seizure rate during baseline	% change in mean daily seizure rate during DBS	mean daily seizure rate during baseline	% change in mean daily seizure rate during DBS
rat 1	60 (± 3)	**-78%***	34 (± 7)	**-100%****
rat 2	25 (± 4)	**-63%***	14 (± 2)	**-100%****
rat 3	13 (± 4)	**-54%***	16 (± 5)	**-68%****
rat 4	4 (± 2)	**+ 75%**	3 (± 1)	**-66%****
rat 5	48 (± 2)	**-38%***	72 (± 2)	**-65%****
rat 6	35 (± 1)	**+ 14%**	37 (± 2)	**-57%****
rat 7	59 (± 4)	**+ 3%**	67 (± 3)	**-22%****
rat 8	26 (± 1)	**-15%**	22 (± 2)	**-5%**
rat 9	41 (± 3)	**+ 11%**	36 (± 1)	**+ 2%**
rat 10	55 (± 2)	**-15%**	44 (± 3)	**+ 2%**
rat 11	37 (± 6)	**+ 5%**	44 (± 3)	**+ 5%**
	n.s.		§	

Table 1: *Response to unilateral and bilateral hippocampal DBS in all rats: Mann-Whitney U test revealed significant decrease in seizure rat in 4/11 rats during unilateral DBS* and significant decrease in seizure rate during bilateral DBS** (P<0.05). Wilcoxon signed rank test revealed significant greater decrease in seizure rate during bilateral hippocampal DBS compared to baseline versus unilateral hippocampal DBS compared to baseline (§) (p=0.03), while seizure frequencies during baseline periods were similar.*

DISCUSSION

In this study, the seizure suppressing effect of unilateral versus bilateral hippocampal DBS was compared in the post SE KA rat model for TLE. The results of this study show that the reduction in electrographic seizure rate is higher during bilateral hippocampal DBS compared to unilateral hippocampal DBS. 7/11 (64%) rats experienced a significant reduction in seizure rate during bilateral hippocampal DBS, whereas only 4/11 (36%) rats experienced a significant reduction in seizure rate during unilateral hippocampal DBS. During bilateral hippocampal DBS seizure freedom was achieved in 2/11 (18%) of the treated rats.

The latent period, the chronic nature of the epilepsy, and the behavior associated with the seizures in the KA model are comparable to the condition in humans with TLE (F.E. Dudek 2006; Mathern et al. 1996; Spencer and Spencer 1994; Williams et al. 2009). The acquired chronic epileptic state in this rat model for TLE makes it a good model to study the effects of anti-epileptic treatments on the occurrence rate of spontaneous epileptic seizures. Several pre-clinical studies investigating the effect of high frequency hippocampal DBS are performed in animal models where seizures are evoked either after kindling stimuli (Cuellar-Herrera et al. 2006; Wyckhuys et al. 2007), or after injection of chemo-convulsants (Akman et al. 2011; Cymerblit-Sabba et al. 2013; Urino et al. 2010). Only a few preclinical studies have investigated the effect of hippocampal DBS on the occurrence of spontaneous seizures in the chronic epileptic state (Kile et al. 2010; Rashid et al. 2012; Wyckhuys et al. 2010).

Stimulation pulses were delivered with Poisson-distributed interstimulus intervals in each hippocampus as we have previously demonstrated superior efficacy with this DBS paradigm in animal experiments (Wyckhuys et al. 2010). Left- and right-sided hippocampal DBS pulses were delivered with a separate stimulator and its own generated poisson distribution with a mean and variance of 1/130s to target the left and right hippocampus with independent stimulation patterns. During bilateral hippocampal DBS the mean decrease in electrographic seizure rate compared to baseline was 45%, whereas this reduction was only 14% during unilateral hippocampal DBS. Previous studies on the effect of DBS in one region of the hippocampal formation on spontaneous seizures reported a reduction in mean seizure rate ranging from no reduction in seizure rate (Bragin et al. 2002) to 90% reduction in seizure rate (Rashid et al. 2012). A previous trial in the same animal model with identical stimulation parameters to our unilateral hippocampal DBS paradigm, except for the stimulation intensity, reported a mean reduction in seizure rate of 26%. Due to technical limitation in the programming of the stimulators, stimulation intensity in this previous trial was more than twice as high compared to stimulation intensity in the current experiment (Wyckhuys et al. 2010). The current

study showed that bilateral hippocampal DBS induced ADs at significantly lower stimulation intensities than unilateral hippocampal DBS, reflecting the higher potency of bilateral hippocampal DBS compared to unilateral hippocampal DBS to affect hippocampal networks. Consequently, the greater reduction in electrographic seizure rate during bilateral hippocampal DBS compared to unilateral hippocampal DBS was obtained with significantly lower stimulation intensities per hippocampus. Although lower stimulation intensities are applied at each individual stimulation tip during bilateral hippocampal DBS, the total current delivered to the network of both hippocampi was higher during the bilateral hippocampal DBS period compared to the unilateral hippocampal DBS period.

All visually observed electrographic seizure onsets recorded with the intrahippocampal depth EEG originated from both hippocampi simultaneously. Due to the limited electrographic coverage of our EEG (LFP) recordings (only hippocampal recordings) and the rapid spread of seizure activity, in this rat model differentiation between left or right-sided onset cannot be performed. In theory, seizures may have initiated either in the left hemisphere or in the right hemisphere or bilaterally. Metabolic mapping of systemic KA induced damage and EEG recordings with higher spatial coverage, with depth EEG recording electrodes implanted bilaterally in the dorsal and ventral hippocampus, amygdala, enthorhinal cortex, striatum and cortical surfaces electrodes placed over the parietal and frontal cortex, showed that systemic KA leads to widespread damage and bilateral seizure onset zones (Lothman and Collins 1981). Lothman and Collins suggested that KA induced seizures are preferentially initiated in the hippocampus and rapidly spread to other limbic structures and cortical regions, because low-dose KA injections led to high frequency peaked oscillations and spiking restricted to the hippocampus whereas higher doses (>4mg/kg) led to electrographic seizures in other limbic structures and cortical regions (Lothman and Collins 1981). This is in accordance with observations in the systemic pilocarpine model demonstrating that the earliest seizure activity was recorded most often in the subiculum and other parts of the hippocampal formation, while later on the seizure activity rapidly spreads over the brain (Toyoda et al. 2013). The reason for the fast and extensive seizure spread in rats is unclear. In rodents, seizure activity could rapidly spread to the contralateral side through the strong interhippocampal connections between left and right hippocampus seizure (Laurberg 1979; Swanson 1977). Another explanation of the rapid seizure spread in rats could be the higher connectivity in rat brains compared to human brains (Toyoda et al. 2013). The rodent brain is composed of fewer neurons than the human brain while the average number of synapses in a rodent brain and a human brain are approximately similar, which reflects a higher connectivity in rodents brains (DeFelipe et al. 2002). Although targeting DBS to the seizure onset zone may herald superior efficacy, the bilateral hippocampal involvement in the KA TLE rat model hampers the possibility to investigate the potentially superior efficacy of ictal onset DBS. From

the results of this study we cannot draw conclusions on whether unilateral DBS could be more efficacious in rats with an ipsilateral seizure onset.

As electrographic seizure duration is unaffected by either unilateral or bilateral hippocampal DBS, it appears that hippocampal DBS decreases the likelihood for seizures to occur during stimulation, but once a seizure starts it remains unaffected by the stimulation. This observation supports the hypothesis that DBS exerts its anti-epileptic effect by increasing the threshold for occurrence of spontaneous epileptic seizures in epileptic networks(Wyckhuys et al. 2010).

Both in the unilateral DBS group (n=4) as in the bilateral DBS group (n=7) with a significant reduction in seizure rate during stimulation, DBS results in a decrease in electrographic seizure rate compared to baseline of 60%. Consequently, the significant difference in mean decrease in electrographic seizure rate can be attributed to the increase in rates with significant a reduction in seizure rate during bilateral hippocampal DBS.

Responders and non-responders are a typical phenomenon associated with anti-epileptic treatments (Brandt et al. 2004; Nissinen and Pitkanen 2007). In a post SE rat model for TLE responder rates (ie. > 50% seizure rate reduction) of approved anti-epileptic drugs currently used in patients vary from 29% up to 100% (Nissinen and Pitkanen 2007). In our experiment 3/11 rats had a >50% reduction in seizure rate during unilateral hippocampal DBS and 6/11 rats had a >50% reduction in seizure rate. In a previous trial in the post SE KA model, the decreases in seizure rate in rats with a significant reduction in seizure rate was 66%, corresponding with the 60% decrease observed in this experiment. Individual differences in seizure rate reduction during hippocampal DBS might be due to small differences in stimulation electrode location and/or differences in epileptic foci between rats.

The observed seizure suppressive effect of both unilateral and bilateral hippocampal DBS was achieved immediately from the first day of stimulation onwards and no outlasting effect of DBS was observed. This was in accordance with a previous study on high frequency hippocampal DBS in the KA rat model (Wyckhuys et al. 2010). This observation is in contrast with the clear outlasting effects of two weeks low frequency DBS of the ventral hippocampal commissure in the amygdala stimulation post SE rat model(Rashid et al. 2012). Immediate increases in seizure rate after hippocampal DBS discontinuation has been reported in patients (Tellez-Zenteno et al. 2006; Velasco et al. 2007). However various patient trials reported increased efficiency of high frequency DBS with longer treatment duration in anterior nucleus of the thalamus DBS (Fisher et al. 2010), responsive focal neurostimulation (Heck et al. 2014) and hippocampal DBS (Vonck et al. 2013). Pre-clinical investigation on 2 week long low frequency DBS of the ventral hippocampal commissure resulted in clear outlasting effects after DBS was stopped. Despite the observed ON-OFF effect in this study, we cannot exclude that better seizure control and/or stimulation outlasting effects could have been achieved with longer stimulation periods.

In clinical trials, bilateral hippocampal stimulation is usually only proposed to patients with bilateral seizure onset. In an uncontrolled observational study of Velasco et al., four patients with bilateral independent foci received bilateral hippocampal DBS. Three of these patients became seizure-free during stimulation and one had a reduction in seizure rate of 85% (Velasco et al. 2007). Other clinical trials in which patients with bilateral independent foci received bilateral hippocampal DBS could not reproduce this high reduction in seizure rate. A small randomized controlled trial in 2 patients resulted in an average reduction in seizure rate of 33% (McLachlan et al. 2010). In other clinical hippocampal DBS studies, responses to bilateral hippocampal DBS varied from an increase in seizure rate in one patient (Cukiert et al. 2014) to a mild reduction in seizure rate of 30-49% in one patient (Boon et al. 2007). The heterogeneity of patients and stimulation parameters in these studies, as well as the lack of a comparative unilateral hippocampal DBS period in the same patients make it impossible to compare efficacy of unilateral and bilateral hippocampal DBS in patients. Nevertheless, our results are in line with the findings of a long term (8.5 years) follow up study of patients with refractory medial TLE treated with hippocampal DBS (Vonck et al. 2013), where it was observed that patients treated with unilateral amygdala/hippocampal DBS experienced a stronger seizure suppression, when their treatment was switched from unilateral to bilateral hippocampal DBS. In this open prospective cohort study, treatment with bilateral hippocampal DBS was proposed to patients with a unilateral focus in whom unilateral DBS failed to decrease seizures by ≥90% after a 2.5-3 year follow-up (6/9). Five out of six patients consented to this change. In 3/5 patients switching to bilateral DBS further improved their seizure control. One patient became seizure free for more than 3 years and two other patients achieved ≥90% seizure reduction after the change in treatment regimen, whereas during unilateral DBS they experienced no reduction in seizure rate. This indicates that also in unifocal TLE bilateral hippocampal DBS might herald better seizure control by combining ictal onset zone and network structure stimulation.

The anatomic differences between rats and humans should be kept in mind when translating our results to the human situation. In rats, there are strong connections between both hippocampi along the full length of their axis (Laurberg 1979; Swanson 1977). These pathways are vestigial in primates and humans. These strong connections in rodents may represent a pathway through which seizure activity can easily spread between both hemispheres, but these pathways do not necessarily represent connections through which the therapeutic effect of unilateral hippocampal DBS can spread to the contralateral side and effectively stop seizure activity. It has been shown that simultaneous stimulation with different stimulation frequencies is potent in disrupting seizure activity (Cymerblit-Sabba et al. 2013) and seizure control can be correlated with desynchronization of brain dynamics (Good et al. 2009).This suggests that multisite stimulation with independent stimulation patterns in strongly interconnected epileptic

networks is potent in disrupting seizure activity by desynchronizing network activity. Multisite stimulation with different frequencies subdivides neuronal networks into separate desynchronized sub-networks that fire at different times. This subdivision of the epileptic network opposes the development of wide spread hyper-synchronous seizure activity (Cymerblit-Sabba et al. 2013).

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Author contributions: BVN set up the experimental design, performed the surgery, was responsible for the daily management of the experiment and analyzed the data. KV is the promoter of PhD student BVN and contributed together with RR, WW, PB and JD to the design of the experiment and interpretation of the results. All the authors have contributed to and have approved the final manuscript.

Competing interests: The authors have no conflict of interest.

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Chapter 6

Long-term hippocampal deep brain stimulation in a rat model for temporal lobe epilepsy contradicts seizures beget seizures hypothesis

Long-term hippocampal deep brain stimulation in a rat model for temporal lobe epilepsy contradicts seizures beget seizures hypothesis

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ABSTRACT

Whether “seizures beget seizures” has been a point of contention ever since Sir William Gowers coined this aphorism more than 125 years ago. There is increasing evidence that hippocampal deep brain stimulation efficiently suppresses seizures, without affecting other mechanisms in the brain causing therapeutic side-effects as opposed to treatment with anti-epileptic drugs. This makes hippocampal DBS a rather straight-forward tool to investigate the hypothesis postulated by Gowers in 1881. To investigate this, we studied the effect of hippocampal DBS on seizure development in the kainic acid (KA)-induced status epilepticus (SE) model for temporal lobe epilepsy (TLE).

Rats were implanted with a quadripolar DBS/EEG-recording electrode in the right hippocampus and a bipolar EEG-recording electrode in the left hippocampus. 24 hours after kainic acid (KA) induced status epilepticus (SE), one group (n=9) was subjected to 22 week long DBS (Poisson Distributed Stimulation, 130 PPS, 100µs PW, 60% of AD evoking stimulation intensity). A SHAM group (n=9) received sham stimulation (SHAM). EEG was recorded continuously during 30 weeks in both groups. Long term treatment (22w) with hippocampal DBS significantly suppressed the increase in seizure rate after SE in the post-KA model for TLE in 4/9 rats and significantly increased this exponential increase in seizure rate in 3/9 rats. 2/9 rats remained unresponsive to the treatment. After switching off the stimulation seizure rate was similar between all rats. Histological evaluation at the end of the experiment revealed no differences in mossy fiber sprouting between DBS treated and SHAM rats.

These results show that affecting seizure rate during the first 22 weeks after SE did not affect the epileptogenic process in the rat KA model for TLE which questions the controversial theory postulated by Gowers in 1881 that “seizures beget seizures”. Although no anti-epileptogenic effect of hippocampal DBS was observed, our results support the idea that hippocampal DBS affects neuronal excitability during stimulation and can hereby affect symptom development in a model for TLE.

KEYWORDS
Deep brain stimulation, temporal lobe epilepsy, epileptogenesis, seizures

INTRODUCTION

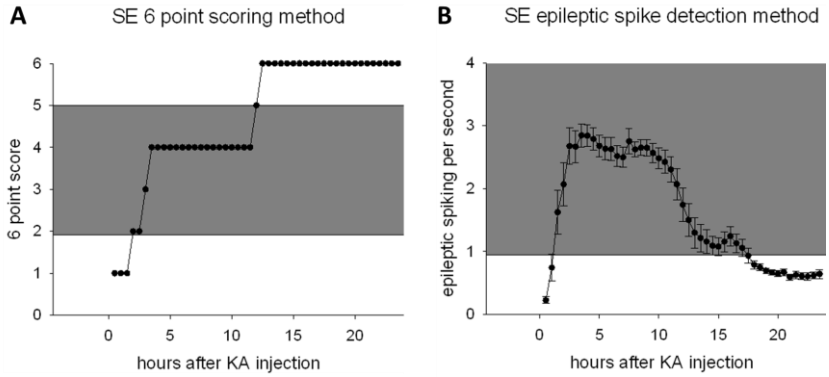
Epilepsy is one of the most common neurological disorders worldwide, affecting over 50 million people (Chang and Lowenstein 2003; Engel 2008). The most common type of epilepsy is temporal lobe epilepsy (TLE) (Panayiotopoulos 2007). TLE is often caused by an initial precipitating event such as febrile seizures, encephalitis, or status epilepticus (SE) that initiates a cascade of molecular and cellular events in the neuronal network, and eventually gives rise to a condition with spontaneous and recurrent seizures, typically originating from temporal lobe regions and of the complex partial type. Whether "seizures beget seizures" has been a point of contention ever since Sir William Gowers coined this aphorism more than 125 years ago (WR 1881). Currently available anti-epileptic drug (AED) treatment for TLE patients, results in an unsatisfactory seizure control in up to 75% of the TLE patients (Spencer 2002) and can give rise to a multitude of side-effects. A promising experimental treatment option for these patients is hippocampal deep brain stimulation (Boex et al. 2011; Boon et al. 2007; Bragin et al. 2002; Cuellar-Herrera et al. 2006; Cukiert et al. 2014; McLachlan et al. 2010; Tellez-Zenteno et al. 2006; Velasco et al. 2007; Vonck et al. 2002; Vonck et al. 2013; Wyckhuys et al. 2007; Wyckhuys et al. 2010b). Open label patient studies and very small randomized controlled trials (RCTs) investigating the efficiency of hippocampal DBS on seizure suppression have shown varying results. These results vary from complete seizure freedom in some patients (Boex et al. 2011; Boon et al. 2007; Cukiert et al. 2014; Velasco et al. 2007) to patients in which hippocampal DBS has no effect on seizure rate (Boex et al. 2011; Boon et al. 2007; Cukiert et al. 2014; Tellez-Zenteno et al. 2006). To resolve this ambiguity larger RCT have been conducted in animal models of TLE. These studies show a significant suppression of seizures during the entire 10 day long stimulation period. The responder rate in these studies varied around 50% (Van Nieuwenhuyse 2014; Wyckhuys et al. 2010b). Despite the varying results in seizure control with hippocampal DBS in these experimental trials, none of these have reported serious side-effects of hippocampal DBS opposed to the frequently observed side-effects seen with the treatment with anti-epileptic drugs. It has been hypothesized that hippocampal DBS can downscale the excitability of neuronal networks without affecting the networks basic functioning, resulting in efficient suppression of epileptic seizures in a rat model for TLE (Wyckhuys et al. 2010b). This makes hippocampal DBS the ideal therapeutic tool to investigate whether suppression of seizures during the epileptogenic process could lead to a state of reduced epileptic activity during and after long-term treatment with hippocampal DBS. TLE begins in many instances in early life with the occurrence of an initial precipitating event. The first epileptic seizure often occurs after a variable latency period following this event and evolves into a condition with recurrent spontaneous seizures. It is unknown in which patients a certain precipitating event

will lead to the occurrence of spontaneous seizures, which impedes us to intervene with potential disease modifying treatments in patients. In this study we investigated the effect of long-term hippocampal deep brain stimulation on seizures during the epileptogenic process initiated by a precipitating kainic acid (KA) induced status epilepticus (SE) in Sprague-dawley rats.

RESULTS

Status epilepticus

KA injections resulted in a self-sustained SE in 18 out of 19 rats. The median latency between the first KA injection and the appearance of the first discrete seizure on the hippocampal EEG was 48 minutes (IQR:20' – 66'). Using the categorical six-point method the SE duration was 10.5 (IQR 10.0 – 11.5) hours, which was similar to the SE duration determined with the spike detection method (median: 11.3 hours; IQR: 10.5 – 16.5 hours). Calculated seizure severity was 84 (IQR: 80 – 93), and during the first 24 hours after SE the total number of epileptic spikes was 131842 (IQR: 124504 – 150968) (fig. 1). These parameters of SE were not different between the rats that were assigned to the DBS group (n=9) or to the SHAM group (n=9) (table 1)



Legend fig 1: *A) median SE progression for all rats according to the six-point method. Status is defined as the time spanning from the first 30' epoch discrete seizures (score 2) appear until the first 30' epoch only relative flat EEG superposed with periodic epileptiform discharges (PEDs) are observed (score 6). B) median SE progression according to the spike detection method (Rats are defined in status from the first 30' epoch the spiking frequency is higher than 1Hz onwards until the last 30' epoch with spiking frequency higher than 1Hz. In both figures the gray zone represents the score/spiking frequency for which the rats are defined to be "in status".*

	DBS (n=9)	SHAM (n=9)
latency 1st seizure in SE	35' (IQR 19' - 75')	57' (IQR 19' - 68')
duration SE (hours) 6 point method	10.5 (IQR 10.4 - 11.6)	10.5 (IQR 10.0 - 11.1)
duration SE (hours) spike detection method	11.5 (IQR 10.9 - 16.6)	10.5 (IQR 9.4 - 15.3)
SE severity	85 (IQR 81.5 - 93.8)	81 (IQR 78.8 - 93.5)
total # spikes	143394 (IQR 128413 - 182605)	129665 (IQR 102626 - 135060)

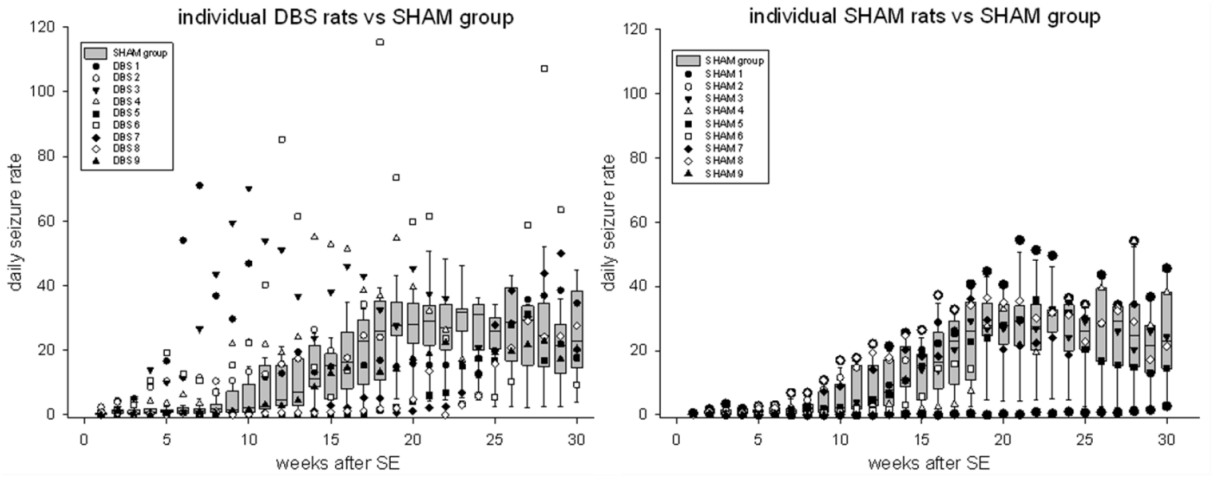
Table 1: *SE parameters are similar between rats in the DBS and SHAM group*

Threshold & electrode impedance

The median threshold to evoke an AD before SE (median:125 µA IQR:75 – 206µA) was lower compared to the median threshold to evoke an AD 4 days after SE (median:275 µA IQR:188 – 306µA) (paired t-test: p=0.027). Impedance of the stimulation electrode in the DBS group remained stable at 89.2 ± 2.4 kΩ throughout the 22 weeks of stimulation. A 22 week long period of hippocampal DBS did not alter the ADT (median:250µA (IQR: 213 – 338µA)) compared to the ADT at day 4 post SE.

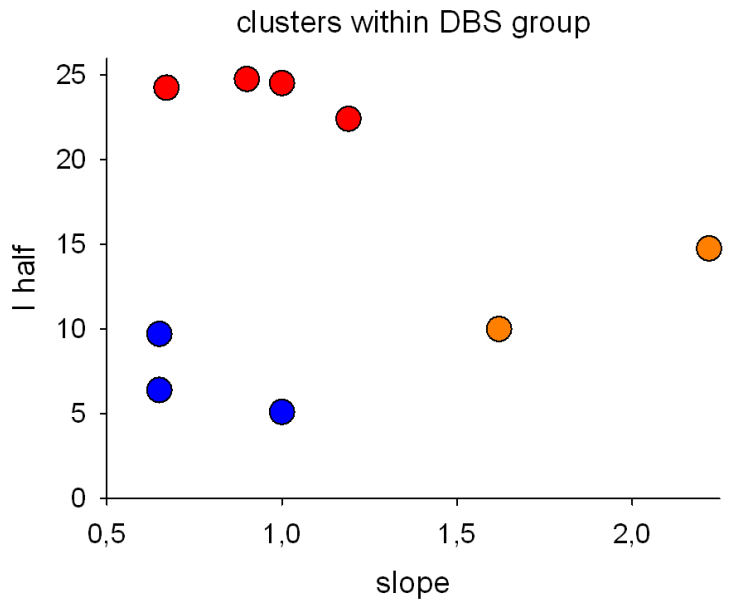
Effect of hippocampal DBS on seizures

EEG was continuously monitored for 30 weeks in 12 out of 18 rats. In 4 rats the EEG monitoring period was limited to 18, 23, 24 and 24 weeks due to the unexpected loss of the head cap ; in 2 rats the EEG monitoring period was limited to 15 and 18 weeks due to unexpected death.
Daily Seizure rate in DBS-treated rats was highly variable (0 – 115 seizures/day) compared to SHAM-treated rats (0 – 54 seizures/day) (fig.2).



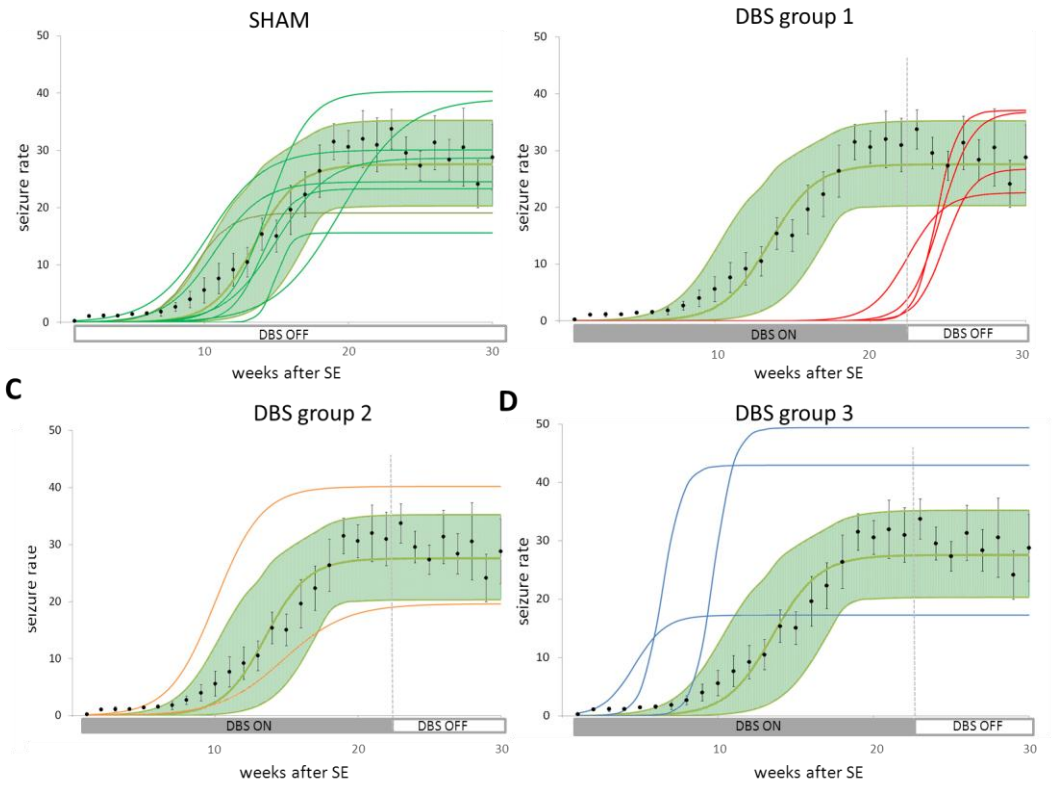
Legend fig. 2: *Seizure rate progression in the DBS group (left panel) vs. the SHAM group (right panel).This figure shows the individual seizure rate progression of all rats compared to the median seizure rate progression in the SHAM group. Boxes represent median and interquartile ranges of the SHAM group data. Whiskers represent 95% CI of the SHAM group data. Individual seizure rate progression is superposed on the boxplots as a scatter plot.*

In order to compare progression seizure rate between rats a Boltzmann sigmoid curve $y(t) = \frac{y_{max}}{(1+ e^{\frac{y(half)-t}{k}})}$ was fitted to the time dependent increase in seizure rate after SE for each rat. Where y(t) is the daily seizure rate calculated for week t after KA injection, y_{max} is the calculated maximum daily seizure rate, y(half) is the time in weeks after KA injections where seizure rate reaches its half maximum value, k is the slope factor of the Boltzmann sigmoid curve. In the SHAM group (fig.4A), 1/9 rats had a non-progressive seizure rate profile, different from the rest of the SHAM group, in which no plateau phase was observed and no Boltzmann curve could be fitted and was not included in further analysis on progression in seizure. To identify the different seizure rate progression profiles in the DBS group, we performed a k-means cluster analysis based on the time in weeks after KA injections where seizure rate reaches its half maximum value and the slope factor of seizure rate progression. Cluster analysis revealed that the DBS group could be subdivided in 3 groups (fig.3).



Legend fig. 3: *3 subgroups could be identified within the group of DBS treated rats. A group with a late but steep increase in seizure rate (red dots – group 1). A group in which seizure rate progression was similar to the SHAM rats (orange dots – group 2).A group with an early but steep increase in seizure rat (blue dots – group 3).*

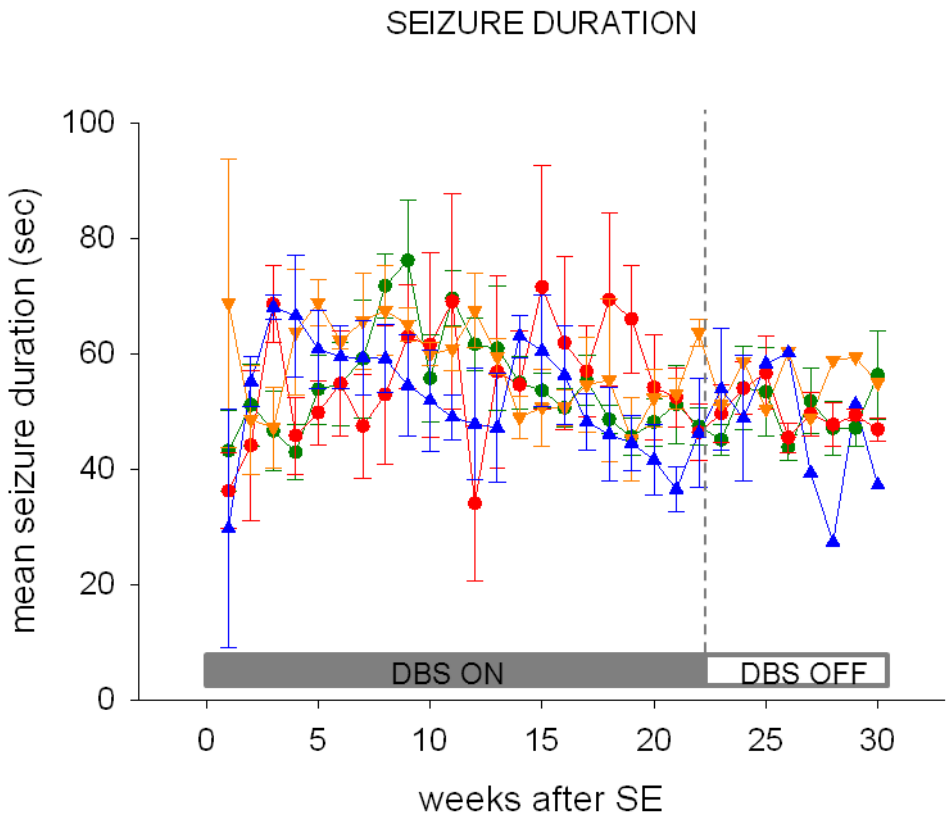
The DBS group could be subdivided in a group (group 1) (4/9), where the increase seizure rate was delayed until DBS was stopped (fig.4B). A group (group 2) (2/9), in which the increase in seizure rate was not different from the seizure rate progression in the SHAM group (fig.4C), and a group (group 3) (3/9) in which the increase in seizure rate occurred earlier compared to the rats in the SHAM group (fig.4D).



Legend fig. 4: *Seizure rate progression in all rats vs. the SHAM group . Green area represents Seizure progression profiles of the fitted Boltzmann function (green solid line) and the SD of the fit (green dotted line fitted to the weekly averages of daily seizure rate of the SHAM-treated rats vs. seizure rate progression of A) the individual SHAM rats, B) the rats in group 1, C) the rats in group 2, D) and the rats in group 3. The duration of the DBS treatment is displayed by the gray bars.*

ANOVA on the fitting parameters of these DBS subgroups and the SHAM group showed that the only significant difference in seizure progression between these DBS subgroups and the SHAM group was the time to half maximum daily seizure rate after KA injection. This was significantly longer for the rats in DBS group 1 (24 ± 1 weeks) compared to the rats in the SHAM group (14 ± 1 weeks) ($p<0.001$), the rats in DBS group 2 (12 ± 2 weeks) ($p=0.002$) and the rats in DBS group 3 (7 ± 1 weeks) ($p<0.001$). The increase in seizures occurred significantly earlier in the rats in DBS group 3 compared to the rats

in the SHAM group and the rats in DBS group 1. No significant differences were observed between any of the DBS subgroups or the SHAM group in neither maximal seizure rate nor slope of seizure rate progression. The mean latency to the first spontaneous electrographic seizure in the SHAM group was 7.4 ± 0.7 days (range: 4.2 – 9.7 days) after the onset of SE. No significant difference could be demonstrated in the latency to the first spontaneous seizure between the SHAM group and any of the DBS groups . Latency to the first electrographic seizure was 6.7 ± 1.2 days for the rats in DBS group 1, 7.1 ± 0.1 days for the rats in DBS group 2 and 5.2 ± 1.0 days for the rats in DBS group 3. The mean duration of spontaneous seizures in the KA model over the 30 week long monitoring period in the SHAM group was 55.6 ± 5.2 seconds. Seizure duration was unaffected during the 22 week long DBS period. During DBS, seizure duration was 55.6 ± 6.9 seconds in DBS group 1, 56.3 ± 2.5 seconds in the DBS group 2, and 51.9 ± 4.5 seconds in the DBS responder group 3. Switching off DBS had no effect on seizure duration. Mean seizure duration during the last 8 weeks of EEG monitoring when DBS was switched off was 49.6 ± 2.5 seconds in the DBS group 1, 53 ± 1.6 seconds in the DBS group 2, and 48.5 ± 5.5 seconds in the DBS group 3 (fig.5).

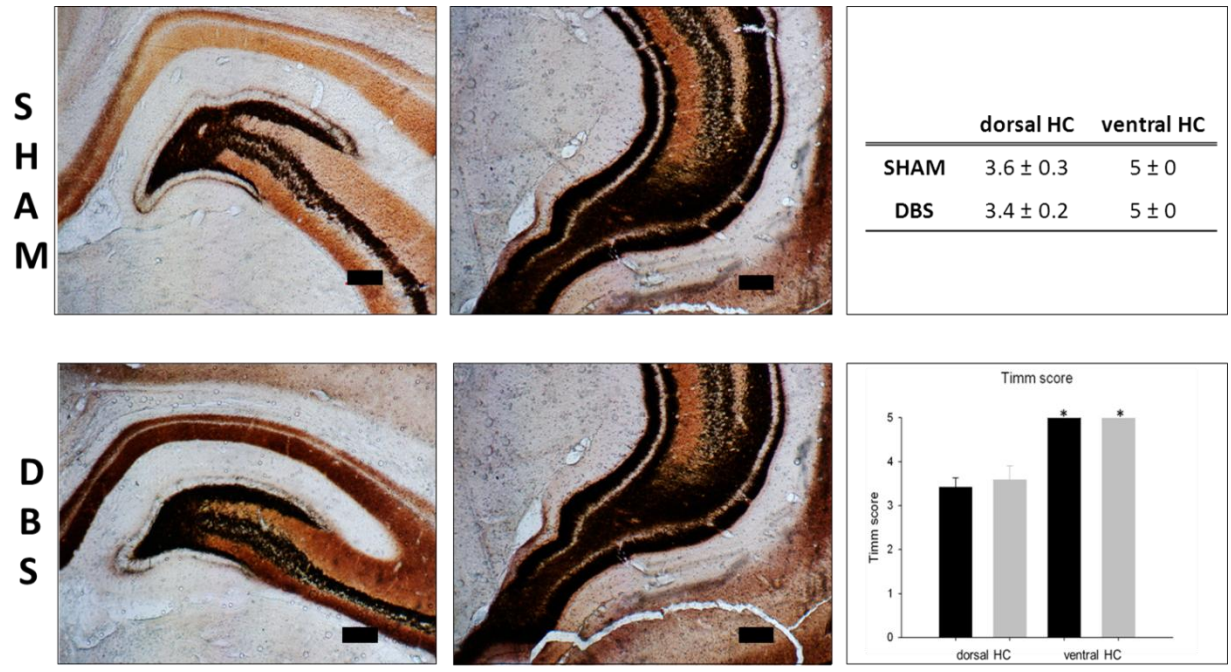


Legend fig.5: *Weekly average of seizure duration in the SHAM (green), DBS group 1 (red), DBS group 2 (orange), and DBS group 3 (blue). Split-plot ANOVA could not demonstrate a within or between group effect of DBS on seizure duration.*

Effect of hippocampal DBS on epileptogenic histopathology changes

Effect on mossy fiber sprouting

Brains of 12 out of 18 rats were processed for Timm staining to asses MFS both in the DBS and the SHAM group. Timm staining showed extensive MFS throughout the entire hippocampus in all rats. No differences in MFS could be observed between the SHAM group and any of the DBS subgroups. Scoring of the MFS in the ventral and dorsal hippocampus both in the DBS and SHAM group, revealed a significant difference between these hippocampal regions. MFS was more extensive in the ventral part of the hippocampus both in the SHAM group (mean Timm score of 5 ± 0) and DBS group (5.0 ± 0) compared to the Timm score in the dorsal part of the hippocampus in SHAM (3.6 ± 0.3) (p=0.006) and DBS group (3.4 ± 0.2) (p=0.002) (fig.6). Inter-rater correlation of the scoring of the 3 investigators was 0.8 for the dorsal hippocampus and scoring for the ventral hippocampus was exactly identical between the 3 investigators.



Legend fig. 6: *Mossy fiber sprouting in SHAM and DBS treated rats. Representative examples of sections (70 µm) of the dorsal part of the HC, and ventral part of the HC stained by the Timm staining protocol for evaluation of mossy fibre sprouting. MFS was present in all rats. MFS was more intense in the ventral HC compared to the dorsal HC both in the SHAM group and in the DBS group. Scale bar is 250 µm.*

DISCUSSION

In this study we evaluated the effect of long-term (22 weeks) hippocampal DBS on the development of epileptic seizures in the intraperitoneal kainic acid rat model for TLE. The post SE KA model is a well validated model for human TLE based on a similar progression and the occurrence of spontaneous seizures with similar semiology as patients with TLE (Hellier et al. 1998; Williams et al. 2009). The progressive increase in seizure rate leading to a chronic epileptic state in this rat model for TLE makes it a good model to study the effects of affecting seizure rate with hippocampal DBS on later seizure rate development (Loscher 2011). Long-term DBS was initiated 24 hours after SE was elicited by intraperitioneal administration of KA. We show that seizure rate is highly affected during long term treatment with hippocampal DBS in the post-KA model for TLE, but does not induce long lasting changes in the underlying pathophysiological processes of epileptogenesis and the seizure rate when stimulation is stopped.

Status epilepticus

Interventions terminating self-sustained SE in animal models have shown that reduced SE duration is correlated with a reduced frequency and severity of seizures during seizure rate progression (Mazarati et al. 2002b; Mazarati et al. 2002a). Evaluation by the categorical six-point method (Lehmkuhle et al. 2009; Treiman et al. 1990; Walton and Treiman 1988) and the spike detection method (Pitkanen et al. 2004) showed that severity and duration of SE was similar between the rats that were assigned to the DBS and to the SHAM group. Observed differences in outcome parameters of seizure progression between rats in the DBS and SHAM group cannot be attributed to differences in the severity or duration of SE.

Hippocampal DBS modulates seizure rate progression

Cluster analysis shows that the rats in the DBS group could be subdivided in 3 groups. In 4/9 rats the seizure rate progression was successfully suppressed until stimulation was stopped (group 1), in 3/9 rats the seizure rate progression was increased until stimulation was stopped (group 3), and in 2/9 rats the seizure rate progression was similar to the rats in the SHAM group (group 2). In our experimental design we could not determine why we observe these differences in response to the treatment. An increase in seizure rate has been described in patients treated with DBS in the anterior nucleus of the thalamus (ANT-DBS) at high intensities (500µA), whereas low intensity (100µA) ANT-

DBS had a seizure-suppressive effect (Covolan et al. 2014). Two open label studies, investigating the effect of hippocampal DBS, described patients with up to 50% increases in seizure rate during DBS. These patients were defined as non-responders (Cukiert et al. 2014; Tellez-Zenteno et al. 2006). Hippocampal DBS is believed to scale down the excitability of neuronal networks without disturbing its basic working mechanisms (Wyckhuys et al. 2010b). It has been reported that therapeutic deep brain stimulation aimed to scale down the excitability of neuronal networks can produce kindling-like effects of the brain in certain conditions manifested as the emergence of new seizures or an increased duration, severity and frequency of pre-existing seizures (McIntyre and Gilby 2009).
Microscopic evaluation of the electrode position confirmed the positioning of the electrodes in the hippocampus. However, a possible explanation for the different responses to hippocampal DBS could be small differences in stimulation electrode orientation or location causing excitation rather than inhibition of the neurons subjected to the electrical field. Bondallez et al. showed that the seizure suppressive capacity of hippocampal DBS was clearly correlated with the location of the active electrode contact being close to the subiculum (Bondallaz et al. 2013). An experimental study where the effect of stimulation dipole orientation on the suppression of neuronal activity in cortical neural network simulations is evaluated showed that orientations of the stimulation dipole axis determined the efficacy to the stimulation to disrupt neuronal activity (Anderson et al. 2007). Despite the different responses observed during hippocampal DBS, our results clearly show that long-term treatment with hippocampal DBS affects seizure rate.

Hippocampal DBS is not anti-epileptogenic and seizures do not beget seizures

Although a clear difference in seizure rate is observed during hippocampal DBS between the different DBS subgroups and the SHAM group at the end of the stimulation period, when stimulation was stopped seizure rate reached a plateau which was not different between SHAM and DBS-treated rats which indicates that effects of DBS on seizures are not sustained after switching of the stimulation. These results contradict the hypothesis of William Gowers that 'seizures beget seizures' (WR 1881). So far no study has shown clear sustained outlasting effect of hippocampal DBS, although an increased efficacy of thalamic and responsive focus DBS with time has been reported in clinical trials (Fisher et al. 2010; Heck et al. 2014). A previous preclinical investigation of high frequency hippocampal DBS in the systemic KA rat model showed an immediate ON-OFF effect of stimulation on spontaneous seizure rate (Wyckhuys et al. 2010b). Besides the absence of any outlasting effect of the treatment, evaluation of MFS in the systemic KA rat model (Buckmaster and Dudek 1997; Sharma et al. 2008) showed no differences at the end of the experiment, no delay in the occurrence of the

first seizure after SE could be observed, and seizure duration was highly stable throughout the experiment. This shows that long-term hippocampal DBS treatment did not have an anti-epileptogenic effect, even though long term treatment with high frequency (130Hz) hippocampal DBS has a strong effects on seizure rate as long as the treatment is continued. The similarity in seizure duration both in the DBS treated as in the SHAM rats showed that when a seizure was started, this seizure remained unaffected by the stimulation. Whereas high frequency hippocampal DBS affects duration of evoked afterdischarges (Cordeiro et al. 2013; Wyckhuys et al. 2010a), our results are in accordance with the previous observation that hippocampal DBS does not affect the duration of spontaneous seizures (Wyckhuys et al. 2010b). Although the occurrence of the first seizure is not affected and hippocampal DBS did not affect the duration of the seizures, our data support the hypothesis that during stimulation hippocampal DBS affects the probability for a seizure to occur. After stimulation is stopped, the probability to have a seizure is similar among rats in the treatment group and in the control group, resulting in a similar seizure rate. Our results show that suppressing or increasing seizure rate by means of hippocampal DBS does not affect epileptogenesis, disproving that “seizure beget seizures”.

CONCLUSION

The ongoing epileptogenesis in rats where seizure rate is highly affected by hippocampal DBS treatment show that affecting seizure rate during the first 22 weeks after SE did not affect the epileptogenic process in the rat KA model for TLE which questions the controversial theory postulated by Gowers in 1881 that “seizures beget seizures”. This present study demonstrates that long term continuous hippocampal DBS treatment affects neuronal excitability during stimulation can hereby affect symptom development in a model for TLE but has no anti-epileptogenic nor evident stimulation outlasting effects.

MATERIALS & METHODS

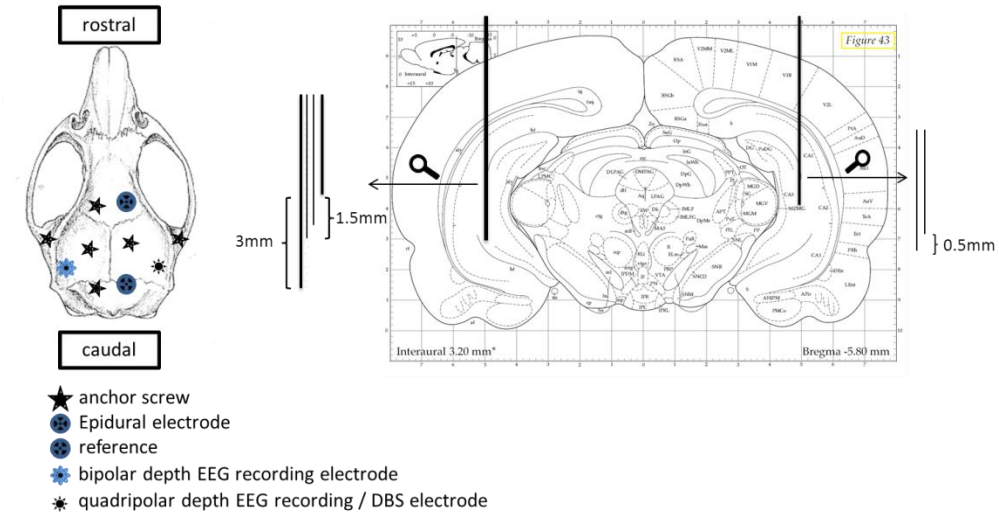
Animals

The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University Hospital (ECP 09 ⁄ 16). Male Sprague-Dawley rats (Harlan, the Netherlands) weighing 200–275 g, were kept under environmentally controlled conditions (12 h normal light ⁄ dark cycles, 20–23°C and 50% relative humidity) with food and water intake *ad libitum*. The animals were treated according to guidelines approved by the European Ethics Committee (decree 86 ⁄ 609 ⁄ EEC).

Surgery

Twenty nine rats were anesthetized with a mixture of isoflurane (5% for induction, 2% during implantation) and medical oxygen. At the start of the surgery, rats were injected subcutaneously with Temgesic (0.03mg/kg) to reduce discomfort during recovery. After exposure of the skull, 10 small burr holes were drilled; six for the positioning of anchor screws (1.6 mm diameter; Bilaney), one for the epidural electrode, one for the reference electrode, one for a bipolar depth EEG recording electrode and one for a quadripolar depth EEG recording/DBS electrode. A custom-made epidural electrode was screwed into the right side of the skull at the height of the frontal cortex. A similarly constructed ground electrode, which was used as recording reference, was placed on the right side posterior to the sutura lambdoidea. The quadripolar depth EEG recording/DBS electrode was custom-made by twisting two polyimide coated stainless steel wires (used for EEG recording) and two Teflon coated platinum-iridium wires (used for delivering DBS pulses). The 2 outer electrode contacts of the quadripolar electrode were made of the Teflon coated platinum iridium wire and had a bare diameter of 76 µm (A-M systems, USA). The inner 2 contacts were made of polyimide-coated stainless steel wire with a bare diameter of 70 µm (CFW, CA, USA). The distance between the two outer tips of the quadripolar electrode was 3000 µm and 500 µm between the two inner electrode contacts, with the deepest recording contact 1500 µm above the lowest stimulation contact (fig 7). The bipolar depth EEG recording electrode was made by twisting together two polyimide-coated stainless steel wires with a bare diameter of 70 µm (CFW, CA, USA). Distance between the recording tips was 500 µm. The quadripolar depth EEG recording/DBS electrode was implanted in the right hippocampus ((AP -5.6, DV -7.4, ML +5.1 relative to Bregma), the bipolar depth EEG recording electrode was implanted in the left hippocampus ((AP -5.6, DV -5.9, ML -5.1 relative to Bregma) (Paxinos C 1998) (fig 7). The electrode leads were connected to a connector that is fixed to the skull

with anchor screws and acrylic dental cement. Immediately after surgery rats were subcutaneously injected with the NSAID Metacam (1mg/kg) to minimize pain and reduce the inflammatory reaction. Lidocaine and Neobacitracine pomade were applied to the wound to lower discomfort during recovery and to avoid bacterial infections of the wound respectively. After surgery, rats were allowed 8 to 18 days of recovery before initiating the experiment.



legend fig. 7: *schematic overview of the configuration of the electrode implantation: a quadripolar EEG recording/DBS depth electrode in the right hippocampus, a bipolar EEG recording electrode in the left hippocampus, 1 epidural electrode above the right frontal hemisphere, 1 reference electrode posterior to the sutura lambdoidea above the right hemisphere and 6 anchor screws. Figure adapted from (Paxinos C 1998)*

Kainic acid injection & status epilepticus

Twenty-nine rats (267 +/- 4 g) received kainic acid (KA) (5 mg/kg; Tocris Bioscience, USA) by intraperitoneal (ip.) injections according to the protocol of Hellier et al. to induce status epilepticus (SE) (Hellier et al. 1998). Seizure activity of all rats was continuously monitored visually and electrographically. The KA treatment was repeated hourly until the animals displayed a stable self-sustained SE for ≥3 hours (i.e., > 10 stage 3,4 or 5 seizures on Racine's scale per hour). Animals exhibiting excessive motor or excessive lethargic behavior were further given reduced dosages (2.5mg/kg) of KA to avoid exorbitant toxicity and mortality (Williams et al. 2009). Of the 29 rats undergoing the KA injection protocol, two rats died during SE.

EEG recording and stimulation set up

EEG signals were recorded via a head stage, carrying unity gain preamplifiers, and a commutator connected to custom-built amplifiers (gain: 510 x; band-width: 0.13Hz – 5.8kHz). A data acquisition card (NI-USB-6259, National Instruments, USA, TX) digitized the EEG signals, which were then stored onto a hard drive for later offline analysis.

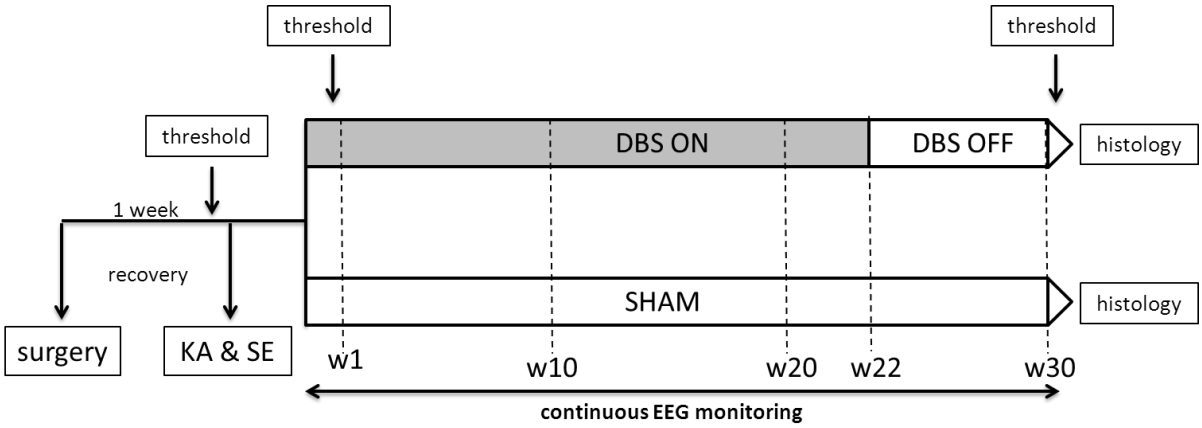
Stimulation pulses were delivered by custom-built constant current stimulator. The stimulation paradigm consisted of a continuous train of bipolar, biphasic, charge-balanced square-wave pulses with a pulse width of 100 μs. Interpulse intervals were Poisson distributed with a mean and variance of 7.7ms (130Hz).

Stimulation intensity was set at 60% of the afterdischarge threshold (ADT), i.e. the minimum stimulus intensity giving rise to epileptic discharges on the EEG. The ADT was measured using 10s pulse trains with parameters identical to those applied for the continuous therapeutic stimulation. There was one minute interval between pulse trains during which the EEG was evaluated for the appearance of epileptic discharges (ADT). Following each pulse train delivery, stimulation intensity was increased with steps of 25 μA starting from 25 μA until the first sign of an AD was detected on the EEG or until 500 μA output current was reached.

Experimental protocol

EEG recording in all rats was started 3 minutes before the first injection of KA and continued for 30 weeks. The EEG recording sessions were stopped weekly during 30' from 9h00 until 9h30 to clean the cages and refresh food and water. Twenty-four hours after the start of the KA injection and the start of the elicited SE, rats were randomly divided into a DBS group, undergoing continuous stimulation with hippocampal DBS for 22 weeks followed by a washout period of 8 weeks, and a SHAM group where no treatment was given during 30 weeks (fig. 8). The ADT was determined twice in each rat i.e. once on the day before SE induction and once 4 days after SE. Stimulation intensity during the first 4 days after SE was 60% of the ADT determined 1 day before SE, and stimulation intensity from day 4 after SE until the end of the 22 week long treatment period was set at 60% of the ADT determined at day 4 after SE. In the DBS group, ADTs were determined again at the end of the 30 week long EEG monitoring period. During the 22 weeks of the treatment period, electrode impedance was measured weekly at fixed moments between 9h00 - 9h30 on the first day of each week with a 1kHz sine wave and <40nA testing current (IMP-2, Bak electronics, Sanford Florida, USA). Eight rats (5 DBS rats and 3 SHAM rats) were left out of the analysis because they lost their head cap (3 DBS and 2 SHAM rats) or died (2 DBS and 1 SHAM rats) before their seizure rate reached a plateau

phase in seizure rate progression. This resulted in a total of 19 rats undergoing the entire 30 week long experiment of which 9 were in the SHAM group and 10 were in the DBS group.



legend fig. 8: *Experimental protocol used in this study. After implantation of stimulation and EEG recording electrodes rats were allowed one week of recovery before they were subjected to SE evoking KA injections. Twenty-four hours after the SE, rats were randomly divided into a DBS group, undergoing continuous stimulation with hippocampal DBS for 22 weeks followed by a washout period of 8 weeks, and a SHAM group where no treatment was given during 30 weeks*

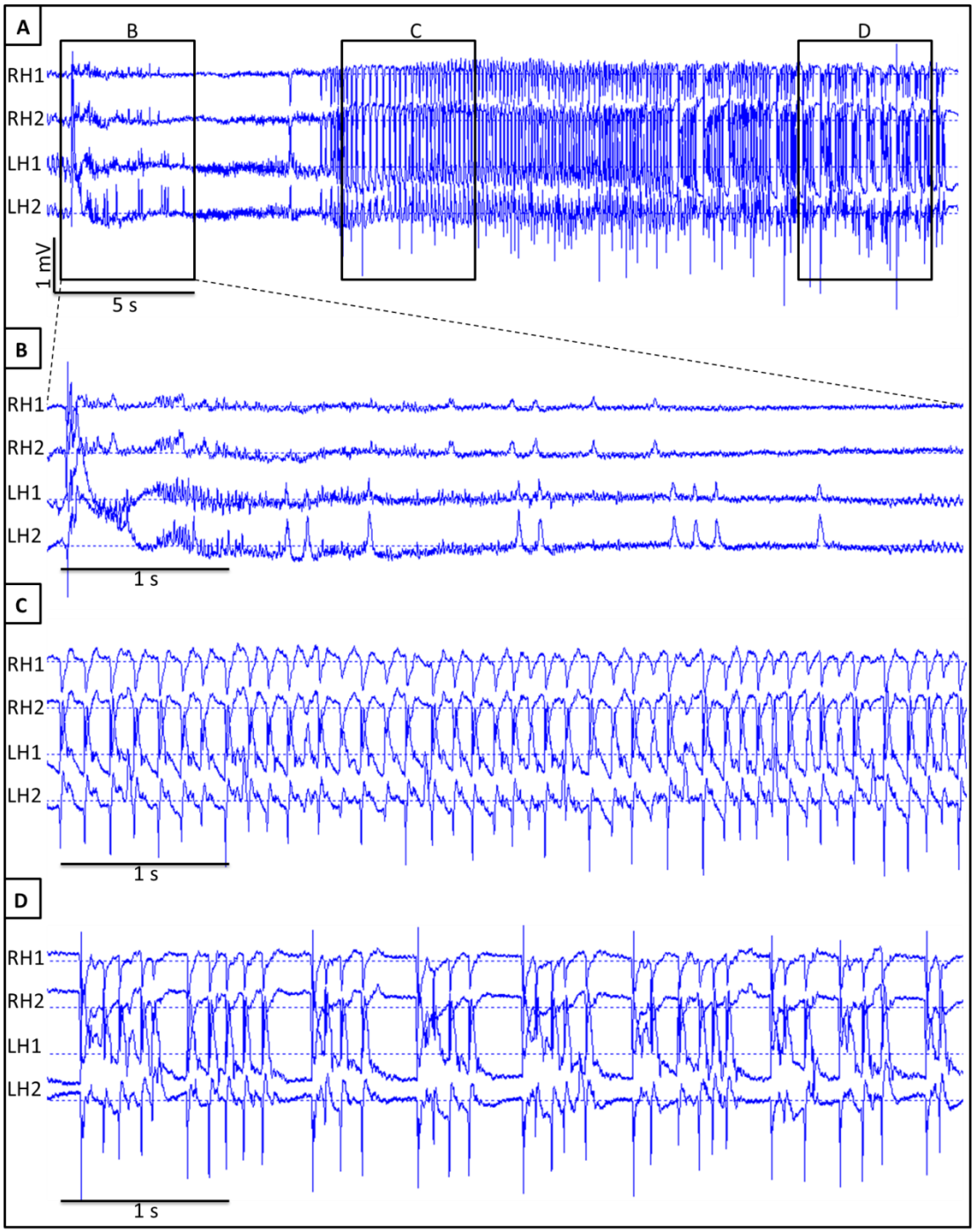
Monitoring of status epilepticus

The EEG recorded during the first 24h after KA injection was divided in 30' epochs and analysed using two different methods; a categorical scoring method and an automated spike detection method.
Based on the occurrence of electrographic patterns like normal or slow isolated EEG spikes (score 1), discrete seizures (score 2), merging seizures (score 3), continuous ictal activity (score 4), continuous ictal activity with flat periods (score 5) and periodic epileptiform discharges (PEDs) superposed on normal flat EEG (score 6), the EEG was scored with the previously published categorical six-point method (Lehmkuhle et al. 2009; Treiman et al. 1990; Walton and Treiman 1988). Total SE severity score was calculated by adding all severity scores for the 30' epochs until score 6 epochs appear. The SE duration was defined as the time between the start of the first score 2 epoch (discrete electrographic seizure) up to the start of the first score 6 epoch (periodic epileptiform discharges (PEDs)). To determine the number of epileptic spikes during SE, an automated spike detection method was used. Before running spike detection, the EEG traces were filtered with a band pass filter (1-50Hz) to remove possible movement artifacts and possible high frequency noise. To detect epileptic spikes a threshold amplitude was set at the mean voltage + 3 times the standard deviation of 3 minutes of filtered EEG taken before KA injection. Because all recorded spikes were positive only positive crossings were counted. However, when due to a small difference in electrode location

negative spikes were observed in an animal, the EEG trace was mirrored before running the spike detection. SE severity was assessed by counting the total number of spikes during 24h after SE (Pitkanen et al. 2004). SE duration was defined as the time during which more than 1 epileptic spikes occurred in 1 second.

Monitoring of spontaneous seizures

Seizures were defined as electrographic episodes characterized by sustained rhythmic epileptic spiking (> 5Hz, amplitude >3 x baseline voltage) for at least 10 seconds. These seizures were often initiated by a large positive or negative potential followed by a decrease in amplitude, which progressed into the described high frequency, large amplitude rhythmic spiking (White et al. 2006; Williams et al. 2009; Wyckhuys et al. 2010b) (fig. 9). Seizure rate progression in the DBS treated rats was compared to SHAM treated rats subjected to identical SE induction, housing and monitoring conditions. Continuous EEG monitoring allowed us to determine the latency to the first spontaneous seizure after SE, and the evolution over time in seizure rate and seizure duration.



Legend fig.9: *A typical electrographic seizure during the EEG monitoring period; RH: right hippocampus, LH: left hippocampus* ***A:*** *electrographic seizure that lasts for 34s.* ***B:*** *Seizure initiation characterized by a large positive or negative potential followed by a period of low signal amplitude.* ***C:*** *Progression of the epileptic spikes into rhythmic, high frequency, large-amplitude EEG spiking.* ***D:*** *Lingering epileptic activity near the end of the seizure.*

Histology

At the end of the experiment, all rats were deeply anesthetized with pentobarbital (100mg/kg, i.p.) and transcardially perfused with a 0.37% Na_2S solution in PBS followed by 4% paraformaldehyde. The brains were removed and post-fixed in a 4% paraformaldehyde solution for at least 24 hours. Then, brains were transferred to a 10%, 20%, and 30% sucrose solution (at least one day in each solution), frozen in ice-cold isopentane and stored at -80°C until sectioning. Coronal sections (70µm) were cut at the level of the hippocampus starting from approximately 2.3 mm posterior from bregma to approximately 6.0 mm posterior from bregma. Sections were collected in 30% ethylene glycol and 25% glycerol in 50 mM phosphate buffered saline (PBS) and stored at -20°C until further processing. Starting approximately 2.3mm posterior from Bregma (Paxinos C 1998), a 1-in-6 series of coronal sections of each rat brain was processed for Nissl staining to confirm electrode location. For Nissl staining the sections were mounted and dried on glass slides, after which they were stained with 0.3% cresyl violet for 5 minutes. After staining, slices were rinsed in distilled water and dehydrated for 1 minute in 96% ethanol and 2 times for 5 minutes in 70% ethanol respectively. Slices were cleared in xylene for two times 5 minute before they were coverslipped with Entellan. In 12 out of 18 rats a second 1-in-6 series of coronal sections was processed for Timm's staining to evaluate the presence of mossy fiber sprouting (MFS). For Timm's staining sections were mounted, dried, dehydrated and developed for 60 minutes in developer solution, that is prepared by mixing 120ml of 50% gum Arabic, 20ml of 2M citrate buffer, 60ml of 0.5M hydroquinone and 1 ml of 17% silver nitrate solution. After rinsing in PBS, sections were again dehydrated and cleared before being coverslipped with Entellan. Brain slices processed for Timm staining were afterwards scored independently by 3 investigators (BVN, RR, MS) with the Timm scoring method of Cavazos et al. (Cavazos et al. 1991). In brief, a score of 0 represents no Timm granules in the supragranular layer. Score 1 represents very light, sparse granules in the supragranular layer. Score 2 represents a very light distribution of granules over a continuous region in the supragranular layer. Score 3 represents a continuous pattern of granules in a band over the supragranular region. Score 4 represents a confluent dense laminar band of granules, not entirely filled. Score 5 represents an entire band of granules in the supragranular layer, extending into the inner molecular layer. To evaluate inter-rate reliability, a split-half reliability analysis was performed to compare the ratings of sprouting by the 3 investigators. The scores of the 3 investigators were averaged; reported values represent the mean and SEM of the averaged scores of the 3 investigators.

Statistics

All data was tested for normality before statistical analysis was performed. In cases where assumptions for parametric testing were not fulfilled, non-parametric testing was used and data is presented as median and interquartile range (IQR). Data fulfilling assumptions for parametric testing are expressed as mean and standard error of the mean (sem). Non parametric Mann-Whitney U test was used to compare SE parameters between the DBS and SHAM group. Changes in afterdischarge evoking thresholds over time were tested with the non-parametric paired Wilcoxon signed rank test. Subdivision of the rats in the DBS group was performed using k-means cluster analysis. Outcome parameters of the DBS subgroups and SHAM group were compared using ANOVA, with post hoc Bonferroni correction for multiple comparisons.

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Chapter 7

Hippocampal deep brain stimulation: mechanism of action

Hippocampal Deep Brain Stimulation induces decreased rCBF in the hippocampal formation of the rat

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ABSTRACT

Deep Brain Stimulation (DBS) is a promising experimental approach to treat various neurological disorders. However, the optimal stimulation paradigm and the precise mechanism of action of DBS are unknown. Neuro-imaging by means of Single Photon Emission Computed Tomography (SPECT) is a non-invasive manner of evaluating regional cerebral blood flow (rCBF) changes, which are assumed to reflect changes in neural activity. In this study, rCBF changes induced by hippocampal DBS are evaluated by subtraction analysis of stimulation on/off using small animal µSPECT of the rat brain. Rats (n=13) were implanted with a multi-contact DBS electrode in the right hippocampus and injected with 370MBq of HMPAO-$Tc99^{m}$ during application of various hippocampal DBS paradigms and –amplitudes and during sham stimulation. Subtraction analysis revealed that hippocampal DBS caused a significant decrease in relative rCBF, both in the ipsi- (the side of the implanted electrode) and contralateral hippocampus. Hypoperfusion spread contralaterally with increasing stimulation amplitude. A clear distinction in spatial extent and intensity of hypoperfusion was observed between stimulation paradigms: bipolar Poisson Distributed Stimulation induced significant hypoperfusion ipsi- and contralaterally ($p<0.01$), while during other stimulation paradigms, rCBF-changes were less prominent. In conclusion, small animal µSPECT allows us to draw conclusions on the location, spatial extent and intensity of the hypoperfusion observed in the ipsi- and contralateral hippocampus, induced by hippocampal DBS. Our study demonstrates an innovative approach to visualize the effects of DBS and can be a useful tool in evaluating the effect of various stimulation paradigms and target areas for DBS.

INTRODUCTION

Deep Brain Stimulation (DBS) is a therapeutic approach that involves the intracranial implantation of one or more electrodes in a specific brain region. By means of an implantable battery and a subcutaneous lead, electrical pulses are sent to the target site to interfere with the neural activity. DBS is a promising treatment for a variety of neurological disorders, such as movement disorders (Benabid, 2003), chronic pain (Hosobuchi et al., 1973) obsessive-compulsive disorder (Nuttin et al., 1999) and drug resistant epilepsy (Boon et al., 2009). Temporal Lobe Epilepsy (TLE) is the most drug resistant form of epilepsy (Kwan and Brodie, 2000). Because there is considerable evidence that the hippocampal formation is involved in seizure initiation in TLE patients (Spencer, 2002; Swanson, 1995), electrical stimulation of the limbic system has been successfully applied to treat drug resistant TLE. Velasco et al. were the first to discover that unilateral DBS delivered through depth electrodes in the temporal area decreased interictal and ictal epileptiform activity in drug resistant TLE patients (Velasco et al., 2000a). Later, these positive results were confirmed in clinical trials (Boon et al., 2007; Velasco et al., 2001; Velasco et al., 2000b; Vonck et al., 2002) and in animal experimental studies (Wyckhuys et al., 2007). Despite these promising results, the precise mechanism of action of DBS and the pathways affected due to hippocampal depth stimulation are unknown. Furthermore, the optimal stimulation parameters are undetermined, hampering its therapeutic potential.

Neuro-imaging by means of Single Photon Emission Computed Tomography (SPECT) is a non-invasive technique to evaluate regional cerebral blood flow (rCBF) changes, which are assumed to reflect changes in neural activity (Hershey and Mink, 2006; Shibasaki, 2008). Consequently, this technique may be a useful tool in visualizing DBS-induced rCBF-changes throughout the brain and evaluate changes induced by different DBS-paradigms. The past 3 years SPECT scanners have been successfully miniaturized to enter the preclinical arena allowing for a high spatial resolution with an acceptable sensitivity in rats and mice. The system used in this study, the Milabs U-SPECT-II, can reach resolutions down to 350 µm in whole body mice scanning and well below 1 mm in rats.

In the current study, four hippocampal DBS paradigms were compared with stimulation-off (sham stimulation) in the rat by means of µSPECT subtraction analysis through coregistration with their individual CT. Additionally, stimulation amplitudes were varied and effect on location, spatial extent and intensity of rCBF-changes were evaluated. Apart from µCT coregistration, µSPECT images were also co-registered with MR-images of the same rat to allow an accurate anatomical correlation.

METHODS

1. Animals

Male Wistar rats (250-300g body weight; Harlan, the Netherlands) were treated according to guidelines approved by the European Ethics Committee (decree 86/609/EEC). The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University Hospital (ECP 09/16). The animals were kept under environmentally controlled conditions (12h normal light/dark cycles, 20-23°C and 50% relative humidity) with food and water *ad libitum*.

2. Surgery

Healthy rats (n=13) were anesthetized with an isoflurane mixture (2-5% isoflurane and medical O_2). After exposure of the skull, 7 small burr holes were drilled; four were used for the positioning of anchor screws, the other three for electrodes. A custom-made epidural electrode (Teflon-coated silver wire, soldered to a screw, 1.57mm diameter, Bilaney, Germany) was placed on the right side of the skull at the height of the frontal cortex. A reference-electrode was placed contralaterally. A multi-contact DBS-electrode was custom-made by gluing together four polyimide coated stainless steel wires (125µm diameter, Bilaney, Germany). Each of the wires was straight-cut at a different length resulting in a quadripolar electrode with 1 mm distance between each of the four tips. This DBS-electrode was inserted stereotactically in the right hippocampus (AP -5.6mm, ML 5.1mm, DV -7.4mm relative to bregma) (Paxinos and Watson, 1998). The electrodes were lead to a connector which was fixed to the screws and the skull with acrylic dental cement.

3. Experimental protocol

After recovery from surgery, rats were handled to get used to the injection protocol (>7 days). Following handling, all rats underwent five µSPECT scans. Each µSPECT-scan was followed by a µCT-scan. Each µSPECT (and consecutive µCT) scan was separated from the next by at least 48h. After termination of all µSPECT-scans, rats were deeply anesthetized with pentobarbital (100mg/kg, i.p.), electrodes (and connector) were removed and rats then underwent a final MR-scan.

Every µSPECT-scan was preceded by either 75 minutes of continuous DBS (one of four paradigms or one of four stimulation amplitudes – see further) or 75 minutes of sham stimulation (=no stimulation). For each rat, the five stimulation paradigms were presented in a randomized order. Stimulation was delivered to the rat by means of a flexible wire allowing the rat to move freely. Additionally, continuous scalp EEG was measured throughout the delivery of stimulation to verify

whether no EEG abnormalities occurred. After one hour of DBS or sham stimulation, rats were, while awake, intravenously injected with 370MBq HMPAO-Tc^{99m}. Stimulation was not interrupted during injection and continued until 15 minutes following injection. After discontinuation of stimulation (or sham), rats were anesthetized with a mixture of isoflurane (2-5% isoflurane and O_2) and placed in a custom-made container that fits onto the bed of the scanner (Fig. 1). The head of the rat was fixed with an alginate paste (Cavex CA37, the Netherlands). Body temperature was kept constant with a heating mat and respiration frequency was measured throughout both µSPECT and µCT scans.

Figure 1: Protocol of one scan day. Before intravenous injection with 370MBq HMPAO-Tc99m, rats received one hour of continuous stimulation with either one of five stimulation paradigms (sham, HFS2, HFS4, PDS2 or PDS4) or one of five stimulation amplitudes (sham, 25µA, 50µA, 100µA, 200µA). During and 15 minutes following injection, stimulation was continued. Then, stimulation was interrupted and rats were anesthetized before initiation of µSPECT (1.5h) followed by µCT.

4. Different DBS paradigms and stimulation amplitudes

Seven out of 13 rats were subjected to one sham stimulation and four DBS paradigms: bipolar High Frequency Stimulation (HFS2), quadripolar HFS (HFS4), bipolar Poisson Distributed Stimulation (PDS2) and quadripolar PDS (PDS4). All stimulation paradigms consisted of a series of biphasic, charge-balanced square-wave pulses with a pulse width of 100µs and an average frequency of 130 Hz. HFS (both HFS2 and HFS4) consisted of stimulus pulses given at a fixed frequency of 130 Hz (interpulse interval of 7.69 ms). In the PDS protocol (both PDS2 and PDS4), the interstimulus intervals were drawn from a Poisson distribution with a mean and variance 1/130s. The mean frequency of the PDS was therefore also 130Hz, so that on average the same number of stimulus pulses were delivered in both PDS and HFS protocols.

For bipolar stimulation (as in HFS2 and PDS2), pulses were delivered between the most superficial and the deepest electrode tip of the quadripolar DBS electrode. For quadripolar stimulation (HFS4 and PDS4), pulses were delivered between the most superficial and third electrode tip and between the second and the deepest electrode tip (Fig. 2).

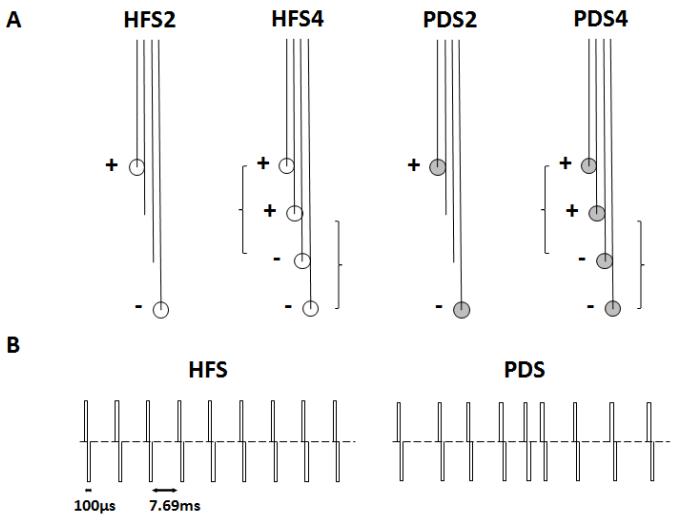

Figure 2: A) Illustration of the four stimulation paradigms delivered through quadripolar electrodes. For bipolar stimulation (HFS2 and PDS2), biphasic stimulation is delivered between the anode (+) and cathode (-). For quadripolar stimulation (HFS4 and PDS4), two times biphasic stimulation is delivered (anode/cathode-couples indicated); B) HFS with a fixed interpulse interval of 7.69ms (130Hz) and pulse width of 100µs and PDS with asynchronous Poisson distributed stimulus intervals with the same mean value as HFS.

Deep Brain Stimulation was delivered by custom-made isolated current sources. DBS was started >1h before and continued during and 15 minutes following injection of 370MBq HMPAO-$Tc99^{m}$. For these seven animals, stimulation amplitude was fixed for all four paradigms at 100µA.
Six out of 13 rats were subjected to one sham stimulation and four stimulation amplitudes of bipolar PDS: 25µA, 50µA, 100µA and 200µA.

5. µSPECT /CT/MRI

The animals received 370MBq HMPAO-$Tc99^{m}$ (Ceretec, GE Healthcare, UK) either during application of hippocampal DBS (various stimulation paradigms and -amplitudes) or during sham stimulation. For optimal registration with µCT, two line sources were placed in oblique positions next to the animal's head (in the alginate paste). These line sources were filled with low activity (0.37MBq) of I^{125}. Static scanning in 18 frames of 5 minutes was performed using the Milabs U-SPECT-II (MILabs, Utrecht, The Netherlands). This µSPECT scanner is equipped with collimators consisting of a tungsten cylinder with 5 rings of 15 pinhole apertures of 1.0 mm diameter. All pinholes focused on a single volume in the center of the tube (van der Have et al., 2009). For imaging rat brain, the animal bed was translated in 3 dimensions using an XYZ stage into 8 different bed positions. A 20 % main

photopeak was centered at 140 keV to reconstruct the Tc^{99m} images while a 100% window was centered around 30 keV for the I^{125} capillaries. The data were reconstructed on 0.75 mm^3 voxels by 3 iterations of 16 OSEM subsets (Vastenhouw and Beekman, 2007). Each time a µCT scan of the animal was acquired with the Gamma Medica Ideas (Northridge, LA, USA) X-O CT in fly-mode acquiring 256 projections (2x2 rebinning) with the tube set to 70 kV and 170 µA and the magnification at 1.3 (FOV=91.08 mm). A general purpose reconstruction mode was used in a 512x512 matrix of 150 µm pixel size. The resultant image was then fused with the µSPECT scan using the capillaries fixing the 6 degrees of freedom. Afterwards the animals were sacrificed, their electrode was removed and a MRI scan was performed using a dedicated rat brain coil (Rapid Biomedical, Rimpar, Germany) on a Siemens Trio 3T (Siemens, Erlangen, Germany). A MPRAGE sequence resulting in 0.281 x0.281 x 0.3 mm resolution was applied with the rats placed head first and prone.

6. Data analysis

First (i) the stimulus-on µCT and the MRI were registered to the stimulus-off µCT using Amide (freeware; http://amide.sourceforge.net) followed by (ii) the fusion of the off/on µSPECT scans with their off/on µCT counterparts thereby using the two line markers (cfr. supra). From the MRI, (iii) with MRIcroN (freeware; http://www.mricro.com) the rat brain is extracted, which is used as a mask for the calculation. Afterwards, (iv) both off/on µSPECT scans are normalized within this MRI brain mask and subtracted from each other in Matlab. Finally, (v) the Z-score, representing the activation map, is achieved through dividing by the standard deviation of the stimulus-off µSPECT (Staelens et al. – unpublished data).

Accordingly, the magnitude of the Z-score can thus be determined for each voxel, indicating the significance of differences in rCBF for stimulation on/off. For each resulting image, we determined the location, the maximal and minimal Z-score and the total number of significant voxels (assuming $p<0.05$ to indicate significant differences; i.e. Z-score > 1.96 or Z-score <-1.96).

Data are expressed as mean and standard error of the mean (SEM). Statistical evaluation of the maximal and minimal Z-score and total number of significant voxels was performed using repeated measures ANOVA.

7. Histology

Histology confirmed that all four electrode tips were located within the hippocampus. Rats with electrode located outside the hippocampus were not included in the analysis.

RESULTS

1. Direction and location of changes in rCBF

Predominantly decreases in rCBF were visualized when the different DBS scans were subtracted from sham stimulation scans. Hyperperfusion was occasionally seen but these changes were never consistent between different rats and/or different scans.

The hypoperfusion was restricted to the different structures of the hippocampal formation including the CA1, CA2 and CA3 areas, the subiculum, the dentate gyrus and the entorhinal cortex in an intensity and stimulation paradigm-dependent manner. These decreases in rCBF were both in the ipsilateral (at the side of DBS) and the contralateral hippocampus (Fig. 3 and Fig. 4). Other brain regions occasionally displayed signal changes during hippocampal DBS including the olfactory lobes, somatosensory cortex and the cerebellum, but the presence and direction of changes in these areas were not consistent among rats, stimulation amplitudes and/or stimulation paradigms.

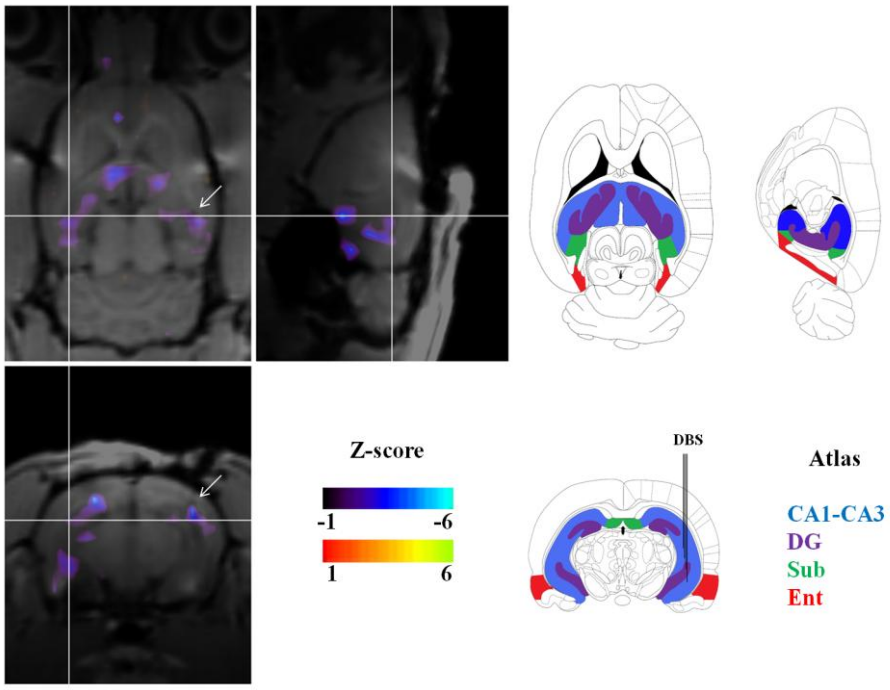

Figure 3: Coronal, sagittal and transverse anatomical scans co-registered with the colored subtraction SPECT data illustrating the rCBF changes induced by DBS (in this example PDS2, 100µA). Colored bars indicate Z-scores for increases (warm colors) and decreases (cold colors) induced by DBS in comparison with sham stimulation. The white arrows indicate the hippocampal DBS electrode artifact. The corresponding sections, modified from the Paxinos and Watson (Paxinos and Watson, 1998) rat brain atlas are shown on the right (CA1-CA3; DG=dentate gyrus; Sub= subiculum; Ent= entorhinal cortex). The different hippocampal structures are colored and the position of the DBS electrode is indicated.

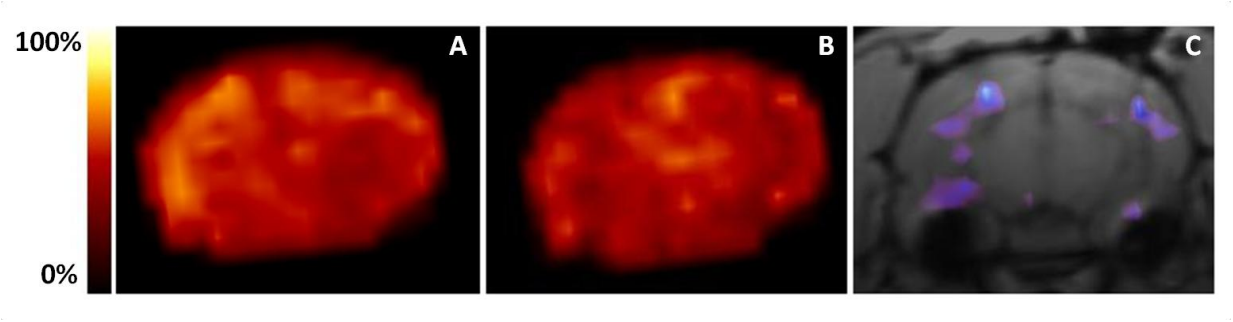

Fig 4: Coronal section of one representative rat during sham stimulation (A) and during 100µA PDS2 (B). Color bar indicates relative intensity for HMPAO-Tc99m. Note the lower tracer uptake in both the left and right hippocampal structure during stimulation in comparison with SPECT taken during sham stimulation. C) Subtraction analysis of both SPECT scans co-registered with MR (stimulation minus sham).

2. Intensity and spatial extent of rCBF changes

Subtraction analysis in the rats (n=7) subjected to the four different stimulation paradigms, revealed that PDS2 caused a significantly stronger hypoperfusion in the hippocampal formation compared with HFS2 and HFS4 ($p<0.05$ and $p<0.01$ respectively; repeated measures ANOVA) as indicated in Fig. 5a. Mean (±SEM) minimal Z-score measured in the hippocampal formation for HFS2, HFS4, PDS2 and PDS4 was -2.07 ± 0.26, -2.26 ± 0.39, -2.90 ± 0.11 and -2.11 ± 0.16 respectively. For all stimulation paradigms, there was no statistical difference between the intensity of rCBF change in the ipsilateral hippocampal formation versus the contralateral hippocampal formation.

Subtraction analysis in the rats (n=6) subjected to PDS2 with four stimulation amplitudes, revealed no statistical difference (repeated measures ANOVA) in minimal Z-score of rCBF changes between 25µA, 50µA, 100µA and 200µA (Fig 5b). The average minimal Z-score for all stimulation amplitudes was -2.82 ± 0.03. Additionally, for all stimulation amplitudes, there was no statistical difference between the intensity of rCBF change in the ipsilateral hippocampal formation and the one in the contralateral hippocampal formation.

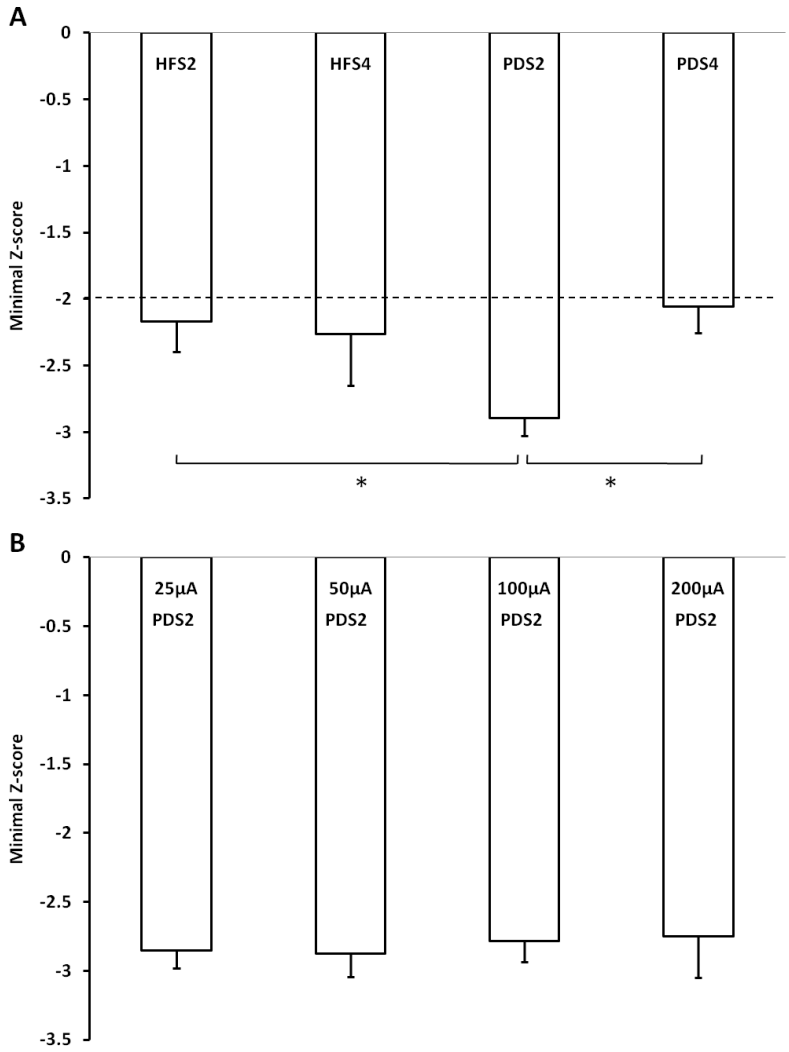

*Fig. 5: Mean (±SEM) minimal Z-score per stimulation paradigm for the whole hippocampal structure illustrating the intensity of hypoperfusion induced by A) HFS2, HFS4, PDS2 and PDS4 and B) PDS2 with 25µA, 50µA, 100µA and 200µA. The dotted line indicates Z= -1.96 which is the threshold for significant (p<0.05) difference between baseline µSPECT-scan and stimulation-scan. Comparison between the four stimulation paradigms reveals a significant stronger hypoperfusion for PDS2 in comparison with HFS2 and PDS4 (*p<0.05, repeated measure ANOVA). Comparison between the four stimulation amplitudes of PDS2 does not reveal significant differences in minimal Z-scores.*

Subtraction analysis in the rats (n=7) subjected to the four different stimulation paradigms, revealed that during PDS2 more significant voxels (Z-score < -1.96) were counted in the hippocampal formation than during the three other stimulation paradigms: 377% more significant voxels due to PDS2 compared with HFS2, 162% more due to PDS2 compared with HFS4 and 660% more due to PDS2 compared with PDS4 (Fig. 6). For all stimulation paradigms, there was no statistical difference

between the volume of hypoperfused brain in the ipsilateral hippocampal formation and the one in the contralateral hippocampal formation.

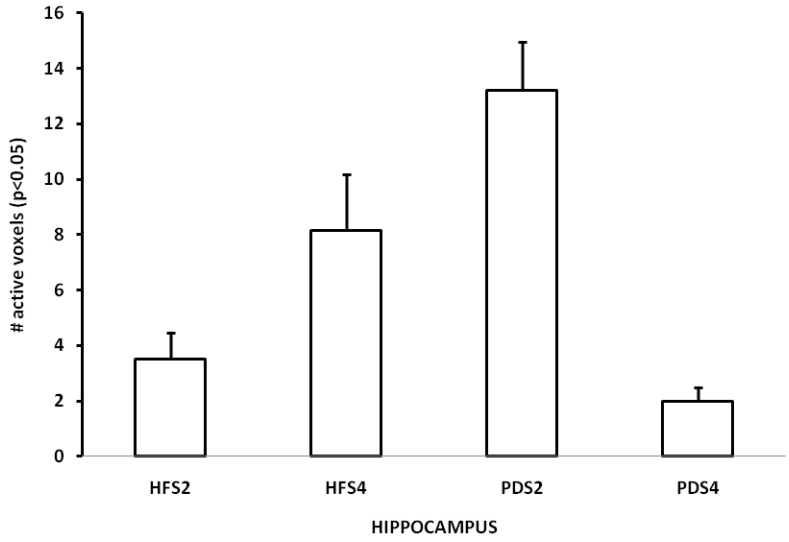

Fig. 6: Mean (±SEM) number of significantly (p<0.05; Z-score < -1.96) active voxels (volume of hypoperfused brain) during four different stimulation paradigms for the whole hippocampal formation.

Subtraction analysis in the rats (n=6) subjected to PDS2 with four stimulation amplitudes, revealed a linear increase (R^2=0.983) in number of significant voxels at the contralateral hippocampal formation with increasing stimulation intensities (Fig. 7). Additionally, a linear decrease (R^2=0.954) was observed in the number of significant voxels at the ipsilateral hippocampal formation with increasing stimulation intensities. For the entire hippocampal structure, the total number of significant voxels was not different between all stimulation amplitudes.

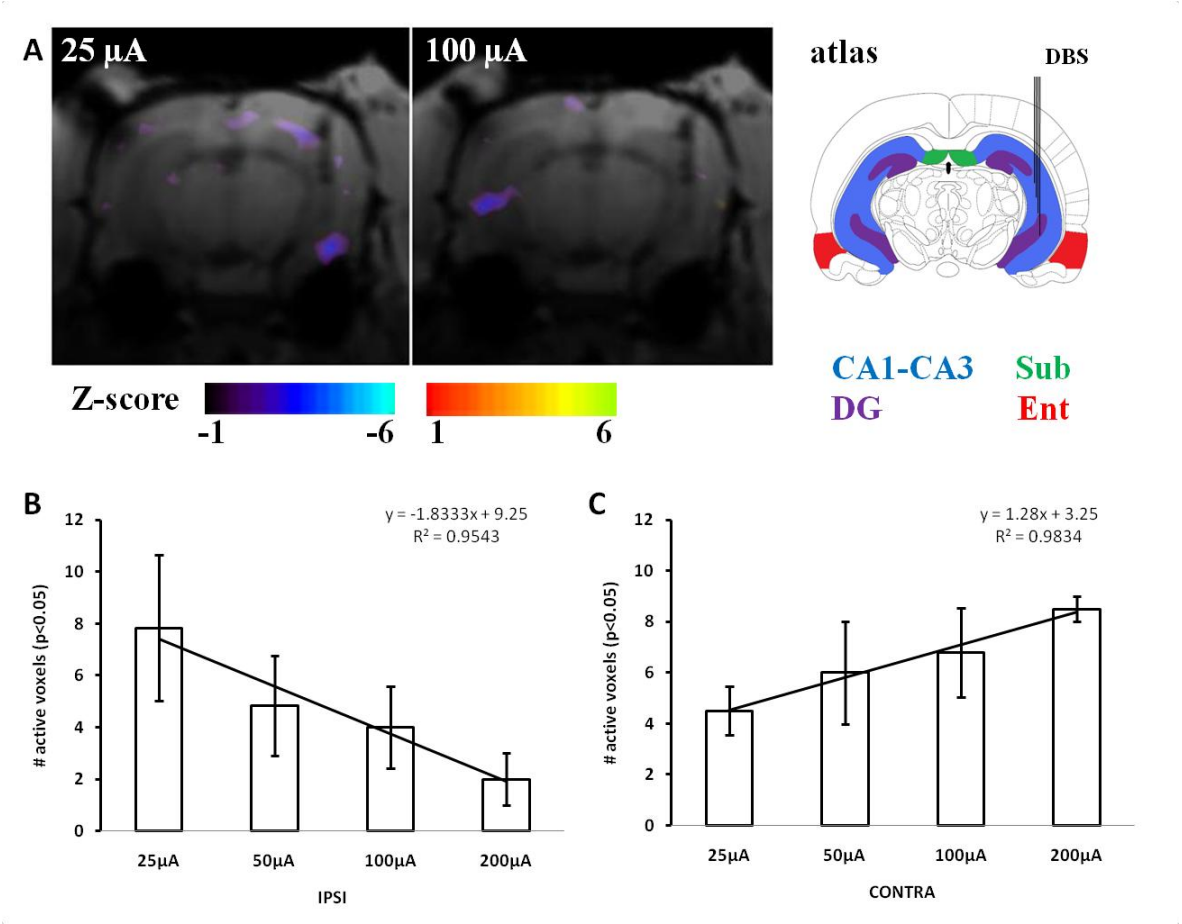

Fig 7: A) Difference in spatial extent of hypoperfusion within the hippocampal formation during PDS2 with different stimulation intensities: 25µA reveals mainly ipsilateral hypoperfusion, 100µA reveals more contralateral. B-C) Number of significantly (Z-score < -1.96) active voxels (volume of hypoperfused brain) during PDS2 with four different stimulation amplitudes for both the ipsilateral (B) and contralateral hippocampus (C). When the number of active voxels for all stimulation amplitudes, and experimental animals is plotted against the current intensity, a clear linear relationship is observed for both the ipsi- and contralateral hippocampus.

DISCUSSION

In this study we demonstrated that unilateral hippocampal DBS caused a significant decrease in relative rCBF, both in the ipsi- (at the side of the implanted electrode) and contralateral hippocampus. Different stimulation paradigms (High Frequency Stimulation and Poisson Distributed Stimulation, both on two and four electrode tips) were evaluated and revealed a clear distinction in spatial extent and intensity of hypoperfusion between the different stimulation paradigms. Bipolar Poisson Distributed Stimulation (PDS2) induced the most intense and widespread hypoperfusion in comparison with the other stimulation paradigms. Next, for PDS2 various current stimulation amplitudes were evaluated. With lower current intensities, relative hypoperfusion was restricted to the hippocampal formation ipsilateral to the electrode tip while with increasing current intensities, more widespread (contralateral) hippocampal tissue was being hypoperfused. The total number of active voxels in the ipsilateral hippocampus decreased with increasing amplitude. In addition, the minimal intensity of hypoperfusion in the hippocampal formation remained constant throughout the different stimulation amplitudes.

Brain perfusion scintigraphy and subtraction analysis using SPECT provides a well-established means of studying changes in rCBF flow in vivo. Intravenously injected Tc^{99m} HMPAO distributes rapidly (2-10 minutes) within the brain, representing perfusion at the time of injection and is assumed to reflect neuronal and interneuronal activity downstream from cell bodies and in distant input pathways (Hershey and Mink, 2006). Therefore, SPECT-measured rCBF changes in both the ipsi- and contralateral hippocampal formation due to DBS can indicate a change in either input to or output from that region or alterations in the local interneuronal activity that is provoked by DBS.

SPECT scanners have been successfully miniaturized to enter the preclinical arena allowing for a high spatial resolution with an acceptable sensitivity in rats and mice. State-of-the-art systems, such as the U-SPECT-II used here achieve resolutions well below 1 mm in rats and down to 350 µm in whole body mice imaging. Further instrumentation research was performed (Beekman et al., 2008) the past two years and a first commercial version of a dedicated rat brain collimator was recently purchased by our group. The performance of this new collimator will first be evaluated with a dedicated physical rat brain phantom (Beekman et al., 2009). We will also study the use of rapid prototyping to modify these physical phantoms to conditions where hypoperfusion is present. A successful deployment of such a dedicated rat brain collimator will allow us to increase the sensitivity versus the specificity of our future experiments by exploiting the enhanced spatial resolution.

The current study revealed predominantly decreases in rCBF in the hippocampal formation due to application of hippocampal DBS. This significant reduction in rCBF is assumed to reflect reductions in local neuronal metabolism and are thus correlates of reduced activity induced by DBS, be it excitatory or inhibitory (Pereira et al., 2007). It is well-established that DBS acts by functional inhibition of the targeted brain region. For the treatment of Parkinson's Disease (PD), stimulation of the subthalamic nucleus (STN) produces clinical effects similar to ablative lesioning (Benabid et al., 1996). Similarly, hippocampal DBS successfully suppresses seizure activity generated in the limbic system both in patients with drug resistant temporal lobe epilepsy as in animal models for TLE (Boon et al., 2007; Velasco et al., 2000a; Velasco et al., 2001; Velasco et al., 2000b; Vonck et al., 2002; Wyckhuys et al., 2007). The exact mechanism of action of DBS is unknown, but the current findings provide important additional evidence that DBS leads to a decreased neuronal activity in the stimulated structure. Active inhibition (e.g. Long-Term Depression) is an energy demanding process and would require increases in rCBF (Ackermann et al., 1984), excluding these processes as possible mechanisms underlying DBS's mode of action. As a possible mechanism of action of DBS, we suggest a role for homeostatic scaling of membrane excitability and/or synaptic strength. Neurons selectively adjust the magnitudes of their functional intrinsic currents (van Welie et al., 2004) and/or modulate their synaptic strength (Turrigiano and Nelson, 2004) in response to the overall level of synaptic activity (Beck and Yaari, 2008). High synaptic activity, as induced by application of DBS, would then lead to downscaling of neuronal activity (van Welie et al., 2004). Consequently, DBS may induce decreases in rCBF in the stimulated structure. Our study design did not permit to evaluate whether one hour of DBS has long-lasting effects on hippocampal functioning. Further studies are needed to determine the mechanisms underlying the effect of DBS and the long-lasting effects following DBS.

Hippocampal DBS is successfully used in the treatment of drug resistant temporal lobe epilepsy (Boon et al., 2007; Velasco et al., 2000a; Velasco et al., 2000b; Vonck et al., 2005). A characteristic feature of temporal lobe seizures is an initial hyperperfusion in the epileptogenic region during the early phase of a seizure visualized during ictal SPECT (Blumenfeld et al., 2009; Chang et al., 2002; Van Paesschen et al., 2003), reflecting the higher neuronal activity during onset. Chronic application of DBS causes the opposite (decreased perfusion in the hippocampal formation), providing evidence that μSPECT may be a possible tool to visualize and evaluate the anti-epileptic properties of this experimental treatment option.

During application of DBS, significant hypoperfusion is seen in all hippocampal structures, both at the ipsilateral and at the contralateral side: CA1, CA2, CA3, dentate gyrus, subiculum and entorhinal cortex. All the regions highlighted in the present study are tightly connected to one another, with

reciprocal connections existing among the entorhinal cortex, amygdala and hippocampus (Beckstead, 1978; Rosene and Vanhoesen, 1977; Witter et al., 1989; Wyss, 1981). Commissural connections (hippocampal or anterior) join the homotopic structures in the two hemispheres. Other brain regions than the hippocampal formation occasionally displayed signal changes during hippocampal DBS but the presence and directions of changes in these areas were never consistent. Occasional changes in distinct brain regions can be caused by slight differences in animal handling or sensory input received by the rat (eg. whiskers are stimulated or not) during or immediately after injection of the tracer (Shimoji et al., 2003).

Despite the promising results of hippocampal DBS in both experimental animals and TLE patients on the suppression of epileptic activity, the optimal stimulation parameters are undetermined, hampering its therapeutic potential. Therefore, in a first part of our study, we systematically investigated the effects of four stimulation paradigms on the generation of functional maps and its quantitative value. Both bipolar and quadripolar HFS and PDS were delivered. Bipolar PDS induced the most intense and widespread hypoperfusion. In a previous animal study, bipolar HFS and PDS were delivered in kainate treated animals with spontaneous seizures (Wyckhuys et al. - unpublished data). Hippocampal bipolar PDS was found to suppress epileptic seizures more effectively than bipolar HFS. These findings may possibly indicate a correlation between the potential of a stimulation paradigm to induce hypoperfusion and its seizure suppressive potential. However, this correlation needs to be further investigated experimentally.

In a second part of our study, four different current intensities (25µA, 50µA, 100µA and 200µA) during bipolar PDS were evaluated. Low current intensity only lead to hypoperfusion of the hippocampal formation ipsilaterally from the DBS electrode. With increasing stimulation amplitudes, more contralateral hippocampal tissue was being hypoperfused. The intensity of rCBF change (the minimal Z-score) remained unaltered for all stimulation amplitudes, the spatial extent (the total volume of hypoperfused tissue) shifted contralaterally with higher stimulation intensities. The linear relationship between the current intensity used to stimulate the tissue and the extension of the induced rCBF-changes shows that higher current intensities lead to larger volumes being stimulated (McIntyre et al., 2004). The effect of DBS on the contralateral side is probably mediated by commissural fibers. These fibers are activated either directly, or via activation of the corresponding neurons. Electrophysiological measurements, such as multi-unit activity and local field potential recordings in both the ipsi- and contralateral hippocampal formation during delivery of DBS would be interesting in determining the underlying mechanisms of the effects observed in our experiment.

In a similar animal experimental study, stimulation with different current intensities and stimulation frequencies in the perforant path was systematically evaluated using fMRI (Canals et al., 2008). Canals et. al also observed a linear relationship between current intensity delivered to the perforant path and spatial extension of the induced BOLD signal. Additionally, they found a frequency-dependent spatial pattern of activation. No different stimulation paradigms beside the different stimulation frequencies were evaluated. This study confirms the potential of small animal neuro-imaging techniques to evaluate DBS paradigms and –amplitudes.

Only a few animal studies have used neuro-imaging techniques to study the effect of DBS, but several human studies are available. Recent subtraction SPECT analysis of on-DBS versus off-DBS in patients with dystonia confirms decreased cerebral blood flow in the frontal lobes, the pre-motor and supplementary motor cortex due to DBS, suggesting the reduction of the hyperactivity causing the clinical symptoms (Katsakiori et al., 2009). In a case-study with pallidal DBS for tardive dyskinesia, a decrease in rCBF was also reported during on-DBS versus off-DBS (Kefalopoulou et al., 2009). Clinical improvement of this patient with bilateral pallidal DBS implies a possible correlation between brain functional imaging findings and the clinical response to DBS. Further, STN-DBS in PD patients revealed bilateral rCBF decrements in motor cortical areas and prefrontal cortex bilaterally compared to presurgical condition as well as compared to PD controls (Cilia et al., 2009). However, the design of the study does not allow to discriminate whether the hypoperfusion is due to STN-DBS, implantation of the DBS electrode or both, or the reduction in medication doses or the effect of the reduction in tremor and motor effects. In our animal study, these confounding factors are ruled out by the fact that all scans were taken after electrode implantation. Furthermore, our study visualizes the 'pure' effect of DBS on brain tissue, and is not biased by the seizure suppressive effects of DBS, as healthy rats are used. The observation of Cilia et al. that STN-DBS leads to reduced rCBF of bilateral motor areas has been an issue of discussion, as other more recent clinical studies have observed increases in rCBF due to STN-DBS rather than decreases of cortical and subcortical areas with STN-DBS.(Hill et al. 2013; Paschali et al. 2013). This discrepancy between these results and the observed hypoperfusion following µSPECT with hippocampal DBS in our study corroborates the idea that hippocampal DBS for the treatment of TLE acts through different mechansims as STN-DBS for the treatment of Parkinsons' disease.

In conclusion, subtraction analysis using high-resolution small animal SPECT reveals that hippocampal DBS induces hypoperfusion both in the ipsi- and contralateral hippocampal formation. Differences in spatial extent and intensity of rCBF changes enables us to discriminate between different stimulation paradigms and –amplitudes. These findings may provide important mechanistic insights into the mechanism of action of DBS and promotes further research on stimulation parameters for DBS using µSPECT in rats.

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Chapter 8

General discussion and conclusions

General discussion and conclusions

In this thesis we have demonstrated that:

- Bilateral hippocampal DBS with independent stimulation pulses in both hippocampi results in higher responder rate compared to unilateral hippocampal DBS in a rat model for TLE. The responder rate during bilateral hippocampal DBS is 7/11 (64%), whereas during unilateral hippocampal DBS only 4/11 (36%) rats respond to the treatment.

- Long-term continuous unilateral hippocampal DBS treatment started 24h after the start of a self-sustained SE affects seizure rate progression in a rat model for TLE, but does not induce long lasting changes in the underlying pathophysiological processes of epileptogenesis. This study undermines the hypothesis that seizures beget seizures.

- In a rat model for TLE unilateral hippocampal DBS induces hypoperfusion both in the ipsi- (i.e. the side of the implanted electrode) and contralateral hippocampal formation. Differences in spatial extent and intensity of rCBF changes enable us to discriminate between different stimulation paradigms and –amplitudes. Low current intensity leads to hypoperfusion of the hippocampal formation ipsilaterally to the DBS electrode only. With increasing stimulation amplitudes, a larger amount of contralateral hippocampal tissue was affected.

Treatment with hippocampal DBS has led to remarkable results in the treatment of drug resistant epilepsy patients, ranging in clinical trials from a moderate reduction in seizure rate of 15% (Tellez-Zenteno et al. 2006) up to a >50% reduction in seizure rate in all treated patients, and even seizure freedom in 50% of some patient series (Velasco et al. 2007). Much of what we know about the MOA of hippocampal DBS, the optimal seizure suppressing stimulation parameters and the neuromodulatory properties of hippocampal DBS on disease progression in TLE comes from empirical experiments on *in vitro* tissue, laboratory animals, or limited numbers of clinical trials.

The systemic repeated low dose KA post SE model was used in this study as the most appropriate animal model displaying histopathological features all stages of human TLE. The post SE model obtained with repeated low-doses of KA injections has a low mortality and results into the occurrence of spontaneous recurrent seizures in all surviving rats after a stable silent period of about 1 week. All rats experienced an exponential increase in seizure rate during the 30 week long EEG

monitoring period and reached a plateau in 8 out 9 rats after 122 ± 9 days. Disadvantages of the model may be the variability in final individual seizure rate and the lack of correlation between SE, seizure rate progression and histopathological findings. But as for all animal models differences between the human and animal situation should be kept in mind when translating data obtained in animal models to the human situation.

Stimulation parameters for hippocampal DBS

One of the goals of this thesis was to tailor the currently used stimulation parameters to the treatment of TLE. Currently available stimulators in the clinic only allow limited programming of stimulation parameters. The use of in house developed programmable stimulators allowed used to explore the effect of rationally choosen experimental stimulation parameters, without technical constraint of possible stimulation parameter. Several stimulation parameters and locations have been explored for the treatment of drug resistant epilepsy with hippocampal DBS. In this section we will focus on the stimulation strategies used to stimulate the hippocampus. Several combinations of stimulation parameters like stimulation frequency, pulse duration, waveform of the pulses, stimulation intensity, interpulse interval and location of stimulation within the hippocampus have been investigated. Up to now there is no consensus on which stimulation parameters or stimulation location would be the most efficient in suppressing seizure activity. In preclinical and clinical trials on hippocampal DBS stimulation frequencies range from low frequency stimulation (LFS) of 1Hz (Velisek et al. 2002; Weiss et al. 1995) up to high frequency stimulation (HFS) of 200Hz (Feng et al. 2014; Vonck et al. 2002). Both short pulse duration of 60 µs (Wyckhuys et al. 2007), long pulse duration of 450 µs (Velasco et al. 2007; Vonck et al. 2013) and pseudo monophasic pulses have been explored (Tyrand et al. 2012). Sinusoidal shaped stimulation pulses have been used in animal experiments (Carrington et al. 2007; Goodman et al. 2005) and square wave pulses (Velisek et al. 2002; Wyckhuys et al. 2010a; Wyckhuys et al. 2010b). Stimulation paradigms with fixed interpulse intervals have been mostly used. Previous research at LCEN3 showed that stimulation of the hippocampus with Poisson distributed interpulse intervals resulted in better seizure control compared to stimulation with fixed interpulse intervals (Wyckhuys et al. 2010b). Like the many stimulation parameters that have been investigated, various structures such as the perforant path (Bragin et al. 2002), ventral hippocampal commissure (Rashid et al. 2012), amygdala (Ullal et al. 1989), the kindling focus (Cuellar-Herrera et al. 2006; Goodman et al. 2005) and the hippocampal formation itself (Wyckhuys et al. 2007; Wyckhuys et al. 2010b) have been targeted. All of these strategies have been reported to suppress epileptiform

activity although there are only few studies that have systematically compared different stimulation parameters.

More research is needed to address this issue of optimizing hippocampal deep brain stimulation. Evaluating the seizure suppressive effect of the many possible combinations of stimulation parameters in rat model for TLE is labor-intensive and hampers progress in the field of optimizing hippocampal DBS stimulation parameters for the treatment of drug resistant epilepsy. Therefore, a biomarker for effective stimulation parameters should be developed. Since the mechanism of action of hippocampal DBS is largely unknown, rational development of effective hippocampal DBS parameters is difficult**.** A characteristic feature of temporal lobe seizures is an initial hyperperfusion in the epileptogenic region during the early phase of a seizure visualized during ictal SPECT (Blumenfeld et al. 2009; Chang et al. 2002; Van Paesschen et al. 2003), reflecting the higher neuronal activity during seizure onset. Our µSPECT study shows that chronic application of DBS causes the opposite (decreased perfusion in the hippocampal formation), providing evidence that µSPECT may be a possible tool to visualize and evaluate the anti-epileptic properties of hippocampal DBS parameters. Hippocampal bipolar 130 Hz Poisson distributed stimulation was found to suppress epileptic seizures more effectively than bipolar 130 Hz stimulation with fixed interpulse intervals (Wyckhuys et al. 2010a). In our µSPECT study we observed that 130 Hz Poisson distributed stimulation caused significantly stronger hypoperfusion in the hippocampal formation compared to bipolar 130 Hz stimulation with fixed interpulse intervals (Wyckhuys et al. 2010b). These findings may possibly indicate a correlation between the potential of a stimulation paradigm to induce hypoperfusion and its seizure suppressive potential. Other experimental animal imaging techniques have shown similar potential to visualize the effects of hippocampal DBS on neuronal activity (Canals et al. 2008; Van Den Berge et al. 2014). Between these changes observed by neuro-imaging in regional cerebral blood flow, blood-oxygen-dependent (BOLD) signal, glucose metabolism, and the effectiveness of stimulation parameters on suppressing seizure activity, the relationship needs to be further investigated experimentally.

Studies systematically comparing stimulation parameters mainly focused on stimulation frequency.

In human TLE patients, Boëx et al. observed that LFS (5Hz) of the amygdala-hippocampal complex increased the epileptogenic interictal activity in 2 out of 3 patients (Boex et al. 2007), while Chkhenkeli et al. reported that LFS of 1-3Hz in the mesiobasal temporal lobe foci suppressed interictal discharges, whereas 5-20Hz stimulation failed to do this (Chkhenkeli et al. 2004). Because of obvious ethical considerations and the heterogeneity of patients subjected to hippocampal DBS, most research on improving therapeutic hippocampal DBS parameters have been conducted in animal models. Wyckhuys et al. showed that HFS (130Hz) is more efficient compared to LFS (5Hz) in reducing evoked seizure activity in the kindling rat model (Wyckhuys et al. 2010a). Shigeto et al.

demonstrated that the capacity of LFS and HFS to evoke epileptiform activity is highest with frequencies between 50Hz and 100Hz and there was no difference between irregular and regular stimulation (Shigeto et al. 2013). Da Silva et al showed that unilateral HFS (130Hz) does not give rise to recruiting responses whereas LFS (6Hz) gave rise to bilateral recruiting responses(da Silva et al. 2013). They interpreted this by stating that high-frequency stimulation was more likely to be effective than low-frequency stimulation regarding the potential inactivation of the hippocampus. These observed intra-operative recruiting responses during hippocampal LFS were also observed in TLE patients albeit unilaterally (Cukiert et al. 2011). These results are in accordance with the observations that high frequency hippocampal DBS can block axonal conduction (Feng et al. 2014; Jensen and Durand 2009). A recent study in the systemic pilocarpine model and focal hippocampal seizure model showed that LFS (<13Hz) in the perforant path was inefficient in terminating ongoing seizures, while HFS (30-250 Hz) could terminate a fraction of epileptic seizures. The most efficient stimulation frequency in terminating seizures was 130 Hz (Cymerblit-Sabba et al. 2013). It has been shown that HFS of 130Hz delivered with a more irregular interpulse interval (Poisson distributed) was as efficient in suppressing seizures compared to HFS delivered with fixed interpulse intervals, but lower stimulation intensities were needed (Wyckhuys et al. 2010b). Cymerblit-Sabba showed that asynchronous stimulation markedly enhanced the antiepileptic effect of neurostimulation. Asynchronous stimulation applied simultaneously in two electrodes more than doubled the fraction of seizures terminated by stimulation and significantly decreased the average duration of seizure in the systemic pilocarpine model and focal hippocampal seizure model (Cymerblit-Sabba et al. 2013). These results provided us with the hypothesis explored in this thesis that stimulation parameters inducing asynchrony within epileptic networks might induce a higher reduction in seizures.

During bilateral hippocampal DBS in our experiment, in which each hippocampus was stimulated with a separate stimulator and its own generated Poisson distribution with a mean and variance of 1/130s, 7/11 (64%) rats experiences a significant decrease in seizure rate, whereas during unilateral hippocampal DBS this was only observed in 4/11 (36%) rats. The anatomic differences between rats and humans should be kept in mind when translating our results to the human situation. In rats, there are strong connections between both hippocampi along the full length of their axis (Laurberg 1979; Swanson 1977). These pathways are vestigial in primates and humans, and are an important difference between the rat brain and the human brain. Furthermore, the rodent brain is composed of fewer neurons than the human brain while the average number of synapses in a rodent brain and a human brain are approximately similar, which reflects a higher connectivity in rodents brains (DeFelipe et al. 2002). The bilateral hippocampal stimulation protocol used in our study in rats may more closely resemble the situation of stimulating more than one region within one hippocampus in humans than stimulating the left and right hippocampus in patients. We hypothesize that multisite

stimulation with independent stimulation patterns in strongly interconnected epileptic networks is more potent at disrupting seizure activity by desynchronizing network activity. Multisite uncorrelated stimulation subdivides neuronal networks into separate desynchronized sub-networks that fire at different times. This subdivision of the epileptic network opposes the development of wide spread hyper-synchronous seizure activity (Cymerblit-Sabba et al. 2013).

Mechanism of action (MOA) of hippocampal DBS

The basic mechanisms of deep brain stimulation are largely unknown, and results on efficacy in preclinical and clinical trials are contradictory. Multiple brain structures have been stimulated to influence laboratory models of seizures, including cerebellum, locus coeruleus, substantia nigra, caudate nucleus, hippocampus, amygdala, hypothalamus, subthalamus, centromedian thalamus, anterior nucleus of thalamus, neocortex and others. Both positive and negative results of deep brain stimulation have been reported, and clinical studies have sometimes proceeded with only limited understanding of the basic mechanisms of brain stimulation. DBS for epilepsy often has moved retrograde, from pilot clinical studies back to the laboratory for validation and modification of stimulation methods. At the level of an individual nerve fiber, electrical stimulation effects are more predictable, but predictability is generally lost when stimulating neuronal networks. The fields of distribution of current can only be estimated, and effects cannot be classified simply as excitatory or inhibitory. Stimulation may have one effect near the electrode and an opposite or different effect at more distant locations (Graber and Fisher 2012). High frequency hippocampal DBS is hypothesized to exert its effect through either a microlesioning effect, local functional inhibition of structures involved in seizure generation or seizure spread or the mechanism of action of DBS maybe related to the effect on projections leaving from the stimulation area to other structures and thereby suppressing neuronal excitability of widespread networks. The underlying mechanisms of this HFS induced inhibition are unknown and many hypotheses exist. These mechanisms do not exclude each other, and the final effect of hippocampal DBS may be achieved by an interaction of different investigated and uninvestigated mechanisms.

The presence of a microlesioning effect independent of the applied electrical stimuli (Vonck et al. 2005) is supported by the observation of prolonged seizure control in patients who underwent invasive recording with conventional electrodes (Katariwala et al. 2001). This theory is however weakened by several studies showing a clear difference between the seizure control during stimulation ON and stimulation OFF condition (Velasco et al. 2007; Wyckhuys et al. 2007; Wyckhuys et al. 2010b). We also observed a clear ON-OFF effect during 10 day long unilateral or bilateral hippocampal DBS. The observed seizure suppressive effect of both unilateral and bilateral

hippocampal DBS was achieved immediately from the first day of stimulation onwards and disappeared the first day after switching of stimulation. These observations are supported by the results of the long-term hippocampal DBS experiment discussed in this thesis in which we observe that the probability for a seizure to occur was affected in 7/9 rats during long-term hippocampal DBS as long as stimulation is continued. After stimulation is stopped, the probability to have a seizure is similar among rats in the treatment group and in the control group. The observed hypoperfusion in limbic structures during the stimulation ON conditions compared to the stimulation OFF condition in our neuroimaging study shows that it is the stimulation itself that causes the observed hypoperfusion. Although a microlesioning effect may contribute to the seizure suppressive effect of hippocampal DBS, our results suggest that the seizure suppressive effect of hippocampal DBS is not only due to the microlesioning effect. Some experiments showed evidence of a local inhibition surrounding the stimulation electrode. Electrical stimulation in a hippocampal slice produced increase of extracellular potassium, a negative DC shift, depolarization block of sodium channels and inhibition of penicillin-induced bursting and picrotoxin induced epileptiform activity. The suppression generated by the monopolar electrode was localized to a region surrounding the stimulation electrode (Bikson et al. 2001; Lian et al. 2003). This in contrast with other experiments that showed more widespread inhibition of neuronal networks caused by hippocampal DBS.

The µSPECT study in this thesis supports the hypothesis of a more widespread effect on limbic structures caused by local stimulation. Unilateral hippocampal DBS causes significant hypoperfusion in all limbic structures, ipsi- and contralateral to the stimulation side. Ramping up of the stimulation intensity results in a shift of the hypoperfusion to the contralateral side. These observations of a widespread involvement of limbic structures caused by hippocampal DBS are supported by the observed reduced glucose metabolism in all limbic structures caused by unilateral hippocampal DBS (Van Den Berge et al. 2014). Furthermore, it has been shown that local hippocampal DBS suppresses penicillin-induced cortical epileptic activity in a rat model for focal neocortical epilepsy (Akman et al. 2011). However, this widespread inhibition of neuronal structures caused by local hippocampal stimulation in rats may be the result of more strongly interconnected neuronal networks compared to in the human brain (DeFelipe et al. 2002; Laurberg 1979; Swanson 1977), and may not necessarily represent the human situation. Inhibition of neuronal network excitability was nicely investigated in a large sheep model with an implanted DBS system. Here it was shown that local field potentials (LFPs) in the hippocampus induced by a stimulation train in the thalamus were suppressed during hippocampal DBS. The suppression of hippocampal LFPs produced by direct hippocampal stimulation reflects a state of reduced excitability. When afterdischarges (ADs) were generated during this state of reduced excitability shorter ADs were observed (Stypulkowski et al. 2013; Stypulkowski et al. 2014).

Many underlying mechanisms may be responsible for local or network inhibition caused by high frequency stimulation. The experiments in this thesis have clearly shown that asynchronous bilateral stimulation parameters are more efficient in suppressing seizure compared to unilateral stimulation parameters, that hippocampal DBS is able to change the probability for a seizure to occur as long as stimulation is sustained, and local stimulation results in network-wide inhibition. These main experimental findings support the following hypothesis on the mechanism of action of hippocampal DBS for the treatment of TLE.

In line with the results observed in our experiments that bilateral DBS, delivered to each hippocampus with a separate stimulator and its own generated Poisson distribution with a mean and variance of 1/130s, it has been shown that simultaneous stimulation with different stimulation frequencies is more potent in disrupting seizure activity (Cymerblit-Sabba et al. 2013) and seizure control can be correlated with desynchronization of brain dynamics (Good et al. 2009). This hippocampal DBS induced desynchronization of brain activity has also been shown in patients (Tyrand et al. 2014). In Parkinsons' disease, the symptoms of the disease result from the loss of dopamine-secreting cells in the substantia nigra. The abnormal motor activity is generated due to increased neuronal synchronization and low-frequency rhythmic oscillations within the basal ganglia and thalamus (McIntyre et al. 2004a). A possible mechanism of action of the regular DBS patterns used in the treatment of Parkinsons' disease is that regular high-frequency stimulation overrides the pathological low-frequency oscillatory activity and replaces it with tonic high frequency output (McIntyre et al. 2004b). In epilepsy however, the symptoms of the disease result from hypersynchronous activity of neurons. Just as regular high frequency stimulation may override the pathologic activity in Parkinsons' disease, stimulation parameters inducing desynchronisation of hypersynchronous neuronal networks may subdivide neuronal networks into separate desynchronized sub-networks that fire at different times. This subdivision of the epileptic network opposes the development of wide spread hyper-synchronous seizure activity.

This hypothesis of subdividing the epileptic network with HFS is in line with the findings of Jensen et al. that HFS blocks axonal conduction (Jensen and Durand 2009) and consequently can induce functional disconnection of structures within a neuronal network (Feng et al. 2013). The mechanisms underlying the HFS induced axonal block are unknown, but may be related to a stimulation induced extension of the refractory period of neurons (Feng et al. 2014).

Another possible mechanism by which hippocampal DBS might exert its effect in the treatment of epilepsy is that DBS increases the threshold for seizure initiation. In this view, the continuously delivered stimuli that are subthreshold to evoke seizure activity to the neuronal network subjects the neuronal network to continuous strong synaptic input. As a consequence of this strong synaptic input homeostatic plasticity mechanisms are activated in response to this increased synaptic input to

compensate for the increased synaptic input and keep their firing rates within the functional range (van Welie et al. 2004). In this way hippocampal DBS shifts the neuronal network to a less excitable state as long as the increased subthreshold synaptic input is maintained. In this less excitable state, a trigger that in the absence of hippocampal DBS would provoke a seizure will not be perceived as such by the neurons. This hypothesis is in line with the ON-OFF effect that is observed with hippocampal DBS in all studies in this thesis.

Neuromodulatory properties of hippocampal DBS

Neuromodulation is defined by the international neuromodulation society as *'the alteration of nerve and brain activity through the delivery of electrical stimulation or chemical agents to targeted sites of the body'*. The idea of neuromodulation stems from its initial direction of a reversible alteration of the nervous system. It is the idea of neural "modulation" as opposed to "ablative" or resective procedures. Treatments are reversible and have the ability to be turned off. The domain of neuromodulation encompasses many neurological disorders such as acute and chronic pain syndromes, movement disorders, dystonia and spasticity, and epilepsy. Information processing in the brain is regulated be means of excitatory and inhibitory postsynaptic potentials, and this information is coded as sequences of action potentials. Similar to the way a cardiac pacemaker or defibrillator corrects heartbeat abnormalities, the goal of neuromodulation therapies is to re-establish normal neural balance and hereby eradicating or suppressing the symptoms associated to the disrupted brain activity. Because neuromodulation therapies are targeted, they can avoid side effects associated with more systemic or irreversible treatments of nervous system disorders. And being easily reversible, they can provide an important degree of therapeutic control for patients and physicians. Long-term follow-up in patients treated with hippocampal DBS has shown an improved response, with longer treatment periods (Velasco et al. 2007; Vonck et al. 2013). This has also been observed in the long-term follow-up of two large multi-center trials on ANT-DBS (SANTE) and responsive focal stimulation (NeuroPace), respectively (Fisher et al. 2010; Heck et al. 2014). This suggests that deep brain stimulation induces changes in the neuronal networks that are continuously subjected to the stimulation and could hereby modify the 'epileptic state'. It is unknown whether these changes could persist when the treatment is stopped and whether long-term treatment with hippocampal DBS could affect the underlying neuropathological features of TLE. Our results show that long-term continuous unilateral hippocampal DBS treatment started 24h after the start of a self-sustained SE affects seizure rate progression in a rat model for TLE, but does not induce long lasting changes in the underlying pathophysiological processes of epileptogenesis. During stimulation long-term hippocampal DBS seems to affect the probability for a seizure to occur. Immediately after

stimulation is stopped, the probability to have a seizure is similar among rats in the treatment group and in the control group. This reversible ON-OFF effect of hippocampal DBS was also observed during the both 10 days of unilateral and 10 days of bilateral hippocampal DBS. This was in accordance with a previous study on high frequency hippocampal DBS in the KA rat model (Wyckhuys et al. 2010b). Immediate increases in seizure rate after hippocampal DBS discontinuation has been reported in patients (Tellez-Zenteno et al. 2006; Velasco et al. 2007). Despite the positive observed neuromodulatory and symptom modifying effects of long-term hippocampal DBS, the observation of 33% of adverse responders with a highly increased probability for seizures during stimulation shows that due to our limited knowledge of the mechanism of action and optimal stimulation parameters of hippocampal DBS caution should be used when starting treatment with long-term hippocampal DBS. Patients experiencing increases in seizure rate during stimulation up to 50% are described in open label patient studies evaluating the effect of hippocampal DBS (Cukiert et al. 2014; Tellez-Zenteno et al. 2006). The presence of adverse responders are in accordance with the observation that repeated electrical stimulation of the brain in certain experimental conditions can produce kindling-like effects in humans (McIntyre and Gilby 2009) which manifests as the new emergence or the increased duration, severity and frequency of seizures. Differences in response to the hippocampal DBS treatment may be due to small differences in electrode position (Bondallaz et al. 2013) or electrode orientation (Anderson et al. 2007) within the hippocampus. Future experiments should be aimed at unraveling the mechanisms by which hippocampal DBS exerts its neuromodulatory effects on neuronal tissue and by doing so optimizing the used stimulation parameters to avoid the adverse DBS response and modulate epileptic networks in such a manner that optimal seizure control is achieved during stimulation. Our results indicate that during hippocampal deep brain stimulation the probability to have a seizure is reduced. As long as stimulation is maintained seizure rate in is affected a rat model for TLE, without affecting the histopathological features of TLE. Once stimulation is stopped no further effect on seizure rate can be observed. These observations question the controversial theory postulated by Gowers in 1881 that “seizures beget seizures”.

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Chapter 9

Future perspectives

Future perspectives

The long-term goal of the research presented in this thesis is to further develop hippocampal deep brain stimulation to be applied in drug resistant TLE patients with high efficiency and, if possible, to render them seizure free. We tried to contribute to these goals by optimizing hippocampal DBS parameters in a rodent model to achieve better seizure control, and by gaining insight in the mechanism of action of hippocampal DBS and the neuromodulatory properties of hippocampal DBS on disease progression in TLE which may result in a more rational approach to determine optimal stimulation parameters and optimal seizure control. The experiments described in this thesis have provided some answers and have created many new questions. In the following section, we will provide a short framework which could be used to further explore the results discussed in this thesis.

Stimulation parameters for hippocampal DBS

We observed that unilateral hippocampal DBS induces hypoperfusion both in the ipsi- (i.e. the side of the implanted electrode) and contralateral hippocampal formation. Hippocampal bipolar Poisson distributed stimulation was found to suppress epileptic seizures more effectively than bipolar high frequency stimulation. These findings may possibly indicate a correlation between the potential of a stimulation paradigm to induce hypoperfusion and its seizure suppressive potential. However, this correlation needs to be further investigated experimentally. To investigate this, the in vivo seizure suppressive effect of different hippocampal DBS parameters should be tested in a less labor intensive and faster animal model for TLE seizures than the KA post SE model. The electrical kindling model may provide a useful tool, as it has already been shown that hippocampal DBS parameters efficient in suppressing spontaneous seizures in the systemic KA post SE model are able to modify seizure characteristics in the kindling model as well (Wyckhuys et al. 2007). To confirm the relationship between seizure suppressive potential of stimulation parameters and induction of hypoperfusion in limbic structures, an experiment should be set up to systematically evaluate the induction of hypoperfusion of a set of experimental stimulation parameters in a group of rats. After kindling of these rats, we could then evaluate the effect of the experimental set of stimulation parameters on AD characteristics. If the relationship between hypoperfusion and the effect on ADs could be confirmed, μSPECT could be used as a fast screening tool for potent stimulation strategies.
Based on the observations made in the experiments discussed in this thesis some stimulation strategies for better seizure control can be suggested.

The observation in the μSPECT study that stimulation parameters with the most widespread hypoperfusion in limbic structures are related with efficient seizure suppression, and the observation that bilateral hippocampal DBS results in a higher amount of epileptic rats with a significant reduction in seizures compared to unilateral hippocampal DBS suggests that stimulation parameters affecting more widespread regions of the limbic structures might comprise a higher seizure suppressive potential compared to more local stimulation parameters. Increasing the region subjected to stimulation can be achieved in 2 manners. First, we can increase stimulation intensity, as higher stimulation intensity creates larger electrical field. However, this approach should be explored with caution, as high stimulation intensities induce epileptiform discharges and even seizures. Increasing the affected region with hippocampal DBS can also be done by strategically placing the electrode within the hippocampal formation. It has been shown that local stimulation of the ventral hippocampal commissure (VHC) with LFS suppresses seizures in a genetic mouse model with seizures and in a post SE rat model of TLE (Kile et al. 2010; Rashid et al. 2012). High frequency stimulation of 100Hz in an acute seizure model (4-AP model) could suppress seizure activity (Chiang et al. 2013). Applying 130 Hz stimulation in the VHC may block/suppress the axonal conduction between both hippocampi and may hereby suppress spontaneous seizures.

The better seizure control with bilateral hippocampal DBS during which each hippocampus was stimulated with a separate stimulator and its own generated Poisson distributed stimulation pattern with a mean and variance of 1/130s compared to unilateral hippocampal DBS and the results of Cymerblit-Sabba that stimulation in the perforant path with different stimulation frequencies is more potent in disrupting seizure activity compared to identical stimulation frequencies (Cymerblit-Sabba et al. 2013) suggest that better seizure control is achieved with multisite stimulation with independent stimulation patterns. An interesting stimulation strategy to investigate in future experiments might be the independent or combined stimulation of the ventral and dorsal part of the hippocampus in the rat. The ventral hippocampus is the rat's equivalent of the human anterior part of the hippocampus and is more involved with emotional regulation, whereas the dorsal hippocampus is the rat's equivalent of the posterior part of the human hippocampus which is more involved in memory functions (Fanselow and Dong 2010). Furthermore, the ventral hippocampus is more excitable compared to the dorsal hippocampus (Akaike et al. 2001; Elul 1964; Gilbert et al. 1985; Racine et al. 1977). In the open label patient trial at Ghent University hospital only the anterior part of the hippocampus is stimulated (Boon et al. 2007; Vonck et al. 2002; Vonck et al. 2013). Stimulation of the ventral and/or dorsal part of the hippocampus with a separate stimulator and its own generated Poisson distributed stimulation pattern with a mean and variance of 1/130s might cause better seizure suppression compared to currently used stimulation parameters and translation of this experimental approach might be further explored in patients.

Mechanism of action (MOA)

High frequency hippocampal DBS has been shown to suppress seizure activity both in animal models and patients with TLE (Boex et al. 2011; Boon et al. 2007; McLachlan et al. 2010; Tellez-Zenteno et al. 2006; Velasco et al. 2007; Vonck et al. 2013; Wyckhuys et al. 2010), but the mechanisms of action of deep brain stimulation are currently unknown. In our experiments we observed a clear ON-OFF effect of the treatment during both 10 day long continuous DBS and 22 week long continuous DBS. Hippocampal DBS did not affect the duration of spontaneous seizures in either of our experiments, which was in accordance with previous reports on the effect of hippocampal DBS on spontaneous seizures (Wyckhuys et al. 2010). Hippocampal DBS seems to alter the probability for a seizure to occur during stimulation by increasing a hypothetical threshold for seizure occurrence, but once a seizure occurs it remains unaffected. The observed ON-OFF effect is in accordance with the hypothesis that high frequency stimulation in the hippocampus induces axonal block by extending the refractory period of neurons (Feng et al. 2014; Jensen and Durand 2009). When HFS is stopped, this axonal block disappears when HFS is stopped and could even result in the occurrence of short epileptiform activity (Feng et al. 2014). Suppressing axonal conduction within the hippocampus causes a functional disconnection of axonal pathways that is reversible and temporary (Feng et al. 2013), which could hereby desynchronize hippocampal neuronal dynamics and disrupt seizure activity (Cymerblit-Sabba et al. 2013). This could explain our observations that bilateral hippocampal DBS in which each hippocampus is stimulated with an individual stimulator with its own generated Poisson distribution stimulation pattern with a mean and variance of 1/130s would results in better seizure control compared to unilateral hippocampal DBS, as bilateral hippocampal DBS would cause axonal conduction block in more widespread areas of the hippocampus and hereby inducing a higher degree of functional disconnection within the limbic structures resulting in more desynchronization of hippocampal neuronal dynamics. However, the appearance of adverse responders during the long-term DBS experiment cannot be explained by an effect of HFS solely on axonal conduction block. Experiments in monkeys have shown that HFS in the subthalamic nucleus (STN) for the treatment of Parkinson's disease has an excitatory effect on axons surrounding the stimulus electrode (Hashimoto et al. 2003). Although it cannot be excluded that HFS in different neuronal structures induces different effects, a reason for these apparent differences in activation could be the effective stimulus amplitudes seen by the surrounding tissue in complex neuronal structures composed of different cell types and cell structures (Butson and McIntyre 2005). This indicates that HFS can induce both excitation and inhibition at the same time dependent of the surrounding tissue. Electrode position and electrode location should be determined with great care when stimulating neuronal tissue, as differences in electrode configuration and location impacts the threshold current needed to elicit

neuronal responses (Butson and McIntyre 2005) and affects the seizure suppressive capacities of stimulation (Anderson et al. 2007; Bondallaz et al. 2013). A possible explanation for the appearance of adverse responders might be small differences in electrode location or electrode orientation, as well as unidentified differences in the intracerebral damage of the tissue surrounding the electrode. Future experiments should include *in vivo* analysis of the implanted electrode position and orientation with neuro-imaging techniques to evaluate the exact position of the stimulation electrode within the hippocampus and identify possible differences in intracerebral damage. Studies employing multi-site evoked potential and/or multi-unit recording within the normal hippocampal networks and epileptic hippocampal networks in awake, freely moving rats are necessary to clarify the role of excitation and inhibition in therapeutic stimulation.

Neuromodulatory properties of hippocampal DBS

In the long-term hippocampal DBS experiment, we clearly showed that seizure rate progression can be modulated by delivering continuous hippocampal DBS pulses to the hippocampal tissue. In the previous section we pointed out the need to evaluate how hippocampal DBS affects neuronal excitability. An interesting research option, to optimize the neuromodulatory properties of hippocampal DBS and avoid the adverse DBS responders we observed, would be to monitor excitability of hippocampal neuronal networks throughout the disease progression in freely moving KA treated rats by means of measuring perforant path stimulation induced dentate gyrus evoked potentials and monitor the changes in EP characteristics throughout this process of developing spontaneous seizures. Long-term hippocampal DBS parameters should in a later study be set and adjusted to counteract the observed changes in EP characteristics. This could potentially lead to optimal suppression of the increasing neuronal excitability during epileptogenesis and hereby suppressing the exponential increase in seizures.

Other interesting future research questions

The effect of hippocampal DBS on cognitive functions

Since the hippocampus is the most involved brain structure in the process of memory and learning (El-Falougy and Benuska 2006), the possible side-effects of hippocampal DBS on memory and learning should be further investigated. The available clinical data suggest that hippocampal DBS has no major effects on memory and learning (Velasco et al. 2007). Recent research has shown that rather than deteriorating or disturbing memory, hippocampal DBS might enhance short-term memory (Luna-Munguia et al. 2012). These possible effects of hippocampal DBS on memory functions should be further investigated.

Identification of DBS responders and non-responders

Responders and non-responders are described in all experiments evaluating the effect of hippocampal DBS on spontaneous seizures. This is in accordance with previous observations both in animal as in patient studies (Boex et al. 2011; Boon et al. 2007; Cukiert et al. 2014; Wyckhuys et al. 2010). In the µSPECT study, all rats experienced widespread hypoperfusion in all limbic regions. However, there were interindividual differences in the spread and intensity of hypoperfusion between rats, which may represent differences in potential seizure suppression of the treatment among different rats. Future experiments should be designed to investigate whether neuro-imaging techniques like µSPECT might provide a tool to identify those rats who will respond to hippocampal DBS with a specific set of stimulation parameters. This could later be used in patients implanted with hippocampal depth electrodes undergoing invasive presurgical evaluation to assess the potential benefit treatment with hippocampal DBS could provide.

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Summary

Epilepsy is a chronic neurological condition, characterized by spontaneous recurrent seizures. These seizures are the consequence of excessive and hyper synchronous electrical activity in the brain. Epilepsy has a high incidence with 50 to 70 new cases per 100000 individuals each year. Current treatment of epilepsy patients is typically based on administration of anti-epileptic drugs (AEDs) in order to restore the imbalance between excitation and inhibition in the brain. In patients with newly diagnosed epilepsy, 47% become seizure free under treatment with a first AED. Treatment with a second AED renders another 13% seizure free, and treatment with a third AED results in an additional 3% of the patients becoming seizure free. This leaves 30-40% of all epilepsy patients who do not respond to conventional AED treatment and are defined as drug resistant epilepsy patients. The most common form of epilepsy is temporal lobe epilepsy (TLE). In this group of patients, the percentage of drug resistant patients even increases to 75%. Systemic administration of AEDs is associated with side-effects such as sleepiness, dizziness, memory – and mood disorders, etc. Information processing in the brain is regulated by means of excitatory and inhibitory postsynaptic potentials, and this information is coded as sequences of action potentials. In this system, the neurotransmitters on which most AEDs act are only the messengers, not the message. The development of new treatments that are based on affecting innate neurophysiological mechanisms of the brain may herald superior efficacy. Furthermore, AEDs act on a timescale of minutes to hours, and cannot replicate the precise timing of electrical information processing in the brain (Montgomery 2010).

A promising, but currently experimental treatment for drug resistant epilepsy that speaks the same "electric language" is hippocampal deep brain stimulation (DBS). Hippocampal DBS is a neurostimulation strategy, where electrical pulses are delivered to hippocampal tissue through a pulse generator that is implanted under the clavicle led via subcutaneous wires to an intrahippocampally implanted electrode. Treatment with hippocampal DBS has led to remarkable results in the treatment of drug resistant epilepsy patients. About 70% of all drug resistant epilepsy patients treated with hippocampal DBS in open label and small randomized controlled trials experience a >50% seizure reduction during stimulation. Although the results of these experimental open label and small randomized controlled trials should be interpreted with caution, the therapeutic potential of hippocampal DBS may increase when the mechanism of action and the most optimal stimulation parameters are further investigated. In parallel with pharmacokinetics and – dynamics studies for drug therapy, the different variables of hippocampal DBS need to be better understood to design an efficient stimulation strategy which can be safely used in patients. The goal of this research project was to narrow this knowledge gap in the use of hippocampal DBS as a

treatment option for epilepsy patients. We investigated the mechanism of action, optimize the stimulation parameters and explore the neuromodulatory properties of hippocampal DBS.

We investigated the mechanism of action of hippocampal DBS by means of a neuro-imaging technique (μSPECT) which allowed us to investigate the changes in regional cerebral blood flow (rCBF) in a healthy brain caused by hippocampal DBS treatment. This study showed that unilateral hippocampal DBS reduced rCBF both in the ipsi- (i.e. the side of the implanted electrode) and contralateral hippocampal formation. Differences in spatial extent and intensity of rCBF changes enabled us to discriminate between different stimulation paradigms and –amplitudes. This showed that low current intensity only led to hypoperfusion of the hippocampal formation ipsilaterally from the DBS electrode, whereas with increasing stimulation amplitudes, more contralateral hippocampal tissue was being hypoperfused.

Since the ultimate goal of our translation research project was to investigate the role of hippocampal DBS in human TLE, we further investigated hippocampal DBS as a treatment for TLE in a rat model for TLE. Typically TLE is caused by an initial precipitating event such as febrile seizures, encephalitis, or SE that initiates a cascade of molecular and cellular events in the neuronal network, and eventually gives rise to a condition with spontaneous and recurrent seizures (i.e. epileptogenesis). We identified the systemic kainic acid (KA) post status epilepticus (SE) rat model for TLE as the most suited animal model as it closely mimics the latent period, the chronic nature of the epilepsy, and the behavior associated with the seizures in human TLE.

In this model, we compared the seizure suppressive potential of bilateral hippocampal DBS in which each hippocampus was stimulated with independent Poisson distributed stimulation patterns with a mean and variance of 1/130s to unilateral hippocampal DBS. This study showed that bilateral hippocampal DBS with independent stimulation pulses in both hippocampi results in a higher responder rate compared to unilateral hippocampal DBS. The responder rate during bilateral hippocampal DBS was 7/11 (64%), whereas during unilateral hippocampal DBS only 4/11 (36%) rats responded to the treatment.

Since Long-term follow up in hippocampal DBS patient trials shows increased response with longer hippocampal DBS treatment duration, long-term DBS may induce long-term changes in excitability of neuronal networks and hereby modify disease progression in TLE. To investigate the disease modifying potential of long-term treatment with hippocampal DBS we started continuous 22 week long treatment with hippocampal DBS 24 hours after the initial SE and monitored seizure rate progression with continuous EEG recordings for a period of 30 weeks. This study showed that long-term continuous unilateral hippocampal DBS treatment can modulate seizure rate progression in a rat model for TLE as long as the treatment is maintained, but does not induce long lasting changes in the underlying pathophysiological processes of epileptogenesis.

In summary, the results of this thesis are the following. Hippocampal DBS induced widespread hypoperfusion in all limbic structures supporting the hypothesis that hippocampal DBS induces network-wide inhibition, and this pattern of hypoperfusion is dependent on stimulation intensity dependent. Future experiments should be aimed at elucidating whether hippocampal DBS-induced hypoperfusion could be used as a screening tool for efficient seizure control with a given set of stimulation parameters. Secondly, stimulation of the hippocampus at more than one location with independent stimulation patterns in strongly interconnected epileptic networks is more potent in disrupting seizure activity compared to stimulation at one location within the network. Future experiments should further explore the role of desynchronizing network activity with hippocampal DBS and its role in the suppression of seizures. Finally, active stimulation during long-term treatment with hippocampal DBS can modulate symptom progression in a rat model for TLE but does not affect the underlying histopathological features of TLE. Future preclinical experiments should be aimed at unraveling the mechanisms by which hippocampal DBS exerts its neuromodulatory effects on neuronal tissue. Monitoring excitability of the DBS affected neuronal networks during long term DBS treatment with electrophysiological measures could reveal how hippocampal DBS affects excitability and subsequently symptom progression in rat models for TLE.

Samenvatting

Epilepsie is een chronische neurologische aandoening die gekenmerkt wordt door spontane herhaaldelijk optredende epileptische aanvallen. Deze epileptische aanvallen zijn het gevolg van het overdreven en hyper synchroon elektrische activiteit van zenuwcellen in de hersenen. De hoge incidentie van epilepsie met 50 tot 70 nieuwe gevallen per 100.000 individuen maakt deze aandoening de tweede meest voorkomende neurologische aandoening. De behandeling van epilepsiepatiënten gebeurt met behulp van anti-epileptische medicatie (AEM) met als doel het onevenwicht tussen excitatie en inhibitie in de hersenen te herstellen. Van alle nieuw gediagnosticeerde epilepsiepatiënten worden de epileptische aanvallen onderdrukt met behulp van een eerste anti-epilepticum in 47% van de gevallen. Behandeling met een tweede anti-epilepticum resulteert in aanvalscontrole bij een additionele 13 % van de patiënten. Bij de behandeling met een derde anti-epilepticum is de winst in aanvalsvrije patiënten slechts 3%. Uiteindelijk blijft een grote groep van 30 à 40% van de epilepsiepatiënten over die niet reageren op conventionele behandeling met AEM. Deze patiënten worden refractaire epilepsiepatiënten genoemd. De meest voorkomende vorm van epilepsie is temporale kwab epilepsie (TLE). In deze groep patiënten loopt het aantal refractaire epilepsiepatiënten op tot 75% van de patiëntpopulatie. Bovendien leidt de systemische toediening van AEM tot de blootstelling van de volledige hersenen aan deze AEM, wat aanleiding kan geven tot een waaier aan neveneffecten zoals slaperigheid, duizeligheid, geheugen-en stemmingsstoornissen enz.

Informatieverwerking in de hersenen is gereguleerd onder de vorm van excitatoire en inhibitoire postsynaptische potentialen. In dit systeem zijn de neurotransmitters waar AEM op inwerkt enkel de boodschappers en niet de boodschap zelf. Dit benadrukt de nood om nieuwe behandelingen te ontwikkelen die rechtstreeks ingrijpen op de “fout gecodeerde boodschap”.

Een veelbelovende maar op dit moment experimentele behandeling voor refractaire epilepsiepatiënten, die “spreekt in de elektrische taal van de hersenen”, is hippocampale diepe hersenstimulatie (DHS). Hippocampale DHS is een neurostimulatietechniek waarbij elektrische pulsen gegeven worden aan hippocampaal weefsel vanuit een pulsgenerator die geïmplanteerd wordt onder het sleutelbeen via subcutane bedrading tot aan een intrahippocampaal geïmplanteerde stimulatie-elektrode. De behandeling van refractaire epilepsiepatiënten met hippocampale DHS heeft reeds tot opmerkelijke resultaten geleid. Ongeveer 70% van alle refractaire patiënten behandeld met hippocampale DHS, waarover gerapporteerd wordt in open label en kleine gerandomiseerde studies met controlegroep, ervaren een reductie in aanvalsfrequentie van >50%. Alhoewel deze resultaten met de nodige voorzichtigheid dienen te worden geïnterpreteerd, reikt het therapeutisch potentieel van hippocampale DHS mogelijks verder dan wat deze veelbelovende

resultaten reeds laten vermoeden aangezien het werkingsmechanisme, de meest optimale stimulatieparameters om aanvalscontrole te verkrijgen, en de neuromodulatoire eigenschappen van DHS tot op heden onvoldoende gekend zijn.

Net zoals voor conventionele medicatie de farmacokinetiek en –dynamiek dienen gekend te zijn, moeten de verschillende variabelen van DHS ook beter begrepen worden om tot een efficiënte stimulatiestrategie te komen voor de behandeling van refractaire epilepsiepatiënten.

Het doel van dit onderzoeksproject is om de kenniskloof in het gebruik van hippocampale DHS als behandeling voor refractaire epilepsie te verkleinen. Hiervoor werd een translationeel onderzoeksproject opgezet waarin onderzoek werd verricht naar het werkingsmechanisme van hippocampale DHS, de optimalisatie van stimulatieparameters voor de behandeling van refractaire epilepsie en de neuromodulatoire eigenschappen van hippocampale DHS.

Het werkingsmechanisme van hippocampale DHS werd onderzocht met behulp van een functionele beeldvormende techniek (µSPECT) die het mogelijk maakt om hippocampale DHS geïnduceerde veranderingen in regionale hersendoorbloeding (rCBF) in kaart te brengen. Deze studie toonde aan dat unilaterale hippocampale DHS een vermindering in de rCBF zowel in de ipsilaterale (d.w.z. de hersenhelft waar de elektrode is geïmplanteerd) als contralaterale hippocampale formatie veroorzaakt. Verschillen in de omvang en intensiteit van deze veranderingen in rCBF stelt ons in staat om verschillende stimulatieparameters en –intensiteiten te onderscheiden. Lage stimulatie-intensiteit leidde enkel tot verminderde rCBF ipsilateraal van de DHS-electrode, terwijl met toenemende stimulatie-intensiteiten de verminderde rCBF steeds meer contralateraal hippocampaal weefsel aangesproken werd.

Aangezien het uiteindelijke doel van dit translationeel onderzoeksproject het onderzoeken van hippocampale DHS in de context van TLE was, werd verder onderzoek uitgevoerd in een rat model voor TLE. TLE wordt over het algemeen veroorzaakt door een zogenaamd initieel incident, zoals koortsstuipen, encefalitis of status epilepticus (SE), wat een proces van verscheidene moleculaire en cellulaire verandering start in het neuronaal netwerk en uiteindelijk aanleiding geeft tot het ontstaan van spontane herhaaldelijk optredende epileptische aanvallen. Deze processen worden omschreven met de term “epileptogenese”. We identificeerden het systemische kainaat (KA) post status epilepticus (SE) rat model voor TLE als het meest geschikte diermodel omdat het zeer goed de latente periode, het chronisch karakter en het gedrag geassocieerd met de epileptische aanvallen in humane TLE imiteert.

In dit diermodel werd de aanvalsonderdrukkende capaciteit van bilaterale hippocampale DHS, vergeleken met unilateral hippocampale DHS. Tijdens bilaterale hippocampale DHS werden beide hippocampi gestimuleerd werden met afzonderlijke Poisson verdeelde stimulatiepatronen met een

gemiddeld interpulsinterval en een variantie van 1/130s. Deze studie toont aan dat bilaterale hippocampale DHS met afzonderlijke stimulatiepulspatronen in beide hippocampi aanleiding gaf tot een betere aanvalsonderdrukkende respons vergeleken met unilaterale hippocampale DHS. Tijdens bilaterale hippocampale DHS vertoonden 7/11 (64%) ratten een significante daling in aantal epileptische aanvallen, terwijl tijdens unilateral hippocampale DHS dit aantal beperkt bleef tot 4/11 (36%) ratten in dezelfde groep ratten.

Aangezien bij lange termijn opvolging van patiënten onder behandeling met hippocampale DHS een betere respons lijken te vertonen met toegenomen duur van de behandeling, zou een langdurige behandeling met hippocampale DHS veranderingen in exciteerbaarheid van de neuronale netwerken kunnen veroorzaken en bijgevolg het ziekteproces van TLE kunnen beïnvloeden. Om dit te onderzoeken werd een experiment uitgevoerd waarin bij proefdieren een SE werd uitgelokt, waarna de helft van de dieren gedurende 22 weken continu behandeld werden met hippocampale DHS. Tijdens dit experiment werd in alle dieren gedurende 30 weken de progressie in het aantal epileptische aanvallen ten gevolge van de initiële SE gemonitord met behulp van continue EEG-registraties. Deze studie toonde aan dat langdurige continue unilaterale hippocampale DHS de progressie in het aantal aanvallen in een rat model voor TLE kan moduleren zoland de stimulatie aangehouden wordt, maar hierbij geen blijvende veranderingen veroorzaakt in de onderliggende pathofysiologische processen van epileptogenese.

Samenvattend tonen de resultaten van dit onderzoeksproject drie zaken. Ten eerste ondersteunt de observatie van de wijdverspreide vermindering in rCBF in alle limbische structuren ten gevolge van hippocampale DHS de hypothese dat hippocampale DHS een functionele inhibitie veroorzaakt in neuronale netwerken, en dat het patroon van verminderde rCBF afhankelijk is van de gebruikte stimulatie-intensiteit. Verder onderzoek moeten uitwijzen of de door hippocampale DHS geïnduceerde vermindering in rCBF gebruikt zou kunnen worden als een screeningsinstrument voor efficiënte stimulatieparameters in de behandeling van refractaire TLE. Ten tweede werd aangetoond dat stimulatie binnen het hippocampaal netwerk op meer dan één locatie met afzonderlijke stimulatiepulspatronen beter in staat is epileptische activiteit te verstoren in vergelijken met stimulatie op één locatie binnen dit netwerk. Verder onderzoek moet gericht zijn op het ontrafelen van de rol van desynchronisatie van neuronale netwerken met behulp van hippocampale DHS en het bijhorende aanvalsonderdrukkend effect hiervan. Ten derde werd aangetoond dat zolang langdurige hippocampale DHS aangehouden wordt de progressie in aantal aanvallen na een initieel incident gemoduleerd kan worden in een diermodel voor TLE, zonder de onderliggende histopathologische kenmerken van TLE te beïnvloeden. Verder onderzoek is nodig om de onderliggende mechanismen verantwoordelijk voor deze door hippocampale DHS geïnduceerde neuromodulatie na te gaan.

Elektrofysiologische metingen van hippocampale neuronale netwerken gedurende langdurige behandeling met hippocampale DHS zou een antwoord kunnen geven op de vraag hoe hippocampale DHS de exciteerbaarheid en bijgevolg de progressie in aantal epileptische aanvallen na een initieel incident beïnvloedt in een proefdiermodel voor TLE.

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Jij bent het die zorgt voor mijn mooiste momenten.
Jij bent mijn zon en mijn maan.

Curriculum vitae

Personal information:

Name: Bregt Balder VAN NIEUWENHUYSE
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Date of birth: 8th of February 1986
Place of birth: Ghent
Tel.: +32 486 25 32 52
E-mail: bregtvn@hotmail.com

Graduate studies:

2004 – 2009: Master in Biomedical sciences – major neuroscience
Ghent University
Graduated with great distinction

Post graduate courses:

2007: Course in Laboratory animal science (FELASA C certificate)
Ghent University
2010: Statistical data-analysis with IBM SPSS
Ghent University
2010: Effective scientific communication
Pricipiae
2011: Effective scientific writing for life sciences research
Oxford University
2012: Advanced power & sample size calculation
Ghent University
2014: General anesthesia and monitoring rat and mouse
Ghent University
2014: Species specific animal welfare in the laboratory environment – rat & mouse
Ghent University

Post graduate position:

2009 – 2014: PhD student
Strategic basic research grant from the "insitituut voor wetenschap door technologie & innovatie" (IWT)
Laboratory for clinical and experimental neurophysiology, neurobiology, and neuropsychology (LCEN3), Department of Neurology (dir. Prof. Dr. Paul Boon)

Awards & grants:

-Participation grant for the 68th annual American Epilepsy Society meeting
December 5 – 9 2014, Seattle, United States

- American Epilepsy Society Young Investigator Award
3th of December 2012, San Diego, USA
Title of the presentation: "Hippocampal deep brain stimulation has anti-epileptogenic potential"

-Participation grant for the European congress on epileptology
September 30 – October 4 2012, London, United Kingdom

- Best poster & abstract at the 14th annual international clinical symposium "Epilepsy and Sleep Update @ Kempenhaeghe.nl"
23th of March 2012, Heeze, The Netherlands
Title of the poster: "Hippocampal deep brain stimulation modifies disease progression in a TLE animal model"

- Best poster and oral presentation at the 2012 Dutch League against Epilepsy SWO-midwinter meeting
10th of February 2012, Amsterdam, The Netherlands
Title of the presentation: "Poisson distributed deep brain stimulation (DBS) in the ventral hippocampal commissure suppresses seizures in the kainic acid rat model"

Publications

Manuscripts in international peer-reviewed journals

1. Hippocampal Deep Brain Stimulation induces decreased rCBF in the hippocampal formation of the rat.
   WYCKHUYS T, STAELENS S, VAN NIEUWENHUYSE B, DELEYE S, HALLEZ H, VONCK K, WADMAN W, BOON P.NeuroImage 2010; 52(1):55-61

2. Suppression of hippocampal epileptic seizures in the kainate rat by Poisson distributed stimulation.
   WYCKHUYS T., BOON P, RAEDT R, VAN NIEUWENHUYSE B, VONCK K, WADMAN W.
   Epilepsia 2010; 51(11):2297-304

3. Real-time detection of epileptic seizures in animal models using reservoir computing.
   BUTENEERS P, VERSTRAETEN D, VAN NIEUWENHUYSE B, STROOBANDT D, RAEDT R, VONCK K, BOON P, SCHRAUWEN B
   Epilepsy Research 2013; 103(2-3):124-134

4. Hippocampal deep brain stimulation reduces glucose utilization in the healthy rat brain.
   VAN DEN BERGE N, KEEREMAN V, VANHOVE C, VAN NIEUWENHUYSE B, VAN MIERLO P, RAEDT R, VONCK K, BOON P, VAN HOLEN R
   Molecular Imaging & Biology 2015;17(3):373-83

5. In search of optimal DBS paradigms to treat epilepsy: bilateral versus unilateral hippocampal stimulation in a rat model for temporal lobe epilepsy.
   VAN NIEUWENHUYSE B, RAEDT R, DELBEKE J, WADMAN W, BOON P, VONCK K.
   Brain stimulation 2015;8(2):192-9.

6. The systemic kainic acid rat model of temporal lobe epilepsy: long-term EEG monitoring and histopathologic features.
   VAN NIEUWENHUYSE B, RAEDT R, SPRENGERS M, DAUWE I, GADEYNE S, CARRETTE E, DELBEKE J, WADMAN W, BOON P, VONCK K.
   Brain Research: submitted

7. Long-term hippocampal deep brain stimulation in a rat model for temporal lobe epilepsy contradicts seizures beget seizures hypothesis.
   VAN NIEUWENHUYSE B, RAEDT R, SPRENGERS M, DAUWE I, GADEYNE S, DELBEKE J, WADMAN W, BOON P, VONCK K.
   Science Translational Medicine: in preparation

8. Modulation of Hippocampal Activity by Vagus Nerve Stimulation in Freely Moving Rats.
   LARSEN LE, WADMAN W, VAN MIERLO P, DELBEKE J, GRIMONPREZ A, VAN NIEUWENHUYSE B, PORTELLI J, BOON P, VONCK K, RAEDT R
   Brain stimulation: submitted

Proceedings in international journals

1. High resolution µSPECT for brain activation analysis in small animals.
   STAELENS S, WYCKHUYS T, DELEYE S, HALLEZ H, VANDENBERGHE S, VAN NIEUWENHUYSE B, VONCK K.
   IEEE Nuclear Science Symposium Conference Record 2009; 2702-2705

2. A multi-modal post processing framework for activation studies of the rat brain.
   STAELENS S, WYCKHUYS T, DELEYE S, HALLEZ H, VANDENBERGHE S, VAN NIEUWENHUYSE B, VONCK K.
   World Molecular Imaging Congress 2009, Abstracts.

Abstracts in international journals

1. High frequency stimulation in the hippocampus reduces the frequency of seizures in kainic acid injected rats.
   VAN NIEUWENHUYSE B, WYCKHUYS T, RAEDT R, VONCK K, BOON P, WADMAN
   Acta Neurologica Belgica 2008; Volume 108 (suppl. 1)

2. Effect of hippocampal deep brain stimulation on blood perfusion evaluated by µSPECT.
   VAN NIEUWENHUYSE B, WYCKHUYS T, RAEDT R, MEURS A, VAN DYCKE A, EL TAHRY R, VONCK K, WADMAN W, DELEYE S, STAELENS S, BOON P.
   Acta Physiologica 2009;195(670):12

3. Hippocampal deep brain stimulation reveals decreased rCBF in the hippocampus of the rat : a mu spect study
   WYCKHUYS T, VAN NIEUWENHUYSE B, STAELENS S, DELEYE S, HALLEZ H, VONCK K, WADMAN W, BOON P.
   Epilepsia 2010; 51(4):16

4. Long term hippocampal deep brain stimulation efficiently reduces seizures in the kainic acid model.
   VAN NIEUWENHUYSE B, RAEDT R, MEURS A, VONCK K, WADMAN W, BOON P.
   Acta Physiologica 2011**;** 203(S 687)

5. Hippocampal deep brain stimulation early during epileptogenesis affects spontaneous seizures in the kainic acid rat model.
   VAN NIEUWENHUYSE B, VONCK K, WYCKHUYS T, RAEDT R, MEURS A, WADMAN W, BOON P.
   Epilepsia 2011; 52(6):160-161

6. Pilot-trial: high frequency, Poisson distributed cortical stimulation in a screening model for epileptic seizures.
   BUFFEL I, MEURS A, RAEDT R, DE HERDT V, EL TAHRY R, VAN NIEUWENHUYSE B, DAUWE I, MOLLET L, SIONCKE L, VONCK K., BOON P
   Epilepsia 2011; 52(6): 159-160

7. Poisson distributed deep brain stimulation (DBS) in the ventral hippocampal commissure suppresses seizures in the kainic acid rat model.

   VAN NIEUWENHUYSE B, PARTHOENS J, WYCKHUYS T, RAEDT R, WADMAN W, BOON P, VONCK K.
   Epilepsy currents 2012; 12(1)

8. The effect of high frequency, Poisson distributed cortical stimulation on cortical excitability in rats. BUFFEL I, MEURS A, RAEDT R, DE HERDT V, EL THARY R, VAN NIEUWENHUYSE B, MOLLET L, WADMAN W, VONCK K, BOON P

   Epilepsia 2012;53(5):175–175.

9. Deep brain stimulation early during epileptogenesis modifies disease progression in temporal lobe epilepsy.
   VAN NIEUWENHUYSE B, VONCK K, RAEDT R, MEURS A, WADMAN W, BOON P.
   Frontiers in Human Neuroscience 2012. Conference Abstract : Belgian Brain Council.

10. Deep brain stimulation early during epileptogenesis modifies disease progression in the hippocampus. VAN NIEUWENHUYSE B, VONCK K, RAEDT R, MEURS A, WADMAN W, BOON P
    Epilepsia 2012;53(5): 26-27

11. Is cortical excitability in rats altered after 1h of high frequency, Poisson distributed cortical stimulation?
    BUFFEL I, MEURS A, RAEDT R, DE HERDT V, EL TAHRY R, VAN NIEUWENHUYSE B, MOLLET L, WADMAN W, VONCK K, BOON P
    Frontiers in Human Neuroscience 2012. Conference Abstract : Belgian Brain Council.

12. Hippocampal deep brain stimulation has antiepileptogenic potential.
    VAN NIEUWENHUYSE B, RAEDT R, VONCK K, MEURS A, WADMAN W, BOON P.

Epilepsy currents 2013; 13(1)

13. PET functional imaging of deep brain stimulation in the healthy rat brain.
    VAN DEN BERGE N, KEEREMAN V, VANHOVE C, VAN NIEUWENHUSYE B, VAN MIERLO P, RAEDT R, BOON P, VAN HOLEN R.
    Clinical Neurophysiology 2014. 125 (S1).

14. Unilateral vs. bilateral hippocampal DBS in a rat model for temporal lobe epilepsy
    VAN NIEUWENHUYSE B, RAEDT R, DELBEKE J, WADMAN W, BOON P, VONCK K
    Epilepsia 2014;55(2):102-103

15. Vagus nerve stimulation decreases hippocampal and prefrontal EEG power in freely moving rats: a biomarker for effective stimulation?
    LARSEN LE, VAN MIERLO P, WADMAN W, DELBEKE J, GRIMONPREZ A, MOLLET L, VAN NIEUWENHUYSE B, PORTELLI J, BOON P, VONCK K, RAEDT R. (2014).
    Frontiers in Human Neuroscience 2014. doi: 10.3389/conf.fnhum.2014.214.00035

16. Consequences Of Kainic Acid-Induced Piriform Cortex Lesions And Therapeutic Potential Of Piriform Cortex Deep Brain Stimulation In The Intrahippocampal Kainic Acid Model.
    SPRENGERS M, RAEDT R, SIUGZDAITE R, VAN NIEUWENHUYSE B, DESCAMPS B, DAUWE I, DELBEKE J, WADMAN W, BOON P, VONCK
    Frontiers in Human Neuroscience 2014; doi: 10.3389/conf.fnhum.2014.214.00029

17. Hippocampal DBS affects disease development in the kainic acid rat model for temporal lobe epilepsy.
    VAN NIEUWENHUYSE B, RAEDT R, SPRENGERS M, DAUWE I, GADEYNE S, DELBEKE J, WADMAN W, BOON P, VONCK K.
    Frontiers in Human Neuroscience 2014. doi: 10.3389/conf.fnhum.2014.214.00013

18. Statistical group-analysis of PET data reveals whole-brain effect of hippocampal deep brain stimulation.
    VAN DEN BERGE N, KEEREMAN V, VANHOVE C, VAN NIEUWENHUYSE B, VAN MIERLO P, RAEDT R, VONCK K, BOON P, VAN HOLEN R
    (2014) Abstract presented at the World Molecular Imaging Congress 2014

19. Hippocampal DBS affects disease development in the KA rat model for TLE.
    VAN NIEUWENHUYSE B, RAEDT R, SPRENGERS M, DAUWE I, GADEYNE S, DELBEKE J, WADMAN W, BOON P, VONCK K.
    Epilepsy currents 2014

20. The piriform cortex in the intrahippocampal kainic acid model: effects of lesions and deep brain stimulation on spontaneous seizures.
    SPRENGERS S, RAEDT R, SIUGZDAITE R, DESCAMPS B, VAN NIEUWENHUYSE B, DAUWE I, DELBEKE J, WADMAN W, BOON P, VONCK K
    Epilepsy currents 2014

Oral presentations at scientific meetings/congresses

1. International state-of-the-art symposium: “New approaches for epilepsy treatment in Europe: back to the future”
   Ghent, Belgium
   May 21-22, 2010
   Oral presentation: Evaluation of short term DBS early in the epileptogenic process

2. SWO Midwinter meeting “New therapies in pharmacoresistant epilepsy”
   Amsterdam, The Netherlands
   February 10, 2012
   Oral presentation: Poisson distributed deep brain stimulation (DBS) in the ventral hippocampal commissure suppresses seizures in the kainic acid rat model

3. Staff meeting neurology department UZ Ghent
   Ghent, Belgium
   May 31, 2012
   Oral presentation: Deep brain stimulation for epilepsy in animal models

4. 2nd Ghent International Epilepsy Workshop
   Ghent, Belgium
   October 26-27, 2012
   Oral presentation: Deep brain stimulation has anti-epileptogenic potential

5. American epilepsy society annual meeting,
   San Diego, United states
   November 30 – December 4, 2012
   Oral presentation: Deep brain stimulation has anti-epileptogenic potential

6. SWO Midwinter meeting “New therapies in pharmacoresistant epilepsy”
   Amsterdam, The Netherlands
   February 14, 2014
   Oral presentation: Unilateral versus bilateral hippocampal DBS in a rat model for temporal lobe epilepsy

7. Neuroscience forum
   Ghent, Belgium
   February 20, 2014
   Oral presentation: Bilateral versus unilateral hippocampal DBS in a rat model for temporal lobe epilepsy

8. Meeting of minds for youth
   Ghent, Belgium
   March 10, 2014
   Oral presentation: Van mens tot cyborg

9. Jaarlijkse wetenschappelijke NVS vergadering Kempenhaeghe
   Heeze, The Netherlands,
   October 09, 2014
   Oral presentation: DBS en lange-termijn neuromodulatoire effecten, dierexperimenteel onderzoek

10. American epilepsy society annual meeting,
    Seattle, United states
    December 5 – December 9, 2014
    Oral presentation: Hippocampal DBS affects disease progression in the rat KA model for TLE

Poster presentations at scientific meetings/congresses

1. Belgian Brain Council
   Ostend, Belgium
   October 24-25,2008
   Poster presentation: High frequency stimulation in the hippocampus reduces the frequency of seizures in kainic acid injected rats

2. Belgian society for Fundamental and Clinical Physiology and Pharmacology
   Ghent, Belgium
   March 7, 2009
   Poster presentation: Effect of hippocampal deep brain stimulation on blood perfusion evaluated by SPECT.

3. Kempenhaeghe Epilepsy and Sleep Update
   Heeze, The Netherlands
   March 26-27, 2009
   Poster presentation: Evaluation of hippocampal Deep Brain Stimulation by µSPECT

4. Ghent University – Wetenschapsdag 2010
   Ghent, Belgium
   March 11, 2010
   Poster presentation: Evaluation of the anti-epileptogenic properties of deep brain stimulation

5. Kempenhaeghe Epilepsy and Sleep Update
   Heeze, The Netherlands
   March 25-26, 2010
   Poster presentation: assessment of the epileptogenesis suppressing features of deep brain

6. International society for monitoring molecules in neurosciences, 13th international conference on *In Vivo Methods*
   Brussels, Belgium
   September 12-16, 2010
   Poster presentation: Evaluation of the epileptogenesis modifying characteristics of deep brain stimulation

7. Physphar 2011, 1st Benelux congress on physiology and pharmacology
   Liège, Belgium
   March 18-19, 2011
   Poster presentation: Long term hippocampal deep brain stimulation efficiently reduces seizures in the kainic acid model

8. Kempenhaeghe Epilepsy and Sleep Update
   Heeze, The Netherlands
   March 24-25, 2011
   Poster presentation: Long term hippocampal deep brain stimulation efficiently reduces seizures in the kainic acid model

9. Ghent University – Wetenschapsdag 2011
   Ghent, Belgium
   April 7, 2011
   Poster presentation: Long term hippocampal deep brain stimulation efficiently reduces seizures in the kainic acid model

10. ENP 2011, 9th dutch endo-neuro-psycho meeting
    Lunteren, The Netherlands
    May 30- June 1, 2011
    Poster presentation: Hippocampal deep brain stimulation early during epileptogenesis affects spontaneous seizures in the kainic acid rat model

11. ILAE-IBE, 29th international epilepsy congress
    Rome, Italy
    August 28 – September 1, 2011
    Poster presentation: Hippocampal deep brain stimulation early during epileptogenesis affects spontaneous seizures in the kainic acid rat model

12. Ghent University – Wetenschapsdag 2012
    Ghent, Belgium
    March 14, 2012
    Poster presentation: Deep brain stimulation early during epileptogenesis modifies disease progression in the hippocampus

13. Kempenhaeghe Epilepsy and Sleep Update
    Heeze, The Netherlands
    March 22-23, 2012
    Poster presentation: Hippocampal deep brain stimulation modifies disease progression in a TLE animal model

14. 10th European congress on epileptology
    Londen, United Kingdom
    September 30 - October 4, 2012
    Poster presentation: Deep brain stimulation early during epileptogenesis modifies disease progression in the hippocampus.

15. Belgian brain and cognition congress 2012
    Liège, Belgium
    October 27, 2012
    Poster presentation: Deep brain stimulation early during epileptogenesis modifies disease progression in temporal lobe epilepsy

16. Knowledge for growth
    Ghent, Belgium
    May 30 2013
    Poster presentation: The effect of hippocampal deep brain stimulation on epileptogenesis and spontaneous seizures

17. Kempenhaeghe Epilepsy and Sleep Update
    Heeze, The Netherlands
    March 27-28, 2014
    Poster presentation: unilateral vs. bilateral hippocampal DBS in a rat model for temporal lobe epilepsy

18. Knowledge for growth
    Ghent, Belgium
    May 8, 2014
    Poster presentation: unilateral vs. bilateral hippocampal DBS in a rat model for temporal lobe epilepsy

19. 11th European congress on epileptology
    Stockholm, Sweden
    June 29 - July 3, 2014
    Poster presentation: unilateral vs. bilateral hippocampal DBS in a rat model for temporal lobe epilepsy

20. Belgian brain council congress 2014
    Ghent, Belgium
    October 4, 2012
    Poster presentation: Hippocampal DBS affects disease development in the kainic acid rat model for temporal lobe epilepsy

Teaching and students

- "Deep Brain Stimulation for the treatment of epilepsy by means of experimental animal studies"
  Master thesis Joke Parthoens (Biology - *Ghent University)* *2010-2011*

- "Deep brain stimulation as a treatment for refractory epilepsy: multifocal vs unifocal stimulation"
  Master thesis Patricia Clement (Biomedical Sciences – *Ghent University*) 2011-2012

- "Deep brain stimulation as a treatment for refractory epilepsy: optimalistion of stimulation location"
  Master thesis Valérie Du Caju (Biomedical Sciences – *Ghent University*) 2011-2012

- "Deep brain stimulation of the ventral hippocampal commissure for the treatment of epilepsy"
  Master thesis Elsemiek Kruijsse (Biomedical Sciences: psychopharmacology & pathophysiology – *Amsterdam University)* 2011-2012

- "Diepe hersenstimulatiie in de ventral hippocampale commissuur van de rat voor de onderdrukking van spontane epilepsieaanvallen – meting & analyse van de geëvokeerde potentialen in de CA3 regio van de hippocampus
  Bachelor thesis Simon Braem (Biology – *Ghent University*) 2012-2013

- "Effects of hippocampal neurostimulation on fear and memory in rats"
  Master thesis Vicky Pauwelyn (biomedical sciences – *Ghent University*) 2012-2013

- "Optimalisation of deep brain stimulation as a treatment for refractory epilepsy: evaluation of the seizure suppressive effect of bipolar stimulation"
  Master thesis Lore Vandaele (biomedical sciences – *Ghent University*) 2012-2013

- "Optimalisation of deep brain stimulation as a treatment for refractory epilepsy: evaluation of the seizure suppressive effect of randomized double bipolar stimulation"
  Master thesis Justien Maes (biomedical sciences – *Ghent University*) 2012-2013

Additional qualifications

Reviewer for Brain Stimulation

Reviewer for Acta Neurologica Belgica

Reviewer for Seizure, the European Journal of Epilepsy

Member of the editorial committee of neuroimmunology and neuroinflammation