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Mapping and zapping

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MAPPING AND ZAPPING

Deep brain stimulation takes hold of epilepsy

by

Frédéric L.W.V.J. Schaper

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Deep brain stimulation takes hold of epilepsy

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Voor mam en pap

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"Here's to the crazy ones…"

Rob Siltanen, Lee Clow, and others

Chapter 1

Introduction

Epilepsia

'To be taken hold of'

EPILEPSY

EPILEPSY IS A BRAIN DISEASE IN WHICH PATIENTS SUFFER FROM RECURRENT spontaneous seizures caused by abnormal excessive or synchronous firing of neural cells.[1] This abnormal neural activity can start in a specific region (focal seizures) or across the brain (generalized seizures). Depending on the location of seizure onset and brain areas affected, patients experience intricate neurological symptoms.[2] For example, seizure onset in the temporal lobe can produce a variety of emotions and sensations including *déjà-vu*, unexpected intense fear or nausea, hallucinations and even religious or spiritual experiences amongst others. Focal seizures in motor regions of the frontal lobe can cause uncontrolled jerks of the arms and legs, and seizures originating from the hypothalamus can even cause abrupt laughter or crying. Instigation or spread of epileptic seizures across multiple brain regions can lead to loss of consciousness and generalization of seizures, typically characterized by tonic-clonic movements of the body and limbs. An almost infinite number of combinations, sequences and intensity of symptoms are possible, which makes seizures a fascinating neurological phenomenon and epilepsy a highly complex disease.

THE BURDEN OF EPILEPSY

The burden of epilepsy is profound. Epilepsy is a surprisingly common disease affecting 1 in every 100 lives (www.epilepsy.com) and more than 70 million people worldwide. In fact, epilepsy is one of the top 5 most common neurological diseases. Persistent seizures carry serious neurological, cognitive, psychological and social consequences. On a societal level, epilepsy has significant economic implications due to chronic health care demands and lost productivity of work.[3,4] In addition to its high prevalence and burden, people living with seizures unfortunately still suffer from stigma, discrimination and even human rights violations leading to difficulties in education and employment (www.who.int).

DRUG RESISTANT EPILEPSY

The majority of patients with epilepsy can achieve satisfactory seizure control or even seizure freedom after chronic treatment with antiepileptic drugs (AEDs). Although effective and noninvasive, AED treatment from a young age throughout adulthood has several disadvantages. AEDs have brain and systemic effects and are not specific to therapeutic target sites nor an epileptogenic lesion or seizure onset zone. Moreover, AEDs can cause disabling side effects on cognitive performance, emotional functioning and energy capacity.[5] Approximately 70% of patients, who attain seizure control, do so with the first or second AED. In 30% of patients, the first two AED regimens do not lead to adequate seizure control and the probability of achieving seizure freedom with further or different AED regimens is modest. If the first two AEDs fail to achieve seizure control, the third one offers only a 4.1% additional probability of seizure freedom. Despite the introduction of more than a dozen new AEDs with differing mechanisms of action over the past 2 decades, there is no robust data to suggest improvement in overall treatment outcomes.[6] Drug resistant epilepsy may therefore be defined as failure to achieve sustained seizure freedom after adequate trials of two tolerated, appropriately chosen and used AED regimes.[7] Therefore, epilepsy, especially of the drug-resistant type, consequently has a high medical, social and economic burden and is to this day a major health problem.[8]

EPILEPSY SURGERY

The single curative option left for patients with drug-resistant epilepsy is an invasive brain surgery often referred to as epilepsy surgery. Epilepsy surgery is aimed at resecting the patient-specific epileptogenic zone to maximize the chance for seizure freedom and minimize the risk for functional deficit.[9,10] Epilepsy surgery is only possible for individuals with focal epilepsy in which the epileptogenic zone can be identified and safely removed. The majority of patients with drug-resistant epilepsy suffer from focal epilepsy and consequently, efforts to treat drug-resistant epilepsy have focused on defining and surgically removing the epileptogenic zone. Since the advent of multidisciplinary epilepsy surgery working groups in the 1970s, numerous diagnostic measures have been employed to identify the epileptogenic zone. The modern comprehensive pre-surgical evaluation for drug-resistant epilepsy consists of at least: detailed accounts of seizure semiology, cognitive and psychiatric evaluations, interictal

electroencephalogram (EEG), optimal high-resolution magnetic resonance imaging (MRI) and long-term video-scalp EEG monitoring. Optional investigations depending on the availability and expertise of the epilepsy center include the Wada test, invasive intracranial EEG using cortical strips or depth electrodes, and non-invasive neuroimaging modalities such as fluorodeoxyglucose (FDG)-positron emission tomography (PET), ictal single-photon emission computed tomography (SPECT), functional MRI, proton magnetic resonance spectroscopy (1H-MRS) and magnetoencephalography (MEG).[11] It is evident that the pre-surgical evaluation for epilepsy surgery includes a scala of diagnostic procedures and can therefore be invasive, long, expensive, emotionally demanding, and disappointing for patients when surgery is not possible. Nevertheless, after clinical consensus on the epileptogenic zone and surgical removal, seizure freedom is reached in 52% of subjects at 5 years after surgery.[12] Although potentially curative, epilepsy surgery is an aggressive and invasive procedure that can induce or aggravate neuropsychological deficits.[13] Since its early beginnings in the 1950's, the principle for surgical treatment of focal epilepsy has remained the same: removing the epileptogenic lesion or zone. While most patients with drug-resistant epilepsy have focal epilepsy, only few qualify for surgical resection or ablation, as the epileptogenic zone cannot be reliably identified or safely removed in the majority of patients. Seizure control in drug-resistant epilepsy therefore remains extremely challenging, highlighting even more the medical need for more scientific knowledge to advance the treatment of drug-resistant epilepsy with an unidentifiable or unresectable epileptogenic zone.

Neuromodulation

Once surgical resection of the epileptogenic zone is not possible or does not result in seizure freedom, neuromodulation is a potential next step. For almost a century, neurosurgeons and neurophysiologists have used brain stimulation to probe and map human brain function. Following important technological discoveries such as the cardiac pacemaker, electrical stimulation by implantable pulse generators (IPGs) made its way into the treatment of brain diseases in the form of neuromodulation. Pioneered by deep brain stimulation (DBS) for movement disorders such as tremor and Parkinson's disease, followed by psychiatric disorders such as obsessive-compulsive disorder, neuromodulation proved to be a valuable addition to the therapeutic armoury of neurologists, neurosurgeons and psychiatrists.[14]

Clinical neuromodulation targets in neurology and psychiatry have predominantly been established by either "serendipity" in cases of sporadic strokes relieving neurological symptoms, or by "trial and error" of experimental brain ablations and stimulations performed in the 1950s-1970s. Scientific evidence from animal studies often lacks and rarely precedes first-in-man or randomized-controlled clinical trials.

Nowadays, neuromodulation is applied to specific brain networks by targeting key nodes whitin these networks. As such, the activity of the stimulated networks is halted, or networks critical for the control of that symptom are engaged, leading to symptomatic relief in neuropsychiatric disease.

Invasive brain stimulation devices tested in randomized-controlled trials[15] which are currently approved in Europe and/or the U.S. for the treatment of focal drug-resistant epilepsy include vagal nerve stimulation (VNS), responsive neurostimulation (RNS) to the seizure focus and DBS to the anterior nucleus of the thalamus (ANT-DBS). In addition, DBS of the hippocampus[16] or centromedian nucleus[17] and chronic subthreshold cortical stimulation (CSCS) to the seizure focus[18,19] have shown encouraging results, but remain relatively experimental.

Non-invasive brain stimulation methods such as transcranial magnetic stimulation (TMS)[20] and transcranial electrical stimulation (TES)[21] are being investigated as potential treatments for epilepsy while they still remain experimental.[22] The location restrictions of its application to rare, superficially located cortical epileptic foci, and the lack of evidence-based neuroanatomical targets or crucial brain networks impede the use of non-invasive brain stimulation as a treatment strategy for epilepsy.

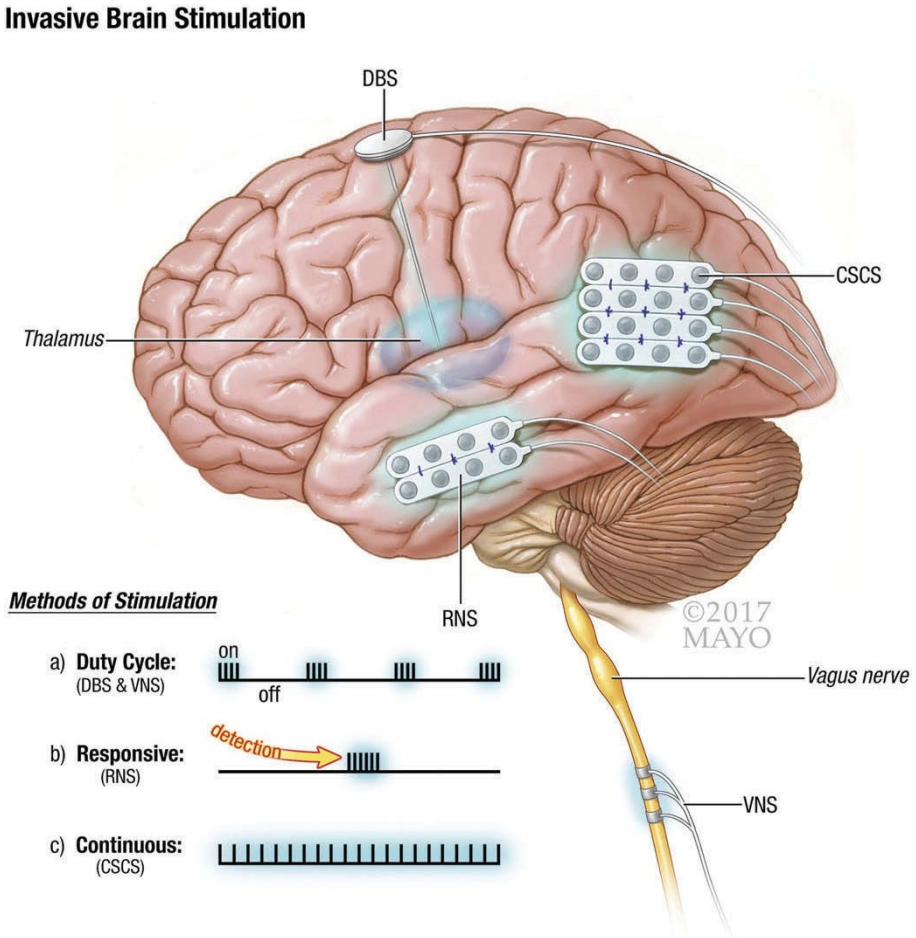

Figure 1.1 Methods of invasive brain stimulation use different stimulation targets and parameters for reducing seizure burden. (**a**) Vagal nerve stimulation (VNS) and deep brain stimulation (DBS) of the thalamus typically use duty cycle stimulation during which stimulation is off the majority of time. (**b**) Responsive neurostimulation (RNS) of the seizure focus only stimulates in response to the detection of putative seizure activity. (**c**) Chronic subthreshold cortical stimulation (CSCS) of the seizure focus stimulates continuously with one set of parameters until altered by a neurologist. (Adapted from Lundstrom *et al.* 2017. Expert Review of Neurotherapeutics[19])

DEEP BRAIN STIMULATION

Deep brain stimulation (DBS) has recently been approved by the U.S. food and drug administration (FDA) and has received a CE mark in Europe for the treatment of drug-

resistant focal epilepsy.[23] DBS in patients with epilepsy involves implanting a depth electrode in the anterior nucleus of the thalamus (ANT) which is connected to a sub clavicular implanted IPG for electrical stimulation. ANT-DBS is designed to stop seizure propagation or modulate structures of the limbic system important in seizure generation and propagation. The Stimulation of the Anterior Nucleus of the Thalamus for Epilepsy (SANTE) trial, a randomized double-blinded controlled trial, demonstrated that bilateral thalamic stimulation in drug-resistant focal epilepsy is a safe procedure that reduces short and long-term seizure frequency and significantly improves quality of life. After 2 years, ANT-DBS resulted in a median 56% reduction in seizure frequency compared to baseline with a 54% responder rate. After 5 years, ANT-DBS resulted in a 69% seizure reduction with a 68% responder rate.[24] While a plethora of case series exists on the safety and efficacy of different DBS targets in drug-resistant epilepsy, only ANT-DBS has been tested in a large randomized-controlled trial and is currently being used in the clinical practice of specialized epilepsy centers across Europe and the U.S. Other studied DBS targets include the cerebellum, caudate nucleus, and subthalamic nucleus among others. However, these studies were largely uncontrolled, underpowered, unblinded, or included populations of heterogeneous epilepsy types and have not been replicated.[25] In Europe, ANT-DBS remains the only approved DBS treatment and thus complements VNS as a last resort for patients with drug-resistant epilepsy.[26]

KNOWLEDGE GAPS

Neuromodulation devices are taking an increasingly prominent position in the treatment of neurological disease. However, little is known on what the optimal brain stimulation device, stimulation target or stimulation paradigm is in order to treat patients with drug resistant epilepsy. Even less is known on the specific brain networks involved in neuromodulation and to their potential role in seizure generation, propagation, facilitation and/or suppression. Similar to the other currently approved neuromodulation devices, such as RNS and VNS, ANT-DBS has shown therapeutic efficacy, but with the moderate responder rates reported in randomized controlled trials, is unlikely to result in seizure freedom and has an unknown mechanism of action. When patients with epilepsy seek seizure control with ANT-DBS, current knowledge gaps on the predictors of response, the correct stimulation site and the brain networks that are potentially crucial to engage for seizure control impede its use as a standard neuromodulation therapy for patients with drug resistant epilepsy.

THE PROBLEM

Patients with drug-resistant focal epilepsy are left with few therapy options if the epileptogenic zone cannot be identified or safely removed. ANT-DBS is a potentially effective neuromodulation therapy for these patients. We are currently on the break of ANT-DBS making its way into the hands of multidisciplinary epilepsy teams worldwide. However, knowledge gaps on the predictors of response, the correct stimulation site and brain networks that are potentially crucial to engage for achieving seizure control impede its application as a standard neuromodulation therapy for patients with drug resistant epilepsy.

AIMS OF THIS THESIS

This thesis aims to define the optimal stimulation site and brain networks that are potentially crucial to engage for achieving seizure control by ANT-DBS in patients with drug-resistant focal epilepsy. To address this aim, we have performed the following animal, human and computational studies:

In *Part 1* on animal studies, we start with **Chapter 2** reviewing the evidence from animal studies on the stimulation paradigms and efficacy of ANT-DBS. Subsequently in **Chapter 3,** we use a translational approach and perform ANT-DBS with a clinically relevant stimulation paradigm in an animal model of epilepsy to investigate its seizure suppressing effects and side effects.

In *Part 2* on human studies, we follow with **Chapter 4** reviewing the rationale, clinical efficacy, safety and the proposed mechanisms of action of ANT-DBS in humans. Next, the results of two human studies are presented that aim to define the optimal stimulation site in ANT-DBS, by leveraging commonly used neurophysiological and neuroimaging tools. In **Chapter 5**, we investigate if single-cell recordings of gray matter can depict the neurophysiological stimulation target. In **Chapter 6** we model the DBS sites in neuroanatomical space and explore the role of white matter in seizure control. We then propose an optimal stimulation site that can be visualized by high-resolution MRI.

In *Part 3* on computational studies, **Chapter 7** introduces a recent technique, termed 'lesion network mapping', which can map brain circuits using causal information from

brain lesions and brain stimulation. In **Chapter 8**, we apply this technique to brain lesions causing epilepsy and DBS sites treating epilepsy to identify a common brain network for lesion-related epilepsy. This brain network could potentially guide future (non)invasive brain stimulation trials for the treatment of drug-resistant epilepsy.

In **Chapter 9**, we close with a general discussion of the studies performed in this thesis and future perspectives for the field.

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Part I

ON ANIMAL STUDIES

"Science is about open-minded skepticism" …

"and skepticism is the easy part."

Mark George and Joseph Taylor

Chapter 2

Deep brain stimulation of the anterior nucleus of the thalamus in epilepsy: evidence from animal studies

Frédéric L.W.V.J. Schaper, Febe Colenbrander, Linda Ackermans, Govert Hoogland, Rob P.W. Rouhl

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Abstract

Deep brain stimulation of the anterior nucleus of the thalamus is a promising efficacious therapy for patients with drug-resistant epilepsy. However, responder rates vary highly among patients and it is unclear if these rates can be increased after surgery by further programming the settings of the pulse generator by the neurologist. An outstanding question for instance is what would be the optimal stimulation site and parameters in order to maximize seizure control. Since there are no published comparative clinical trials to date, we review the evidence from animal studies on the different stimulation paradigms in deep brain stimulation of the anterior thalamus for epilepsy.

DEEP BRAIN STIMULATION OF THE ANTERIOR NUCLEUS OF THE THALAMUS (ANT-DBS) is a promising, efficacious therapy for patients with drug-resistant epilepsy. While it has been reported that in 68% of the patients the long term seizure frequency decreases more than 50%, these responder rates are variable.[1] Possible causes for the differences in therapeutic response among patients are epilepsy type and electrode placement. Postoperatively, the neurologist can adapt the stimulation parameters to maximize the seizure suppressing effect and tailor neurostimulation treatment to the patient. However, the efficacy of different stimulation parameters in ANT-DBS has not been tested in comparative clinical trials. Here, we therefore summarize the available evidence gathered from animal studies on the effect of variable stimulation parameters of ANT-DBS on seizures.

EVIDENCE FROM ANIMAL STUDIES

Effects in acute models of epilepsy

In one of the first animal studies on ANT-DBS in rodents, the authors investigated if ANT-DBS can influence the amount of pentylenetetrazol (PTZ) necessary to induce seizures. Continuous high frequency stimulation with 100 Hz during seizure induction resulted in a 100% increase of PTZ dose necessary to induce a clonic seizure.[2] Another research group following the previous study design, repeated this study, but now using pilocarpine for seizure induction.[3] Similarly, ANT-DBS stimulated rats only had seizures after higher dosages of pilocarpine. In the kainate model for seizure induction, continuous ANT-DBS shortly after administration of kainate resulted in a lower seizure frequency (from 46 to 6 seizures per hour).[4] These first studies showed that high frequency ANT-DBS during chemically induced seizures could increase the seizure threshold.

Effects in chronic models of epilepsy

Only few studies perform ANT-DBS in animal models of chronic epilepsy to study the effects of stimulation on seizure frequency. In a pilocarpine model of chronic epilepsy in 20 rats, continuous stimulation with a frequency of 130 Hz and current intensity of 100 μA for 5 days reduced seizure frequency by 52%. However, this reduction was not statistically significant. In contrast, when increasing the current intensity to 500 μA, seizure frequency increased.[5] In an earlier study, continuous and intermittent ANT-DBS of mean 5.3 days with a frequency of 100 Hz and current intensity of 100-300 μA,

resulted in increased seizure frequency as well.[6] A possible explanation could be that in this study kainate was used to induce chronic epilepsy as opposed to pilocarpine in the previous study. Evidence on the efficacy of ANT-DBS in animal models of chronic epilepsy thus is scarce and inconclusive.

Uni- or bilateral stimulation

Two studies have compared unilateral versus bilateral stimulation. Zhang *et al.* performed ANT-DBS in amygdala kindled rats with chronic epilepsy.[7] Amygdala kindling is a procedure in which epileptogenesis is induced by unilateral electrical stimulation of the amygdala. The reduction of seizure frequency was 2 times higher after bilateral compared to unilateral ANT-DBS (frequency of 150 Hz, current intensity of 450-800 μA for 15 minutes). Zhong *et al.* used the same animal model and showed that bilateral low frequency stimulation (frequency of 1 Hz, current intensity of 200-500 μA for 15 minutes) significantly reduced seizure frequency, while ipsilateral ANT-DBS had no effect on seizures.[8] Although the stimulation parameters differ between the studies, bilateral stimulation, as commonly used in clinical practice, is shown to be more effective than unilateral stimulation.

The role of stimulation parameters

The effect of frequency (low vs. high frequency ANT-DBS) was interrogated in three studies. In the study conducted by Mirski *et al.*, high frequency stimulation with 100 Hz doubled the PTZ threshold to induce seizures compared to low frequency stimulation with 8 Hz. Even without administration of PTZ, low frequency stimulation evoked seizures.[2] In a similar study, induction of seizures after pilocarpine was independent of the stimulation frequency (130 Hz vs. 30 Hz).[9] However, neither of the studies were performed in chronic epilepsy models. In another study, low frequency stimulation with 1 Hz reduced both the frequency and severity of seizures in mice that experience spontaneous recurrent seizures after kainate injections, while high frequency stimulation with 100 Hz had no anti-convulsive effect.[10] The importance of the stimulation intensity has only been investigated in one animal study where in a pilocarpine model of chronic epilepsy, stimulation with 100 μA reduced seizures by 61% while stimulation with 500 μA increased seizure by a factor of 5.[5]

Thus, both high and low frequency ANT-DBS can reduce seizures and higher stimulation intensities can worsen seizures. However, thus far there is no scientific consensus towards these settings and it is even proposed there is a need to use tailored stimulation parameters in animal studies.[11]

Discussion

Considering the clinical application of ANT-DBS for patients with drug-resistant epilepsy, it is critical to explain the discrepancies in seizure reduction among patients, and optimize therapeutic efficacy. Drawing evidence from the current animal studies complements our understanding but can only partially explain this phenomenon. A possible avenue for improving the efficacy of ANT-DBS is the investigation of different stimulation parameters. However, research into the effect of various stimulation parameters has not been thorough. The described studies used different animal models or seizures of epilepsy, obstructing scientific translation. Currently, animal studies support the finding that bilateral stimulation, as performed in clinical practice, is superior to unilateral stimulation. Additionally, murine studies show that both low and high frequency stimulation have reduced seizures, while only high frequency DBS is used in clinical practice. To reduce the longevity of the battery, clinical ANT-DBS is performed with a cycle mode of 1 minute on and 5 minutes off stimulation. However, no animal studies have been performed with this cycle mode as used in patients. There is thus no evidence from animal studies to support the use of cycled ANT-DBS. Nevertheless, animal studies into the effect of DBS with different stimulation parameters can spark new ideas for DBS programming in patients with epilepsy, e.g. continuous stimulation with different frequencies. Based on these animal studies, neurologists could thus modify the stimulation parameters by tailoring frequency or intensity of stimulation. This is not only important for the treatment of seizures, but also to minimize potential side effects of ANT-DBS such as memory problems, agitation and sleep disturbances.

Conclusion

Many questions by neurologists on optimizing outcome after ANT-DBS by programming of the pulse generator remain unanswered. It is still unclear if every patient with drug-resistant epilepsy is a suitable candidate for ANT-DBS and if treatment of specific types of epilepsy requires particular stimulation parameters or even a specific stimulation site. To answer these outstanding questions and ultimately advance DBS for epilepsy towards a patient-tailored therapy, the combined efforts of translational animal researchers and clinical researchers is essential.

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Chapter 3

Cycled deep brain stimulation of the anterior nucleus of the thalamus improves spatial memory in a rat model of epilepsy

Frédéric L.W.V.J. Schaper, Soleil Garcia Brito, Sarah A. Hescham, Vincent C.J. van de Vlasakker, Erik Gommer, Vivianne van Kranen-Mastenbroek, Johan S.H. Vles, Yasin Temel, Linda Ackermans, Rob P.W. Rouhl, Govert Hoogland

In preparation

Abstract

Deep brain stimulation of the anterior nucleus of the thalamus (ANT-DBS) is a new treatment option for patients with drug resistant epilepsy. Yet, systematic case-control studies aimed at improving the efficacy and reducing side effects face ethical and experimental design challenges, and thus remain scarce. Here, we investigated efficacy and side-effects of cycled ANT-DBS applied continuously during seven consecutive days in a rat model of epilepsy. We assessed seizure frequency, short-term memory, anxiety and locomotion. In the limited number of animals that completed the study, we did not observe an effect of ANT-DBS on seizure frequency, but rather improved spatial memory performance.

INTRODUCTION

DEEP BRAIN STIMULATION OF THE ANTERIOR NUCLEUS OF THE THALAMUS (ANT-DBS) is a new treatment modality for patients suffering from drug resistant epilepsy. The choice for the ANT as suitable DBS target is based on a limited number of studies, showing that ANT-DBS reduces seizure frequency in patients[1,2] and prevents the induction of seizures in animals.[3,4] This empirical evidence led to a randomized clinical trial of ANT-DBS in 110 patients with drug resistant epilepsy (called the SANTE study) that reported long-term safety, seizure frequency reduction and improvement in quality of life.[5] Consequently, this therapy received CE marking and was approved by the U.S. food and drug administration as a possible treatment option for drug resistant focal epilepsy.[6] Although the results of the SANTE study seemed promising, it also showed strong inter-individual differences in treatment efficacy. Predictors of response for ANT-DBS are currently lacking.[7] In addition, side effects, such as depression, memory impairment, anxiety and sometimes even an increase in seizure frequency are observed.[8-11]

Animal models of chemically-induced seizures using pentylenetetrazol[3], pilocarpine[4] or kainate[12] have shown that ANT-DBS can increase the threshold for seizure induction. Only a few studies applied ANT-DBS in animal models of epilepsy, i.e. exhibiting spontaneous recurrent seizures, thus with high external validity.[13-15] Evaluation of treatment-induced behavioural changes to investigate side effects in these models is equally rare.[15] To date, these preclinical studies have shown contradictory outcomes reporting seizure frequency changes varying from a mean 52% reduction in the pilocarpine model[13], a mean 259% increase in one kainate model study[12] and a 30-50% decrease in another.[15] This may be partly explained by the applied stimulation parameters. Remarkably, no animal studies have tested the efficacy of cycled ANT-DBS of 1 minute on - 5 minutes off mode as used in clinical practice, but previous studies only included short and continuous stimulation paradigms. Hence, there still is a need for translational preclinical evidence on the efficacy and side effects of cycled ANT-DBS in animal models of epilepsy.

Here, we report on a rat model of epilepsy that allows the assessment of chronic ANT-DBS under controlled conditions. In this study, we addressed the hypothesis that cycled ANT-DBS reduces seizure frequency and has behavioural side effects on short-term memory and anxiety in this model.

METHODS

Animals

Twenty, adult male Sprague Dawley rats (Charles River, Sulzfeld, Germany) with a weight of approximately 300 g enrolled this study. Figure 3.1 shows a timeline of the procedures that the animals were submitted to. Animals were housed individually in Makrolon® cages under a reversed 12 h light : 12 h dark cycle (lights on 19:00h) with *ad libitum* food and water. This experiment was approved by the Animal Experiments and Ethic Committee of Maastricht University and complied with the Experiments on Animals Act according to the Dutch law.

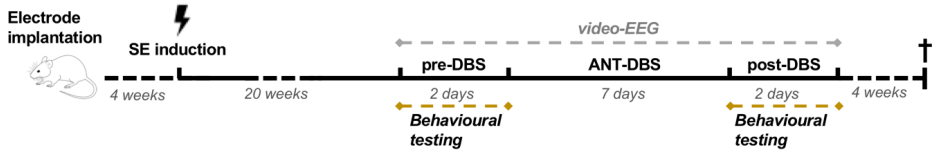

Figure 3.1 Overview of the study design and timeline. ANT-DBS was performed continuously for seven days using a stimulation cycle of 1 min on, 5 min off with the following stimulation parameters: 140 Hz, 100 µA, 100 µs monophasic pulse width. Behavioural testing (object location task, object recognition task and open field) was performed at pre- and post-DBS time points. *Abbreviations*: SE, status epilepticus; ANT, anterior nucleus of the thalamus; DBS, deep brain stimulation.

Electrode implantation

A detailed description of the stereotactical procedures and electrode design can be found elsewhere.[16] To reduce perioperative pain, rats received 0.1 mg/kg buprenorphine hydrochloride s.c. (Temgesic, Schering-Plough Inc., Amstelveen, The Netherlands) 30 min before implantation. General anesthesia was induced with 4-5%- and maintained with 1-2% isoflurane (IsoFlo®, Abbott Laboratories Ltd, Berkshire, Great Britain). Rats were mounted in a stereotactic frame (Dual Manipulator Lab Standard Sterotact, Stoelting Inc., Wood Dale, III, USA), after which the skull was exposed and the following three types of electrodes were implanted: 1. For DBS, custom-made bipolar platinum-iridium electrodes were bilaterally implanted in the ANT (coordinates relative to Bregma: posteriorly -1.5 mm, laterally +/- 1.5 mm, ventrally -5.2 mm). 2. As EEG reference, a Teflon®-coated stainless steel wire connected to a stainless steel plate was fixed with a screw on the midline anterior to Bregma and in line with the maxilla. 3. For EEG recordings and status epilepticus induction, a platinum iridium bipolar twisted

electrode (MS303/8-B/SPC, Plastics One, Roanoke, Virginia, USA) was implanted in the left hippocampal CA3 area (coordinates relative to Bregma: posteriorly -4.7 mm, laterally -5.0 mm, ventrally -5.0 mm). Electrodes were fixed to the skull using dental cement (Paladur, Heraeus Kulzer GmbH, Hanau, Germany).

Status epilepticus induction

Four weeks after electrode implantation, self-sustained limbic status epilepticus (SSLSE) was induced in all rats, i.e., 1 h hippocampal stimulation as described previously.[17,18] Briefly, a DLS100 stimulus isolator and a DS8000 Digital Stimulator (WPI, Sarasota, FL, USA) were used to deliver stimuli to the hippocampal electrode. Stimuli were administered for 9 min (stimulation cycle: 10 s on, 1 s off; stimulation parameters: 50 Hz, 400 μA, 1 ms biphasic pulse), followed by 1 min no stimulation. This 10 min protocol was consecutively repeated until status epilepticus (SE) was observed based on the EEG recording with concomitant behaviour. A maximum of six 10 min protocols were administered. Video-EEG was performed until the subject was returned to their home cage the following day.

Video-EEG recordings and DBS

Twenty weeks after SE induction, video-EEG recordings started to assess the frequency of spontaneous recurrent seizures.[17,19-21] During these recordings, rats were freely moving in a Perspex box (40 cm wide x 40 cm long x 80 cm high) with a sawdust covered bottom and *ad libitum* food and water. The electrodes were connected to an Octal Bio Amp and Powerlab (ADInstruments, Oxford, UK) through custom made electricity cables, which were rotatably connected to a swivel. Video-EEG recordings were made before (40 h), during (7 consecutive days) and after (40 h) ANT-DBS. Animals received 7 consecutive days of cycled ANT-DBS (24h/day). Due to the stimulation cycle (1 min on, 5 min off), animals were stimulated for 4 h daily (stimulation parameters: 140 Hz, 100 μA, 100 μs monophasic pulse width[17]), and video-EEG recordings were performed for 20 h daily using a table mounted webcam (Logitech, HD pro Webcam C920).

Seizures

Seizure frequency was assessed by two independent observers. Observer 1 (FS) evaluated video recordings for the occurrence of generalized seizure behavior according to Racine's scale stage 4-5.[22] To reduce the evaluation time, a selection of video data was reviewed. This selection was based on abnormalities in the power spectrum (e.g. electrodecremental events, high frequency spiking or synchronous activity between 15

and 45 Hz) of the hippocampal EEG (filtered between 0.95 and 45 Hz). When seizure behavior on video was concomitant with typical epileptiform discharges on the hippocampal EEG (Figure 3.2), it was scored as a seizure. Observer 2 (VvdV) evaluated only seizure behavior in the complete video recording (24 hours each) of 21 randomly selected video-EEG recording days out of the total 55 video-EEG recording days (i.e., 5 animals * 11 video recording days per animal). The inter-observer variability in assessment of seizure frequency was expressed by a Pearson's correlation coefficient.

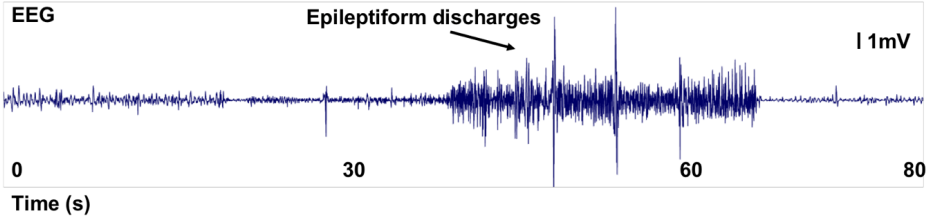

Figure 3.2 Example of typical epileptiform discharges in the hippocampal EEG during a generalized seizure.

Behavioural testing

To investigate the effects of ANT-DBS on behaviour, animals were subjected to an object location task (OLT) for spatial memory, an object recognition task (ORT) for recognition memory and an elevated zero maze (EZM) for anxiety-related behavior before (pre-DBS) and after ANT-DBS (post-DBS).[17,23]

The circular arena used for both, the OLT and ORT, had an 83 cm diameter and 40 cm polyvinyl chloride high walls of which half was grey and the other half transparent. In the first trial (T1), two identical objects are symmetrically positioned to each other and 10 cm away from the wall. A rat was placed in the arena by facing the middle of the wall to consequently explore for 4 min before it was returned to its home cage. Four different sets of standardized objects were used in a balanced manner to minimize potential bias due to preferences for particular objects. After an interval of 1 h, one object was replaced by another object with a different shape (ORT) or one object was displaced 15 cm (OLT) and the animal was reintroduced in the arena for 4 min (T2). The time spent exploring each object was recorded during T1 and T2. Object exploring behaviour was defined as: sniffing at the object and pointing towards the object within a distance of 2 cm. Climbing on the object was not considered exploratory behavior. Performance on discrimination between the old and the novel object in the ORT and the displaced object in the OLT, was defined by the discrimination index (d2). D2 was calculated as

follows: (time spent at the novel/displaced object – time spent at the old object) / (time spent at the novel/displaced object + time spent at the old object).
The EZM consisted of a circular runway with diameter of 98 cm and a path width of 10 cm, placed 70 cm above the floor and divided in two open parts without walls and two parts with 50 cm high black walls. Animals entered the runway in an open part and the time spent in the open and enclosed parts was recorded during a 5 min trial. To exclude effects of locomotion on this test, animals were introduced in the center of an open field (OF) of 1 m x 1 m with 40 cm high transparent walls and a dark floor. The total distance moved during a 10 min trial was measured. Automated tracking of animals in the EZM and OF was performed using Noldus EthoVision XT tracking software (Noldus Information Technology, Wageningen, The Netherlands).

DBS electrode localization

After post-ANT-DBS behavioural testing, rats were sacrificed by administration of pentobarbital (120–180 mg/kg depending on body weight, i.p.) and transcardial perfusion with 0.9% NaCl, followed by perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.6). Next, brains were removed, postfixed in the same perfusion solution (1 day, 4°C), cryoprotected by subsequent incubation in 10% sucrose in 0.1 M phosphate buffer (1 day, 4°C) and 20% sucrose in 0.1M phosphate buffer (2 days, 4°C), and finally snapfrozen with CO_2 and stored at -80°C. To verify electrode positions, 30 µm cryosections were cut in the coronal plane, mounted on gelatin coated glass slides, stained with haematoxylin-eosin and photographed under bright field microscopy. DBS electrode tips were localized by one observer (FS) in reference to the rat brain atlas of Paxinos and Watson (Elsevier 2006).

Statistical analyses

Seizure frequency and performance on behavioural tests pre- and post-DBS were compared using a paired sample, two-tailed t-test. Interobserver reliability of seizure frequency scoring was calculated using a Pearson correlation coefficient. A $p<0.05$ was considered statistically significant.

RESULTS

Animals

In total, five rats completed the full experiment and were included in the analyses. Seven rats died during SE and three rats were resistant to SE induction. In the course of the experiment, five animals were sacrificed because they had reached a humane endpoint, i.e., three rats lost their electrode construct, one rat suffered from a persistent scratch wound around the electrode construct and another rat experienced progressive weight loss.

ANT-DBS electrode localization

Post-mortem histological analysis showed that all ANT-DBS electrodes had indeed been implanted in the ANT. In 3 animals, the bilateral electrodes had been implanted in the ventral ANT and in 2 animals they had been implanted in the dorsal ANT (Figure 3.3). There were no signs of histological damage.

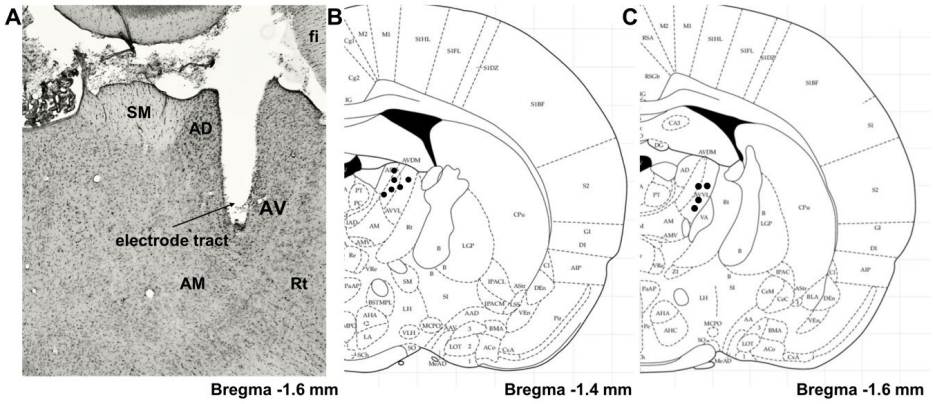

Figure 3.3 Photomicrograph showing the electrode tract in the right ANT in a haematoxylin-eosin stained section at -1.6 mm relative to Bregma (**A**). Schematic representation of the location of the electrode tips (black circles) in the ANT at -1.4 mm (**B**) and -1.6mm (**C**), relative to Bregma. Abbreviations: AM, anteromedial; AV, anteroventral; AD, anterodorsal; Rt, reticular thalamic nucleus; SM, stria medullaris of the thalamus; fi, fimbriae. Modified from the rat brain atlas of Paxinos and Watson (Elsevier 2006).

Seizure frequency

The interobserver variability of seizure frequency scoring as assessed by the video-EEG (FS) and video-only (VvdV) observations in 55 randomly selected video-EEG recordings showed a Pearson correlation coefficient of 0.94 (p<0.0001). On a group level, the seizure frequency counts by observer 1 did not differ significantly when pre- and post-ANT-DBS were compared (Figure 3.4). On an individual level and in the course of ANT-DBS, the seizure frequency strongly varied (Figure 3.5). Roughly, three patterns of changes in seizure frequency may be recognized during ANT-DBS: 1. a decrease (rat 4), 2. an increase followed by a decrease (rats 1 and 5) and 3. a gradual increase in seizure frequency (rats 2 and 3). Interestingly, animals in which the electrodes were implanted more ventrally (rats 1, 4, and 5) showed a slightly better treatment effect than animals in which the electrodes were implanted more dorsally (rats 2 and 3).

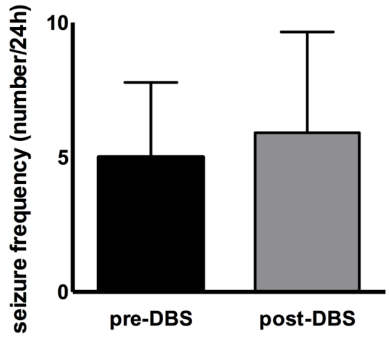

Figure 3.4 Seizure frequencies pre- and post-ANT-DBS on a group level. Frequencies are expressed as mean + standard error of the mean of n=5 animals.

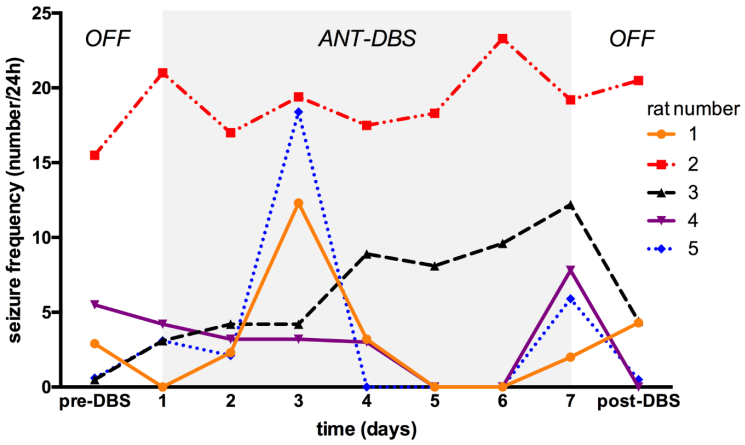

Figure 3.5 Seizure frequencies pre-, during and post-ANT-DBS on an individual subject level.

Behavioural testing

Performance in the OLT significantly improved at the post-ANT-DBS time of assessment, compared pre-ANT-DBS testing (85% increase, p=0.0265, Figure 3.6A). In contrast, performance in the ORT remained unchanged when pre- and post-ANT-DBS measurements were compared (Figure 3.6B). In the EZM, the time spent in the closed arms did not differ when pre- and post-ANT-DBS testing were compared (Figure 3.6C). Also, the mean distance moved in the OF did not differ when pre- and post-ANT-DBS testing were compared (Figure 3.6D).

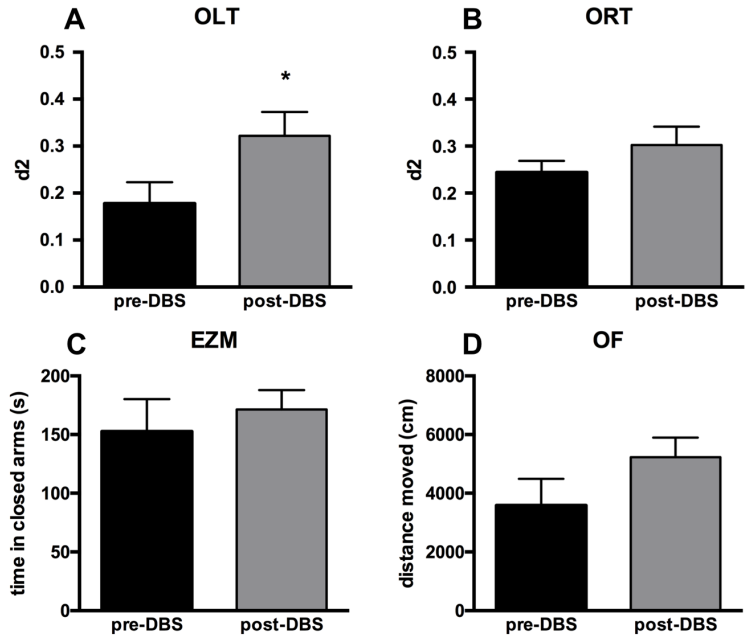

Figure 3.6 Pre- and post-ANT-DBS performance on recognition (ORT; **A**) and spatial memory (OLT; **B**), anxiety (EZM; **C**) and locomotion (OF; **D**) during behavioural testing. Data are expressed as mean + standard error of the mean of n=5 animals. * indicates a $p<0.05$.

Discussion

To the best of our knowledge, this is the first study that applied cycled ANT-DBS for 7 consecutive days using clinically relevant stimulation parameters in a rat model of epilepsy to test treatment efficacy and behavioural side effects. In the limited number of animals that completed the study, we did not observe an effect of cycled ANT-DBS on seizure frequency. In this animal model of epilepsy, characterized by reduced spatial memory[17], cycled ANT-DBS improved spatial memory, yet recognition memory, anxiety and locomotion remained unchanged.

ANT-DBS in models of epilepsy

Evidence on the efficacy and side effects of ANT-DBS in models of epilepsy is scarce. In the pilocarpine model of epilepsy, Hamani *et al.*[4] found that ANT-DBS with a low current (100 μA) resulted in a non-significant seizure frequency reduction, yet high

current (500 μA) ANT-DBS increased the seizure frequency significantly by 2.8-fold. Our study complements these results by showing that stimulation with a low current (100 μA) can improve spatial memory in the SSLSE model[17], while similarly having limited effects on the seizure frequency. Another study by Wang *et al.*[15] reports alleviation of memory impairment and a 50% reduction of spontaneous recurrent seizures in the intrahippocampal kainate mouse model of epilepsy only by low (1 Hz)- but not by high (100 Hz)-frequency ANT-DBS. In their study, ANT-DBS was performed continuously for 30 min daily during seven days, mice behaviour was tested in the OLT and ORT before and after DBS and seizure frequency was evaluated. Next to an improved spatial and recognition memory after low-frequency ANT-DBS, they observed an antiepileptic effect of low-frequency ANT-DBS. This was concluded based on a reduction of interictal spikes and high frequency oscillations. However, high-frequency ANT-DBS had the opposite effects. Low-frequency ANT-DBS may thus be a critical stimulation parameter to control seizures and improve cognition. This is furthermore supported by evidence from experimental stimulation studies in sheep[24] and epilepsy patients.[25] This scarce amount of data suggests that optimal stimulation parameters may further improve the treatment efficacy and thus deserve further investigation in animal models of epilepsy and randomized controlled clinical trials in humans.

ANT-DBS and spatial memory

The role of the ANT in spatial memory has mainly become apparent by ANT lesion and stimulation studies. In humans and animals, lesions of the mammillothalamic tract and ANT can result in prominent symptoms of memory loss.[26,27] Stimulation studies in animals have shown that electrical stimulation of the ANT can modulate spatial memory, presumably through its key connections to the hippocampus within the Circuit of Papez.[28]

Several studies have investigated the effects of ANT-DBS in animal models of memory impairment, such as Alzheimer's disease[29,30] and experimental dementia.[23] Hamani *et al.*[31] reported that 1 h of continuous ANT-DBS applied one month before behavioural testing, reversed corticosterone-induced working memory deficits in a delayed non-matching to sample task, suggesting long-term neuroplastic effects. In wild-type rats, they[32] showed that continuous stimulation at high current (500 μA) disrupted spatial memory and reduced the spontaneous firing rate in the hippocampus. A low current (100 μA), as used in our study, did not change spatial memory and increased the spontaneous firing rate in the hippocampus.

Despite considerable research efforts, little is known about the mechanism(s) of action of DBS.[33,34] The ANT is connected with the hippocampus through reciprocal direct connections and indirect connections along the mammillary bodies and the fornix.[28] Since the hippocampus is essential for spatial memory[35], our findings indicate that cycled ANT-DBS may have a circuit-wide effect. Other research suggests that hippocampal firing[32] and EEG rhythm[15], adenosine release[36,37], acetylcholine release[38,39] and neurogenesis[31] may play a role in cognitive improvement by ANT-DBS. Whether the beneficial effect on spatial memory following ANT-DBS in the present study is mediated by electrical, neurochemical, neuroplastic effects or another mechanism of action remains to be determined.

Limitations

Due to the unexpectedly large loss to follow-up, the power of the current study is limited. Moreover, the design of a within subject evaluation cannot exclude a lesional effect caused by the electrode implantation. However, as electrodes were implanted long before the EEG recordings were performed, it seems unlikely that microlesions in the ANT are a cause of the observed changes in seizure frequency and behaviour. This study furthermore demonstrates that even in a controlled experimental setup, studies of long-term DBS in epilepsy models show a substantial inter-individual variability similar to other studies.[13] Some of these limitations may be overcome by designing future studies on ANT-DBS in animal models of epilepsy to apply continuous long-term and daily EEG monitoring, automated seizure detection algorithms for focal and generalized seizures and neurophysiological outcome measures of cortical excitability.

Conclusion and future perspectives

Cycled (1 min on, 5 min off; stimulation parameters: 140 Hz, 100 μA, 100 μs monophasic pulse width), chronic ANT-DBS in the SSLSE model of epilepsy improves spatial memory and does not affect recognition memory, locomotion and seizure frequency. Future studies on chronic ANT-DBS in animal models of epilepsy are needed to select the stimulation parameters that result in both seizure control and cognitive improvement.

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Part II

ON HUMAN STUDIES

"One of the great challenges in life is knowing enough to think you're right, but not enough to know you're wrong."

Neil deGrasse Tyson

Chapter 4

Deep brain stimulation of the anterior nucleus of the thalamus for drug-resistant epilepsy

Tim A.M. Bouwens van der Vlis, Olaf E.M.G. Schijns, Frédéric L.W.V.J. Schaper, Govert Hoogland, Pieter Kubben, Louis Wagner, Rob Rouhl, Yasin Temel, Linda Ackermans

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Abstract

Despite the use of first-choice anti-epileptic drugs and satisfactory seizure outcome rates after resective epilepsy surgery, a considerable percentage of patients do not become seizure free. ANT-DBS may provide for an alternative treatment option in these patients. This literature review discusses the rationale, mechanism of action, clinical efficacy, safety, and tolerability of ANT-DBS in drug-resistant epilepsy patients. A review using systematic methods of the available literature was performed using relevant databases including Medline, Embase, and the Cochrane Library pertaining to the different aspects ANT-DBS. ANT-DBS for drug-resistant epilepsy is a safe, effective and well-tolerated therapy, where a special emphasis must be given to monitoring and neuropsychological assessment of both depression and memory function. Three patterns of seizure control by ANT-DBS are recognized, of which a delayed stimulation effect may account for an improved long-term response rate. ANT-DBS remotely modulates neuronal network excitability through overriding pathological electrical activity, decrease neuronal cell loss, through immune response inhibition or modulation of neuronal energy metabolism. ANT-DBS is an efficacious treatment modality, even when curative procedures or lesser invasive neuromodulation techniques failed. When compared to VNS, ANT-DBS shows slightly superior treatment response, which urges for direct comparative trials. Based on the available evidence ANT-DBS and VNS therapies are currently both superior compared to non-invasive neuromodulation techniques such as t-VNS and rTMS. Additional in-vivo research is necessary in order to gain more insight into the mechanism of action of ANT-DBS in localization-related epilepsy which will allow for treatment optimization. Randomized clinical studies in search of the optimal target in well-defined epilepsy patient populations, will ultimately allow for optimal patient stratification when applying DBS for drug-resistant patients with epilepsy.

INTRODUCTION

EPILEPSY IS A COMMON CHRONIC NEUROLOGICAL DISORDER characterized by spontaneous recurrent seizures and affects around 70 million patients worldwide.[1] Of these patients, over 30% will suffer from persistent seizures despite (optimal) anti-epileptic drug (AED) regimens.[2] Drug-resistant epilepsy is defined as a failure of two adequate trials of AEDs that are appropriate for the person's disease.[3] The pathogenesis of drug-resistant epilepsy is not completely understood. However, both biological mechanisms and environmental factors are known to contribute to the development of drug resistance.[4] Persistent epileptic seizures and long periods of incomplete seizure control have profound social, physical, and psychological consequences leading to a decline in quality of life and impose a financial burden.[5] Moreover, patients with epilepsy are at risk for sudden unexpected death (SUDEP) which is a prominent cause for the elevated mortality-ratio in chronic epilepsy. Its annual incidence ranges from 0 to 10 per 1000 in epilepsy surgery candidates.[6] Risk factors include frequent generalized tonic-clonic seizures, AED polytherapy and an early onset of drug-resistant epilepsy.[7]

If the epileptogenic focus or network can be localized and if the benefits outweigh the risks, resective surgery is effective when compared to medication treatment alone.[8,9] A meta-analysis estimated that 67% of epilepsy patients showing MRI abnormalities treated with surgery were seizure free at 1 year vs. only 55% in patients with absent MRI abnormalities.[10,11] Patients who cannot benefit from curative, resective surgery, can be referred for neuromodulation therapy, e.g., vagal nerve stimulation (VNS) or deep brain stimulation (DBS). In the case of DBS, several anatomical targets have been identified for neuromodulation of drug-resistant intractable epileptic seizures including the centromedian nucleus (CM) of the thalamus, the hippocampus, and the anterior nucleus of the thalamus (ANT), the latter of which gained widespread attention after the publication of the SANTE trial, a large double-blind, randomized trial in 110 patients with localization-related epilepsy.[12,13]

Here, we present a review of DBS of the anterior nucleus of the thalamus in patients with drug-resistant epilepsy and discuss its rationale, clinical efficacy, safety, tolerability, and mechanism of action. Further, we will discuss future steps of identifying DBS as a third line treatment modality in drug-resistant epilepsy, within the spectrum of neuromodulation techniques.

METHODS

Literature for this review was identified searching Medline, Embase, and the Cochrane Library databases from the date of the first available article until September 2017. The following keywords were queried either individually or combined: deep brain stimulation, epilepsy, anterior nucleus of the thalamus, complications, and mechanism of action. The search was limited to studies published in English.

RATIONALE

ANT anatomy and function

The ANT is situated in the rostral end of the dorsal thalamus and is separated from the rest of the dorsal thalamus through a Y-shaped internal medullary lamina. The ANT consists of the anteroventral (AV), anterodorsal (AD), and anteromedial (AM) subnuclei. As part of the limbic circuit of Papez, the ANT receives input from the hippocampal subiculum either directly via the fornix or indirectly via the mammillothalamic tract from the mammillary bodies (MB). Other afferents to the ANT originate from the anterior and posterior cingulate cortex, retrosplenial cortex, and the inferior parietal lobule.[14] Many of these cortical connections are reciprocal. Its putative functions comprise the involvement in relay of visceral and emotional information to prefrontal areas (AM), the modulation of alertness and as a component of an 'extended hippocampal system' in different aspects of learning, episodic memory and in spatial navigation (AD). The majority of neurons in the AV subnucleus fire synchronous with the hippocampal theta frequency, which has been implicated in spatial cognition.[15,16]

ANT and epilepsy

The recognition of the putative role of the ANT in epilepsy, emerged from several animal studies in the second half of the twentieth century. In a model of focal cortical epilepsy in Rhesus monkeys, ipsilateral lesions in the ANT led to a significant decrease in frequency and duration of seizure generalization.[17] Further, pharmacologically mediated inhibition of the ANT in guinea pigs with bilateral injection of the γ-aminobutyric acid (GABA) agonist muscimol showed suppression of high-voltage synchronous EEG activity and behavioral components of pentylenetetrazol (PTZ) induced seizures, in a dose-dependent manner.[18] Correspondingly, bilateral ANT

injections of γ-vinyl-γ-aminobutyric acid (vigabatrin), a suicide inhibitor of the enzyme GABA-transaminase, produced significant protection against PTZ-induced tonic-clonic seizures in rats.[18,19] The functional role of the ANT in PTZ-induced seizure propagation was therefore hypothesized to be a relay nucleus to mediate paroxysmal activity between its associated subcortical structures and cerebral cortex. This was supported by earlier findings that lesions in the mammillothalamic tract significantly attenuated EEG activity and lethal effects of PTZ. Furthermore, chronic stimulation or single shock of either the fornix, MB, mammillothalamic tract or the ANT, induced cortical EEG discharges including seizure like activity.[20,21] The latter of which implicated involvement of the Papez circuit in seizure propagation.

Subsequently, it was reported that specific electrical stimulation of the MB resulted in a protective effect against seizures.[22] In agreement with neuroanatomical identified MB-ANT connections, bilateral high frequency stimulation at 100 Hz (300-500 μA) of the ANT in rats doubled the dosage of PTZ required to elicit clonic motor seizures, but did not alter the expression of low dose PTZ-induced cortical bursting. High frequency stimulation of the ANT leads to EEG desynchronization, rendering the cortex less susceptible to seizures.[22,23] In contrary, low frequency stimulation with 8 Hz proved to be a proconvulsant stimulus, as it lowered the threshold for early EEG paroxysmal bursts.[23] Although these findings support the concept of ANT mediation of cortical-subcortical interactions in PTZ-induced seizures, the specific synaptic or membrane mechanism of electric stimulation remained incompletely understood. The necessity of bilateral ANT stimulation was affirmed in a pilocarpine model of secondarily generalized seizures in rats. Whereas unilateral anterior nucleus thalamotomy elicited no effect on pilocarpine-induced propensity or latency of developing seizures and status epilepticus, bilateral ANT stimulation significantly delayed the time to status epilepticus.[24]

ANT stimulation in drug-resistant epilepsy: efficacy and safety in the pre-SANTE era

The first clinical case series with thalamic lesioning for the control of epilepsy date back to 1967.[25] Due to its involvement in seizure propagating circuitry (corticothalamic, mammillary, and the Papez circuits) Cooper and Upton, hypothesized in 1985 that "stimulation of the anterior nucleus of the thalamus should produce suppression of abnormal neural discharge within the limbic system".[26,27] In 1987, they described the bilateral ANT stimulation in six patients with drug-resistant complex partial seizures, which resulted in significant clinical control of the seizures in four of these patients. Subsequent to Cooper and Upton, several studies reported on the efficacy of bilateral

ANT-DBS in drug-resistant epilepsy patients. The studies published in the pre-SANTE era are summarized in Table 4.1.[28-32]
The pre-SANTE studies show a variable treatment efficacy, which may be explained by the significant differences between the studies, including seizure type, follow-up and ANT-DBS stimulation parameters. Regarding the latter, initial stimulation parameters were based on experimental evidence, experience with stimulation of the central median nucleus of the thalamus for epilepsy and STN-DBS for Parkinson's disease.[31,32] All studies collectively concluded that ANTDBS is a safe and well tolerable procedure, with minimal adverse events. Only one study reported a case of wound infection requiring system removal. Similar to the experience of DBS in movement disorders, the authors discuss a microthalamotomy effect, defined as a reduction in or abolition of symptoms with insertion of DBS alone. Hodaie et al. observed no additional seizure reduction after stimulation initiation and no increase in seizure frequency after stimulation cessation. In contrast, Kerrigan et al. report on an acute exacerbation of seizure frequency after discontinuation of stimulation, reversed by resuming stimulation. Osorio et al. did not observe the microthalamotomy effect.

SANTE TRIAL

These encouraging results culminated with the publication of a randomized double-blind controlled trial of Stimulation of the Anterior nuclei of Thalamus for Epilepsy (SANTE) which enrolled 110 patients with localization-related epilepsy.[12] One month after bilateral ANT implantation, patients were randomly assigned to a regime of stimulation (n = 54, 145 Hz, 5 V, 90 μs, 1 min on/5 min off) or no stimulation (n=55, 0 V). After the 3 months blinded phase, all patients received stimulation. At month13, all patients entered long-term follow-up in which stimulation parameters and AEDs varied freely. The long-term (5 years) efficacy and safety of this trial was reported in 2015.[34]

Efficacy

At the end of the blinded phase, the stimulation group showed a relative greater estimated reduction of seizure frequency compared to the non-stimulated group with a difference of 29% (p=0.0023). Secondary outcome measures: 50% responder rate, Liverpool Seizure Severity Scale (LSSS), and the Quality of life in Epilepsy (QoLIE-31) did not significantly differ at the end of the 3-month blinded phase. However, compared

to baseline, all measures showed significant improvement at the end of the unblinded phase. At month 13 and 25, the median seizure frequency reduction was 41 and 56%, respectively, with corresponding 50% responder rates of 43, 54, and 67% at 37 months. Self-reported seizure severity decreased by 40% in the stimulated group compared to 20% in the control group (p=0.047). Both LSSS and QoLIE-31 significantly improved at 13 and 25 months.[12]

Long-term follow-up at 5 years showed a gradual increase of the mean percentage seizure reduction to 69%, with 11 participants reporting seizure freedom for at least 6 months. The 50% responder rates improved to 68% at 5 years.[34] The authors convincingly refute the confounding effect of discontinuation in the trial, due to poor response on improved outcome in terms of seizure reduction.

However, the increased response rate may be influenced by these drop-outs during the 5-year follow-up period. In addition, the gradual prolonged increase (1-5 years) of the beneficial effect of ANT-DBS may also be influenced by additional AED regiment changes, tailoring of stimulation parameters and/or progressive improvement with stimulation.[35,33]

Safety and tolerability

Reported adverse events (AE) at any time after implantation were most commonly hardware related (22.7%) consisting of paresthesia (18.2%), implant site pain (23.6%), implant site infection (12.7%), and electrode misplacement (8.2%). Procedural related AE such as intracerebral hematoma occurred in 4.5% of the patients, none of which were symptomatic.

There were no observed deaths during the operative month or 3-month double-blind phase. In total there were seven deaths during the study: one due to suicide, two definite, and two possible SUDEP, one due to cardiorespiratory arrest, and one died from liver cancer. None of which were considered to be device related by the authors.

Of the 105 participants entering the long-term follow-up, 30 discontinuations were reported (including six deaths, one before device implantation). Of these, 14 comprised device explants (implant site infection (2), device ineffectiveness (7), neuropsychological disorder (3), meningitis (1), and an undesirable change in stimulation (1)).

Although neuropsychological test scores for mood and cognition did not differ between the control and stimulated groups at the end of the blinded phase, significantly more patients in the stimulated group reported on AE relating to depression (14.8%) and memory impairment (13%) compared to the control group (1.8%, 1.8%). Depression related symptoms were reported in 32.7% and memory impairment in 27.3% of the patients during long-term follow-up. At 5-year follow-up several components of the

neuropsychological examination showed gradual improvement from baseline including attention, executive function, depression, tension/anxiety, total mood disturbance, and subjective cognitive function. This paradoxical outcome regarding self-reported depression related symptoms and objective neurobehavioral testing was recently addressed by an in-depth and long-term analysis.[36] During the 7-year open label period, patients with prolonged ANT stimulation showed no cognitive decline or worsening of depression scores. In contrary, higher scores in executive functions and attention were observed at 7 years.[36]

ANT STIMULATION IN DRUG-RESISTANT EPILEPSY: EFFICACY AND SAFETY IN THE POST-SANTE ERA

In the years after the publication of the SANTE trial, several case series further reported on the efficacy and safety of ANTDBS in drug-resistant epilepsy, presented in Table 4.2.[37-39] A case series of Piacentino et al. who qualitatively describe a cohort of six individual ANT-DBS patients is not included in this table. However, they report on a mean seizure reduction rate of more than 50%in patients with temporal lobe epilepsy.[40] AE reported by these case series comprise of implant site infection (three) of which two required hardware removal, and severe stimulation induced agitation requiring stimulation cessation. In concordance with the SANTE trial, one patient reported an increase in seizure frequency of 200% compared to baseline following stimulation initiation. With regard to the responding patients, Krishna et al. describe three patterns of seizure control (1) sustained (>50%) seizure frequency reduction without stimulation initiation (prolonged insertional effect) (2) immediate stimulation effect: an increase in seizure frequency reduction immediately associated with stimulation initiation and (3) delayed stimulation effect: a decrease in mean seizure frequency with continued stimulation after initial failure of seizure reduction.

Of note, an insertional effect was observed in 56% of the patients. Interestingly, in a case series reporting on seizure outcome after battery depletion, one patient with ANT-DBS and 3 years of continuous stimulation did not show a change in seizure frequency 6 months after battery depletion, either implicating a prolonged insertional effect or definite epileptic network modulation, or reflecting the natural course of epilepsy.[41] Lee et al. only observe a prolonged stimulation effect, as their study design rules out the possibility of a prolonged insertional effect, in which short-term outcomes remarkably associated with long-term seizure control. Regarding long-term cognitive functioning, Oh et al. report on slight improvement on fluency tasks and delayed verbal memory.

Table 4.1 Pre-SANTE clinical studies showing the efficacy of ANT-DBS in intractable epilepsy. GTCS generalized tonic-clonic seizures, CPS complex partial seizures, DA drop attacks, SGTC secondary generalized tonic-clonic seizures, SPS simple partial seizures, TS tonic seizures, HMS hypermotor seizures, AMS automotor seizures. Also reviewed in.[33]

Authors, year	N	Seizure type	Stimulation parameters	Follow-up (months)	Mean seizure (%) reduction at last follow-up
Cooper et al., 1987	6	CPS	60–70 Hz, 3.5–3.8 V, 300 μs	42	n/a
Hodaie et al., 2002	5	GTCS, CPS, DA, CPS, SGTC	Hz, 10 V, 90 μs, 1 min on/5 min off, alternating left and right sides	12-21	54% (24-89%)
Kerrigan et al., 2004	5	SPS, CPS, SGTC	100 Hz, 1–10 V, 90 μs, 1 min on/10 min off, 5 min offset	6-36	48% (57-98%)
Osorio et al., 2007	4	CPS, SGTC, DA, SPS	175 Hz, 4.1 V, 90 μs, 1 min on/5 min off	36	75.6% (53-92%)
Lee et al., 2006	3	TS, DA, HMS, AMS, SGTCS	130 Hz, 1.5–7 V 90 μs, 1 min on/5 min off, alternating left and right sides	2-30	75.4% (50-90.6%)
Lim et al., 2007	4	GTCS,CPS, SPS SGTCS	90–110 Hz, 4–5 V, 60–90 μs, continuous	33-48	49% (35-76%)

Table 4.2 Post-SANTE clinical studies showing the efficacy of ANT-DBS in drug-resistant epilepsy. CPS complex partial seizures, SGTC secondary generalized tonic-clonic seizures, DA drop attacks, MC myoclonic, GTCS generalized tonic-clonic seizures

Authors, year	N	Seizure type	Stimulation parameters	Follow-up (months)	Mean seizure (%) reduction at last follow-up
Krishna et al., 2012	16	CPS, SGTC, DA, SPS, GTSC	100–185 Hz, 2.4–7V,90μs, 1 min on/5 min off	51.6	11.5% (– 400–99%)*
Lee et al., 2012	15	CPS, GTCS	100–185 Hz, 1.5–3.1 V, 90-150 μs, continuous	24-67	70.51 (0–100%)
Oh et al., 2012	9	CPS, SGTC	10–0185 Hz, 1.5–3.1 V, 90–150 μs, continuous	22-60	57.9% (35.6–90.4%)

*Median decrease seizure frequency for the whole cohort (n=16)

TREATMENT RESPONSE

Although the efficacy and safety of ANT-DBS in drug resistant epilepsy patients was convincingly shown in the SANTE trial, questions remain about the variability of responsiveness to treatment. In addition, two patients displayed a paradoxical response to ANT-DBS: one patient in the SANTE trial suffered from 210 brief partial seizures corresponding to the on-off cycle of stimulation in the blinded phase and one patient had a 200% increase of seizure frequency reported by Krishna et al. The variability of the treatment effect of ANT-DBS may be partially explained by the localization of the seizure onset zone, as patients with a seizure origin in one or both temporal lobes showed a greater response to ANT stimulation when compared to extratemporal, or multiple seizure onset-zones.[12,33] Another explanation may be sought in the influence of the anatomical position of the active electrode on clinical outcome, as this could generate differential activation patterns by preferential stimulation of different subnuclei. In the SANTE trial, the DBS electrodes were placed presumably using a direct targeting method, therefore solely relying on its relative anatomical position of the ANT within the thalamus, and comparisons to the Schaltenbrand and Wahren atlas (SWA). The position of the active electrode within the ANT was verified visually on a post-operative magnetic resonance imaging (MRI). The role of micro-electrode recording (MER) in targeting the ANT and improving clinical response is unknown and not routinely applied. Interestingly, although not found to be clinically relevant, a post-hoc analyses of the SANTE study participants revealed that almost 10% of the electrodes were not within the limits of the ANT[42] via.[43]

Recent proposed 3T MRI short tau inversion recovery and 1.5T T1 weighted magnetization prepared gradient echo (MPRAGE) images allow for visual delineation of the ANT. The imaging protocols are capable of clearly visualizing the anatomical boundaries of the ANT (mammillothalamic tract and the external medullary lamina).[44,45] As these imaging protocols were unavailable at the start of SANTE trial, this may provoke uncertainty about the exact location of the active electrodes. Particularly when considering the significant volumetrical and microstructural changes of the thalamus associated with increasing age; especially the anterior thalamus including the anterior-ventroanterior and dorsomedial nucleus.[46] In addition, ipsilateral thalamic atrophy has been demonstrated in patients with temporal lobe epilepsy but not in extratemporal and idiopathic generalized epilepsy.[47] Patients with mesial temporal lobe epilepsy show specific atrophy of the ANT, medial dorsal nucleus, and the medial pulvinar nucleus, with a concomitant decrease of thalamohippocampal connected volume.[48] Recent cohorts of ANT-DBS patients revealed a better clinical response when the active

electrode was located within the anterior aspect of the ANT.[37,49] Furthermore, non-responding to responding conversion was observed in four out five patients after re-programming the IPG to activate the most cranial contact.[49] Clear preoperative visualization of the ANT therefore is likely to reduce the variability of responsiveness and may therefore further increase treatment effectiveness. Another cause of treatment variability may be sought in defining the optimal trajectory to the ANT. A transventricular approach is more susceptible to lead misplacement due to penetration of the lateral ventricles. However, other neurosurgeons advocate a transventricular approach as they observe an increased feasibility in reaching the ANT and less hardware related events.[49]

MECHANISM OF ACTION

Despite its widespread clinical use, the exact mechanism of action of electrical stimulation on the central nervous system remains poorly understood. Initial hypothesis about the mechanism of DBS were based on the similarity between ablative procedures and high frequency stimulation with regard to treatment effect. High-frequency stimulation was therefore thought to function as a reversible lesion by inhibiting neurons near the stimulating electrode.[50-52] However, progressive understanding revealed that electrical fields have differential effects on different neuronal structures.[53] High-frequency thalamic DBS results in regions of both activation (axonal, within 2 mm of the electrode) and suppression (subthreshold, more than 2 mm of the electrode), where activation generates axonal output at the stimulus frequency.[54] Consequently, DBS may override or "hijack" the neural circuitry by blocking pathological activity and replacing efferent output.[51] Further evidence that ANT-DBS induces network modulation rather than simply inducing a local functional lesion arose from EEG and fMRI data. Indeed, ANT-DBS results in a pattern of cortical activation corresponding to the hodology of the ANT and therefore includes the Papez circuitry. Furthermore, the differential distribution of cortical activation is hypothesized to be dependent on the relative anatomical location of the active electrode within the ANT. Of note, cortical activation patterns are strongly dependent on stimulation amplitude and susceptible for considerable inter-and intra-individual variation.[55,56]

Other mechanisms by which ANT-DBS may remotely modulate neuronal network excitability is through local molecular hippocampal alterations. Unilateral ANT stimulation in kainic acid (KA) induced seizures in rats provoked decreased levels of glutamate and aspartate and an increase of GABA concentration in the ipsilateral CA3

region of the hippocampus.[57] This phenomenon has also been observed in the hippocampi of rhesus monkeys with mesial temporal lobe epilepsy induced by KA, indicating that ANT-DBS remotely inhibits KA-induced excitatory hyperactivation.[58] Secondly, chronic ANT-DBS may exert protective effects on hippocampal neurons and enhance the regeneration of neuronal fibers.[59,60] Hippocampal neuronal cell apoptosis has been correlated with seizure frequency as was found in resected sclerotic hippocampi in patients with mesial temporal lobe epilepsy.[61] Vice versa, the number of neuronal cells negatively correlates with seizure frequency.[62] Although controversial, approaches to reduce neuronal cell loss may decrease seizure frequency.[63,64] ANT-DBS has been shown to increase neurogenesis in the chronic stage of ANTDBS in KA-induced seizures in rats as shown by an increased expression of Ki-67 and DCX.[65] The model of prolonged neurogenesis could further explain the observation of an increased efficacy of ANT-DBS in the long-term follow-up of the SANTE trial. Lastly, ANT-DBS may further induce neuroprotective effects by reversing the hippocampal pro-inflammatory state. In KA-induced seizures in rats, ANT-DBS induced a normalized gene expression of pro-inflammatory cytokines such as IL-1βand IL-6 and therefore prevent subsequent neuronal injury in the hippocampalCA1.[66,67] The involvement of inflammatory mediators in seizure susceptibility and epileptogenesis has been extensively recognized.[68,69]

Another mechanism which may underlie the therapeutic effect of ANT-DBS is through influencing glucose metabolism. In patients with temporal and frontal lobe epilepsy, an ipsilateral thalamic and hippocampal interictal glucose hypometabolism is often observed on FDG-PET, and its severity is correlated with a prolonged course of epilepsy.[70-72] Interestingly, a mouse model of chronic inhibition of brain energy metabolism showed that epileptiform activity could be induced by intracerebroventricular injection of a non-metabolizable glucose analog.[73] Strikingly, bilateral ANT stimulation promotes energy metabolism in the anterior thalamic region, thalamus and the hippocampus as measured by FDG-PET in rats.[74] Local ANT stimulation, induced increased glucose metabolism and may therefore reverse the predisposing thalamic hypometabolism and attenuate its deterioration. Further, ANT-DBS inhibits energy metabolism in the cingulate cortex and the frontal cortex.[74] The ANT-DBS induced hypometabolism was the most prominent in the motor cortex, which therefore through inhibition may increase the seizure threshold and thereby directly contribute to the anti-epileptic action of ANT-DBS. In contrast, bilateral ANT chemical lesioning did not show an increased glucose uptake in the bilateral anterior thalamic region nor did it induce neuronal energy metabolism changes in distant brain areas.

ANT-DBS WITHIN THE SPECTRUM OF NEUROMODULATION FOR EPILEPSY

In addition to the ANT, several other brain structures have been targeted with stimulation for epilepsy and have been addressed in randomized controlled trials (RCTs).[75-84] The intracranial targets include the centromedian thalamic nucleus, cerebellar cortex, hippocampus, nucleus accumbens, and responsive ictal onset zone stimulation. These RCTs have been systematically reviewed in a recently updated Cochrane meta-analysis.[85] In short, in addition to ANT-DBS (mean difference (MD): −17.4% compared to sham stimulation), a statistical significant reduction in seizure frequency was found for responsive ictal onset zone stimulation (−24%; multi-focal epilepsy) and hippocampal DBS (−28.1%; temporal lobe epilepsy), with comparable adverse events in terms of frequency and severity.[85] However, no statistical significance was provided in terms of seizure freedom, responder rate or quality of life.[85]
To date, there are no trials comparing intracranial stimulation to either lesser invasive modalities such as vagus nerve stimulation (VNS), transcutaneous-VNS (t-VNS) and repetitive transcranial magnetic stimulation (rTMS) or best medical practice. Two RCTs report on a similar or slightly inferior treatment response for VNS (MD -12.7% and −18.4%) when compared with intracranial targets.[86,87] A direct comparison between VNS and ANT-DBS concerning treatment outcome should be made with caution as almost half (44.6%) of the SANTE study population received VNS implantation prior to ANT stimulation.[12] With regard to rTMS, a recent Cochrane review concluded that, although reasonable evidence suggests that rTMS is effective at reducing epileptiform discharges, strong evidence is lacking for the efficacy of rTMS for seizure reductions in drug resistant epilepsy.[88] Furthermore, add-on therapy with t-VNS (25Hz) has not been proven superior compared to active controls (1 Hz) after 20 weeks in patients with drug-resistant epilepsy.[89]

CONCLUDING REMARKS AND FUTURE DIRECTIONS

ANT-DBS for drug-resistant epilepsy is a safe and well-tolerated therapy, where a particular emphasis must be given to monitoring of depression and memory function. ANT-DBS is an efficacious treatment modality, even when curative procedures or lesser invasive neuromodulation techniques failed. When compared to VNS, ANT-DBS shows slightly superior treatment response, which urges for direct comparative trials.

Based on the available evidence ANT-DBS and VNS therapies are currently both superior compared to non-invasive neuromodulation techniques such as t-VNS and rTMS. Despite its clinical efficacy, ANT-DBS for drug-resistant epilepsy still faces great challenges. Optimization of the procedural DBS protocol including imaging techniques, surgical procedure, and algorithms for adaptation of stimulation parameters could aid to reduce the treatment response variability. Additional research will have to provide for better understanding of normal physiological neuronal networks compared to epileptogenic networks in order to gain more insight into the mechanism of action ANT-DBS in localization-related epilepsy. Ideally, further in-depth knowledge of epileptogenic networks may explain for the differential response of DBS of different anatomical targets in different seizure types.[12,75,90-93] Furthermore, adaptive (seizure-dependent) ANT-DBS may increase the efficacy, efficiency and selectivity of this treatment as is observed in focal responsive cortical stimulation and subthalamic nucleus DBS for Parkinson's disease.[94,95] Randomized clinical studies in search for the optimal target in well-defined epilepsy patient populations will ultimately allow for optimal patient stratification when applying intracranial neuromodulation therapy for drug-resistant epilepsy patients.

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Chapter 5

Single-cell recordings to target the anterior nucleus of the thalamus in deep brain stimulation for patients with refractory epilepsy

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Abstract

Deep brain stimulation (DBS) of the anterior nucleus of the thalamus (ANT) is a promising treatment for patients with refractory epilepsy. However, therapy response varies and precise positioning of the DBS lead is potentially essential for maximizing therapeutic efficacy. We investigate if single-cell recordings acquired by microelectrode recordings can aid targeting of the ANT during surgery and hypothesize that the neuronal firing properties of the target region relate to clinical outcome. We prospectively included 10 refractory epilepsy patients and performed microelectrode recordings under general anesthesia to identify the change in neuronal signals when approaching and transecting the ANT. The neuronal firing properties of the target region, anatomical locations of microelectrode recordings and active contact positions of the DBS lead along the recorded trajectory were compared between responders and nonresponders to DBS. We obtained 19 sets of recordings from 10 patients (five responders and five nonresponders). Amongst the 403 neurons detected, 365 (90.6%) were classified as bursty. Entry into the ANT was characterized by an increase in firing rate while exit of the ANT was characterized by a decrease in firing rate. Comparing the trajectories of responders to nonresponders, we found differences neither in the neuronal firing properties themselves nor in their locations relative to the position of the active contact. Single-cell firing rate acquired by microelectrode recordings under general anesthesia can thus aid targeting of the ANT during surgery, but is not related to clinical outcome in DBS for patients with refractory epilepsy.

INTRODUCTION

DEEP BRAIN STIMULATION (DBS) IS A PROMISING TREATMENT FOR patients with refractory epilepsy who are ineligible for resective epilepsy surgery. After several case and pilot studies that showed a reduction of seizure frequency,[1–6] the SANTE study was the first double-blinded, randomized, controlled trial for DBS of the anterior nucleus of the thalamus (ANT) in 110 refractory epilepsy patients. In this pivotal study, median seizure frequency reduced by 41% at 1-year follow-up and by 69% at 5-year follow-up, with 16% of patients remaining seizure-free for at least 6 months.[7,8] Additional reports have confirmed the efficacy of DBS of the ANT for refractory epilepsy since the SANTE study.[9-12] However, the therapeutic effect varies considerably among patients. While progressive improvement with neurostimulation is observed[8] and tailoring of stimulation parameters can influence the clinical response,[12] it remains unclear why certain patients respond to DBS of the ANT while others do not. Suboptimal targeting of the DBS lead likely plays a vital role in the observed differences in therapy response.

Single-cell recordings by microelectrodes during surgery may assist target identification and guide optimal placement of the DBS lead to improve clinical outcome.[13] Given the recent technological advances in the field of DBS,[14] DBS target identification and prediction of therapy response are areas where computational-based technologies could assist.[15] This is illustrated by the development of commercially available software systems that map computer models and neurophysiological data on patient's MR images to guide DBS programming (https://clinicaltrials.gov/ct2/show/ NCT03353688). Other systems even assist neurosurgical targeting by automatically detecting the entry and exit of the subthalamic nucleus in real-time using microelectrode recordings during DBS surgery for Parkinson's disease (https://clinicaltrials.gov/ct2/show/NCT03363-724). As opposed to the subthalamic nucleus,[13,16,17] little is known about the neuronal firing properties of the ANT and their relation to clinical outcome in DBS for patients with refractory epilepsy.[18,19] The clinical utility of microelectrode recordings during DBS surgery for refractory epilepsy is therefore unclear. This is in part due to a limited number of subjects investigated and a variation of transventricular and extraventricular surgical trajectories used in clinical practice. Moreover, differences in neuronal firing properties of the target region have not been related to clinical outcome previously.

In this study, we investigate whether single-cell recordings acquired by microelectrode recordings along an extraventricular trajectory could aid targeting of the ANT during surgery and we hypothesize that the neuronal firing properties of the target region relate to therapy response in DBS for patients with refractory epilepsy.

METHODS

Patients

Between 2011 and 2014, we prospectively included 10 consecutive refractory epilepsy patients with partial onset seizures scheduled for DBS surgery at Maastricht University Medical Center, the Netherlands. All patients failed to respond to trials of at least two reasonably tolerated and adequately chosen antiepileptic drug regimens.20,21 We only included patients who were considered ineligible for resective epilepsy surgery after general work-up by an expert panel (including video-EEG monitoring), or patients in whom previous epilepsy surgery or vagal nerve stimulation was not effective. Patients were excluded if they exhibited any of the following: psychiatric co-morbidities such as severe depression or psychosis, severe pulmonary disease, uncontrolled hypertension and blood coagulation disorders. Patient characteristics are presented in Table 5.1.

Ethics

The study was approved by the Medical Ethical Testing Committee of Maastricht University Medical Center (ID: METC 14-4-126).

Imaging

As part of standard clinical practice, all subjects had a pre-operative 3-T MRI (Philips, Eindhoven, The Netherlands) or 1.5-T MRI in case of an implanted vagal nerve stimulator for stereotactic planning of the electrode trajectory. The sequences used were a 3D T1 with gadolinium, axial T2 and a T1 inversion recovery. Post-operatively, a CT or a 1.5-T T1 MRI was performed to localize the DBS lead.

Table 5.1 Patient characteristics.

Patient	Therapy response	Gender	Age at surgery (years)	Epilepsy duration (years)	Seizure type	Seizure focus	Resective surgery/VNS	Baseline seizure frequency/ change to baseline in % at 1-year FU	Active contact at 1-year follow-up Left/Right
1	R	Male	41	20	CP	Right temporal lobe	Left temporal lobe/No	3/Seizure free	1/1
2	R	Female	35	33	SP, CP, TC	Bilateral temporal lobe	No/ON	7.7/-87%	0 - 1/0 - 1
3	R	Male	65	51	CP	Left frontal lobe	Left temporal lobe/Removed	23/-52%	0 - 1/0 - 1
4	NR	Male	46	23	CP	Right parietal lobe	No/Removed	4.7/+28%	1/1
5	R	Male	48	39	SP, CP	Bilateral temporal lobe	No/NoX	0.5/Seizure free	1/1
6	NR	Male	36	13	CP, TC	Left temporal lobe	No/ON	X7.7/-35%	1/1
7	R	Female	30	12	SP, CP	Right temporal lobe	Left temporal lobe/ON	10/Seizure free	1/1
8	NR	Male	40	30	CP	Left temporal lobe	No/No	18.5/-42%	1/1
9	NR	Male	38	29	SP, CP, TC	Multifocal epilepsy	No/ON	7.5/+20%	1/1
10	NR	Male	40	29	T, CP, TC	Right parietal lobe	No/Removed	268/+34%	1/1

R = responder; NR = nonresponder; SP = simple partial; CP = complex partial; T = tonic; TC, tonic–clonic; FU = follow-up; and VNS = vagal nerve stimulation.

Surgical procedures

Surgical procedures were performed under general anesthesia with remifentanil and propofol. A Leksell stereotactic frame (Model G, Elekta Instrument, Stockholm, Sweden) was mounted onto the skull of the patient. Subsequently, a per-operative stereotactic CT scan of the head with stereotactic frame was acquired and fused with the pre-operative MR images using Framelink software (Medtronic, Fridley, USA). The planned target was the center of the ANT. We planned an extraventricular approach to target, typically transecting the internal capsule (IC) and other thalamic nuclei before entering the ANT. Along this trajectory, we performed microelectrode recordings (MicroMacroElectrode, ISIS MER, Inomed, Emmendingen, Germany) to identify the neuronal signals when approaching and transecting the target region. For more details on our stereotactic surgical procedures, we refer to previous publications.[22,23]

Microelectrode recordings

Single-cell potentials were recorded at a sampling frequency of 20 kHz or 25 kHz, with a bandpass filter of 160–5000Hz, for at least 30 s at each step starting with 1-mm intervals from 10mm above target and 0.5-mm intervals from 5mm above target until maximally 5 mm below target (Figure 5.1). The macrocontact was the reference contact for microelectrode recordings at the microtip. In only one patient, the lateral trajectory was chosen in one hemisphere as the central trajectory did not show typical spiking activity. After microelectrode recording, the final DBS lead (Model 3389, Medtronic, Fridley, USA) was implanted along the same trajectory with contact 1 (contact 0 is the most distal contact) typically situated at target. Subsequently, the leads were connected to an internal pulse generator (Activa PC, Medtronic, Fridley, USA). To relate neuronal firing properties to therapy response, we analyzed the microelectrode recording trajectories used for DBS lead implantation.

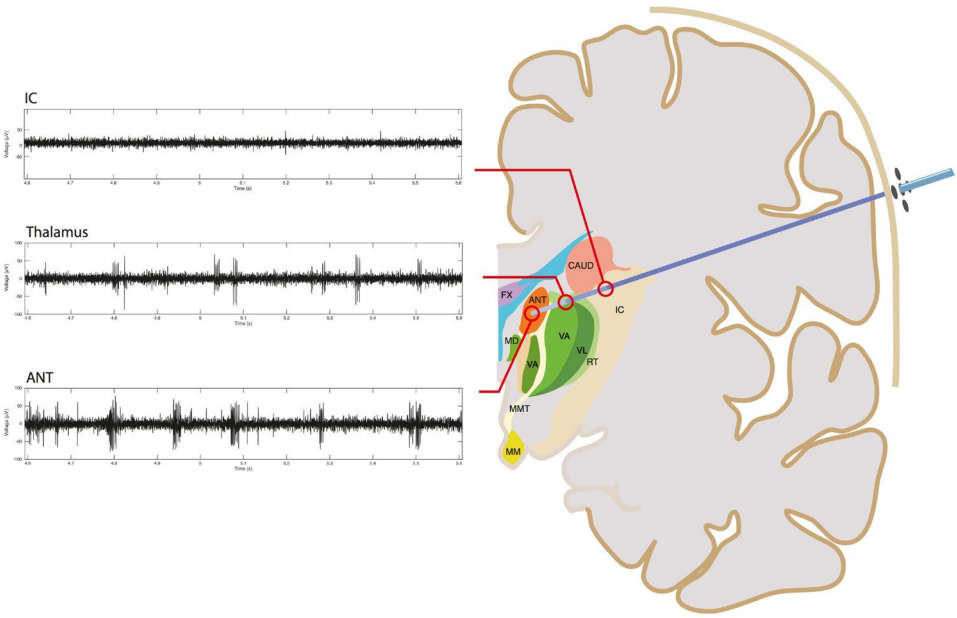

Figure 5.1 Schematic representation of an extraventricular trajectory to the ANT with representative microelectrode recording signals of internal capsule, thalamus and ANT region at −10 mm, −5 mm and at target, respectively. Note that in the IC almost no spikes are present, in the thalamus a typical bursty signal and in the ANT a bursty signal with a high firing rate are observed. Abbreviations: ANT, anterior nucleus of thalamus; MM, mammillary bodies; IC, internal capsule; MMT, mammillothalamic tract; CAUD, caudate nucleus; FX, fornix; RT, reticular tract; VL, ventrolateral nucleus of thalamus; VA, ventral anterior nucleus of thalamus; and MD, mediodorsal nucleus of thalamus.

The anatomical locations of the microelectrode recordings were verified by fusing pre-operative MR and post-operative CT images. Landmarks for identifying the boundaries of the ANT on preoperative 3-T MRI were the mammillothalamic tract, external medullary lamina, third ventricle and lateral ventricle. The microelectrode recording trajectory was reconstructed using Framelink software (Stealthstation, Medtronic, Fridley, USA) via the coordinates of planned target and skull entry point. Along this trajectory, two observers (Yasin Temel, Rob P. W. Rouhl) manually confirmed and reached consensus for the location of microelectrode recording at every depth relative to target to ensure the recording was taken from the IC, thalamus or ANT. The mean depths of entry into the thalamus, ANT and exit out of the ANT region were subsequently calculated. The distance of the active contact along the implantation trajectory was derived from the final position of contact 0 relative to target during surgery, considering a 2-mm spacing between the centers of successive contacts for the Medtronic 3389 lead model.

Deep brain stimulation

The neurosurgeon (Yasin Temel) evaluated the location of the DBS lead for minor post-surgical movement on fused pre-operative and post-operative images. Stimulation of the contact closest to the center of the ANT started 5-8 weeks after surgery. Primary stimulation parameters were identical to those in the SANTE study[7,8] and consisted of monopolar stimulation with a frequency of 145Hz, a pulse width of 90 μs, an intensity of 5V and cycling mode of 1min on and 5min off. Stimulation parameters were tailored at the discretion of the treating neurologists (Louis Wagner, Albert J. Colon) during regular follow-up moments, which are generally performed by adapting the current amplitude, pulse width and/or frequency to control the volume of tissue activation.[24] After 1 year, the treating neurologist assessed the clinical response to DBS therapy. Responders were defined as patients exhibiting a seizure frequency reduction of ≥50% compared to baseline and nonresponders as patients exhibiting a seizure frequency reduction of <50% compared to baseline. We classified five patients as responders and five patients as nonresponders to DBS. Patient characteristics, seizure frequency at baseline and active contacts are presented in Table 5.1.

Data analysis

We obtained 19 sets of recordings from nine bilateral and one unilateral microelectrode recording trajectories from 10 patients. One patient only had microelectrode recording on the left hemisphere to reduce the risk for intracranial bleeding accompanied with a dense collection of vessels in the target region at the right hemisphere. Microelectrode recordings were processed with MATLAB and Statistics Toolbox R2012a (Mathworks, Natick, USA). First, the recorded signal was bandpass filtered (350-5000Hz) with the filtfilt command to isolate the action potential component of the signal. Mechanical artifacts in the microelectrode recordings were automatically identified.[17] Signals with artifacts were not included for further analysis. Spikes were detected from the filtered data using an envelope noise-detection method.[25] The spikes were then sorted into single neuronal units using Haar wavelet coefficients[26] as input features for an expectation-maximization clustering algorithm. Next, we classified the firing pattern of every neuron based on their discharge density histogram.[27] In short, the spike train was first binned using the mean interspike interval as the bin width. The histogram of the number of spikes per interval was statistically compared with the Poisson, normal and bimodal distributions to determine if the firing pattern was random, regular or bursty. For each neuron, we then calculated the mean firing rate (MFR) and, for bursty neurons, the mean burst rate (MBR) and MFR within bursts (MFRib). Microelectrode recordings

with an insufficient number of spikes for classification (<100 spikes or <10 s of spike activity) were classified as unknown.
The firing characteristics for each recording were summarized by mapping them onto the corresponding microelectrode recording location, labeled as distance relative to target, where location 0 corresponds to the planned target. The distributions of each of the three firing patterns along the trajectory were constructed by calculating the number of recorded cells from all patient recordings that showed regular, random, bursty or unknown firing patterns at a certain depth. To facilitate comparisons between different sets of recordings from diverse patients, the mean firing and burst rates along each trajectory were first normalized by dividing them by the maximum firing or burst rate recorded along the trajectory in a specific patient. Firing characteristics were then averaged for each recording depth across all recordings at that depth for the total population of responders and nonresponders. Curves were then smoothed using a three-point moving average.

Statistical analysis

Group comparisons of anatomical locations of microelectrode recordings were conducted using the two sample Kolmogorov-Smirnov test on the empirical distribution functions along the trajectory. For the percentage of bursty cells, MFR, MBR and MFR within bursts, a linear mixed model was used to test whether these firing characteristics varied with the patient group and/or depth to target and any interaction between these two factors. An unpaired Student's t-test was used to compare the distance between the active contact and the location of the peaks in MFR, MBR and MFR within bursts between responders and nonresponders. Firing characteristics and distances to target are presented as mean±standard error (SE), unless otherwise stated. All statistical tests were performed in MATLAB and a p-value <0.05 was considered statistically significant.

RESULTS

Neuronal firing properties

The electrophysiological characteristics of all single cell recordings are summarized in Table 5.2.

Table 5.2 Electrophysiological characteristics of single cell recordings in mean ± standard error across all depths from all the subjects.

Electrophysiological characteristic	Mean ± SE
Spike ratea (Hz)	20.9 ± 0.9
Spike amplitudea (μV)	55.4 ± 0.2
Burst rateb (Hz)	1.6 ± 0.1
Spike rate within burstsb (Hz)	447.9 ± 5.7
Number of spikes per burstb	3.6 ± 0.1
Burst durationb (ms)	9.6 ± 0.3
Interburst intervalb (ms)	890.8 ± 47

[a]Averaged over $N = 403$ neurons. [b]Averaged over $N = 365$ bursty neurons.

Amongst the 403 neurons detected, 365 (90.6%) were classified as bursty with a mean±SE number of spikes per burst of 3.6±0.1, a burst duration of 9.6±0.3 ms and an interburst interval of 890.8±47 ms. Based on the anatomical verification of the planned trajectory on pre-operative MR images, the mean point of entry into the thalamus was at −7.5±0.5 mm relative to target, entry into the ANT at −3.8±0.2 mm and the mean exit out of ANT was at +2.3±0.4 mm. A typical thalamic bursty firing pattern was seen when entering the thalamus and ANT (Figures 5.1 and 5.2). Considerably lower numbers of neurons were detected above −6 mm relative to target in the region of the IC and below −2 mm after exiting the ANT (Figure 5.2). Entry into the ANT was characterized by an increase in the MFR while exit from ANT was characterized by a decrease in the MFR, MBR and MFR within bursts (Figure 5.3). The MFR [$F(23, 437)=8.64$, $p<0.0001$], MBR [$F(23, 330)=5.93$, $p<0.001$], and MFR within bursts [$F(23, 329)=14.55$, $p<0.0001$] all significantly changed with depth along the trajectory. Moreover, MFR, MBR and MFR within bursts consistently reached a peak around −2 mm to target. Both in the total population (Figure 5.3) and in an individual subject (Figure 5.4), we identified a profile of an increase in firing rate when entering the thalamus, followed by a zone without spikes and a subsequent increase in firing rate when entering and decrease when exiting the ANT. After review of the individual patient data, we found that 15 out of 19 trajectories fulfilled this profile with an approximate firing rate range of 10–60Hz in ANT recordings. Other firing characteristics such as spike amplitude, spikes per burst, burst duration and interburst duration did not markedly change along the trajectory and were not further analyzed.

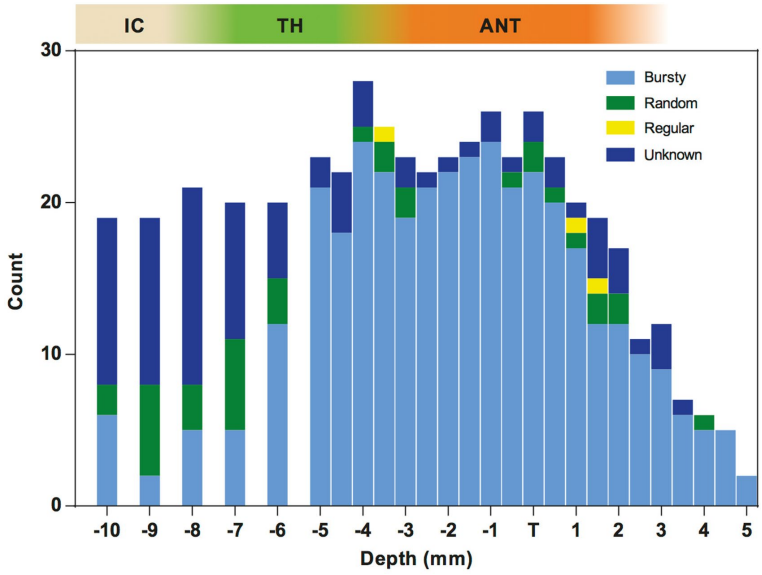

Figure 5.2 Histogram of the total neuron count and distribution of different cell types by their firing characteristics (bursty, regular, random or unknown) along the trajectory. Note the increase of proportion of bursty cells when approaching the thalamus and ANT. Here IC=internal capsule; TH=thalamus; and ANT=anterior nucleus of the thalamus.

Responders versus nonresponders

There was no significant difference between the distributions of percentage of bursty cells for responders and nonresponders. Further investigation of distributions of MFR, MBR and MFR within bursts revealed no significant difference along the trajectory (Figure 5.5) between the two groups. There were no interaction effects between depth and group. Anatomical locations of microelectrode recording did not differ between responders and nonresponders, since the probability of a recording being taken from the internal capsule, thalamus or ANT along the different depths of the trajectory did not significantly differ. We additionally evaluated if active contact placement near the peak of the firing characteristics could be related to clinical response. However, we did not find a difference in the distances of the active contact to the peak of MFR, MBR or MFR within bursts between responders and nonresponders (means ranged from 0.7 mm to 2.4 mm). To evaluate if the probable location of the epileptogenic zone relates to the nature of the neuronal signals along the trajectory, we compared the neuronal firing properties between patients with temporal and extra-temporal onset of seizures (Table 5.1), but no differences were found.

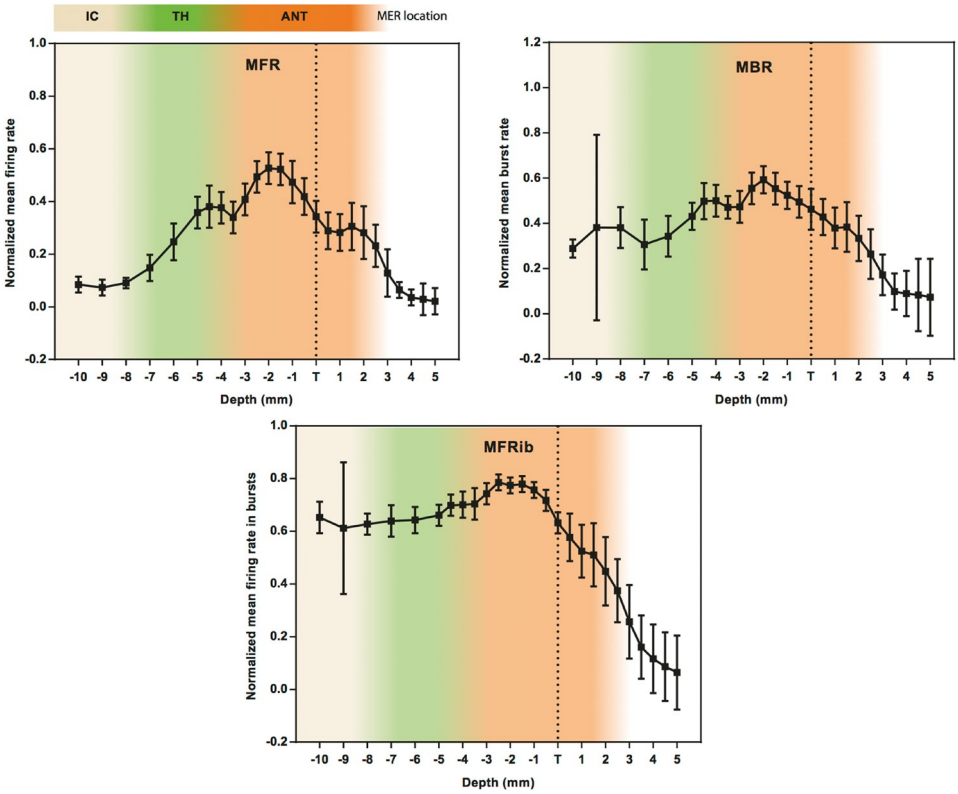

Figure 5.3 Neuronal firing properties along the trajectory for all subjects. (a) Normalized MFR, (b) normalized MBR and (c) normalized MFRib. Note the increase in MFR when approaching the target and the decrease when crossing the target. Error bars represent standard errors of the mean.

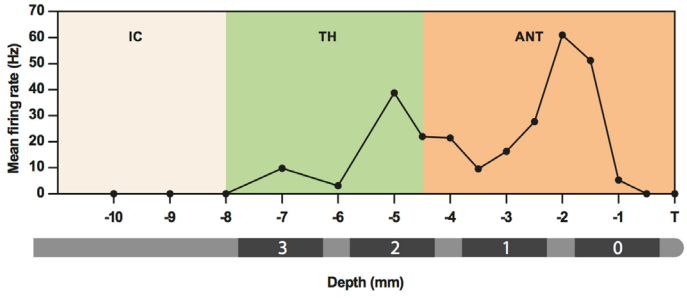

Figure 5.4 Individual subject example (case 2: right hemisphere) of the mean firing rate along the recorded trajectory to target and a schematic representation of DBS lead placement. Note the increase in mean firing rate when entering and decrease when exiting the ANT. Following microelectrode recordings, the DBS lead was implanted along the same trajectory and the center of contact 0 was positioned at –1mm to target.

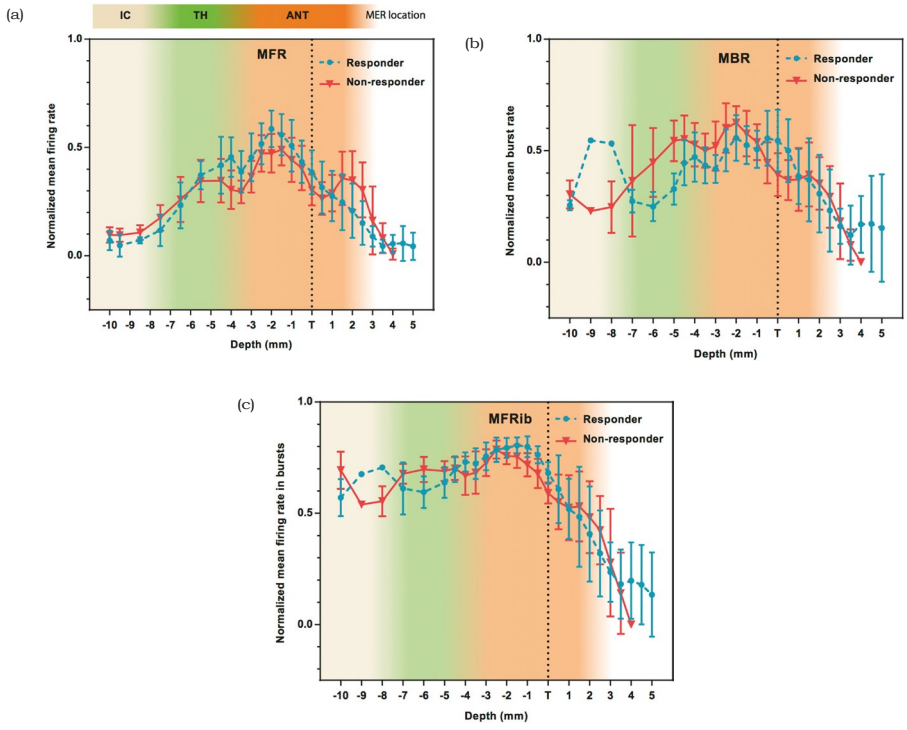

Figure 5.5 Neuronal firing properties along the trajectory for responders (blue dashed lines) and nonresponders (red solid lines). (a) Normalized MFR, (b) normalized MBR and (c) normalized MFRib. Error bars represent standard errors of the mean.

Discussion

Single-cell recordings acquired by microelectrode recordings during DBS surgery were obtained from 10 patients with refractory epilepsy. A total of 19 extraventricular microelectrode recording trajectories were used to analyze clinically relevant parameters for defining the target region and were subsequently compared between responders and nonresponders to DBS. We found an incremental increase in firing rate when entering the ANT and a decrease in firing rate when exiting the ANT. Comparing the trajectories of responders to nonresponders, we found differences in neither the neuronal firing properties themselves nor their locations relative to the position of the active contact. Single-cell firing rate detected by microelectrode recording under general anesthesia can aid in targeting the ANT during surgery, but is not related to therapy

response in DBS for patients with refractory epilepsy. Other factors such as patient characteristics (e.g. location of seizure onset and epilepsy etiology), location, duration and parameters of stimulation likely play a role in therapy response.

Single-cell recordings could be implemented in machine learning models for computational tools that assist the neurosurgical team with DBS target identification. This model can, in theory, be built by taking a set of inputs (e.g. features related to the patient) and mapping them onto the desired output (e.g. location of the ANT or therapy response to DBS). The performance of the model and its engineering potential depend on the selection of relevant and discriminating features.[28] In this study, we therefore conducted a thorough exploration of microelectrode recording data to identify electrophysiological features that may correlate with the location of the ANT and/or relate to therapy response to DBS. Explored features include the neuronal firing pattern (regular, bursty or random), mean firing rate, burst rate, firing rate within bursts, burst duration, interburst duration, spikes per burst and spike amplitude. Amongst these features, we found that only the firing pattern and mean firing rates varied in a systematic manner along the trajectory. As such, we focused our analyses on these parameters and compared these between responders and nonresponders to DBS. Our results show that a clear peak in the firing rate precedes the intended DBS target defined on pre-operative MR images. The simplicity of this feature, which is expected not to be computationally intensive, means that localization of the ANT during surgery may be possible via real-time analysis of firing rate during DBS surgery. Subsequently, the trajectory for definitive lead implantation can be chosen and lead implantation can be guided for optimal placement of the DBS electrode contacts relative to the neuronal signals of the ANT. The clinical utility of this technique would be to more accurately place the DBS electrode within the anatomic and electrophysiological defined ANT. Although we found a change in firing rate along the trajectory, this feature did not relate to therapy response. This is possibly due to the dimensions of the electrode and coarser effects of DBS compared to the distribution of neuronal signals recorded with microelectrode recordings. Considering the intensity of stimulation is generally around 5V, we estimate that the volume of tissue activation will cover the ANT and peak in firing rate in both responders and nonresponders. A more specific target localization, using computational tools that combine electrophysiological data, MR images and volume of tissue activation models, could enable more focal stimulation to consume less battery, prevent side effects and expand our knowledge on the clinical importance of the ANT as an anatomic and electrophysiological target in DBS for epilepsy.

Hodaie et al.[18] first described the electrophysiological properties of the human ANT during DBS surgery. In contrast to our extraventricular approach under general anesthesia, the authors used a transventricular trajectory to the ANT and performed

microelectrode recordings under local anesthesia in five subjects with intractable epilepsy. They found bursting activity in the ANT and characterized most bursts as low-threshold calcium spike (LTS) mediated bursts, which are generally only observed during sleep. Although we did not specifically analyze LTS bursts, our burst characteristics (number of spikes per burst and burst duration) obtained under general anesthesia are similar (Table 5.2) and do not markedly change along the trajectory. LTS bursts may thus be due to an altered electrophysiological state in epilepsy or a result of long-term anticonvulsant use. As there were no recordings in normal control subjects and no concurrent EEG recordings were performed, the authors note that it cannot be ruled out that bursting activity is normal in awake patients.[29] LTS bursts were however not specific to the ANT, as they were additionally found in the nucleus cucularis and dorsal dorsomedian nucleus of the thalamus. Moreover, the percentage of LTS bursts found was similar at different depths along the trajectory since they were frequently found after exiting the estimated ventral border of the ANT. Although the authors describe an interesting finding for a potential role of thalamic bursting in facilitating seizure propagation, the lack of specificity and minimal change in LTS bursts along the trajectory might preclude its use to target the ANT.

Along an extraventricular trajectory, we report a change in firing characteristics when entering and exiting the ANT. The parameter that most prominently depicted the ANT was the firing rate, which increased until it reached a peak around −2 mm to target and subsequently decreased. A change in firing rate along an extraventricular trajectory to the ANT was previously reported in two subjects by Möttönen et al.[19] The authors describe a zone without spikes at the lateral aspect of the ANT and attributed this to white matter lamina between the ANT and other thalamic subnuclei as defined by 3-T MRI. Our results support this finding, as we see a decrease in mean firing or spike rate before entering the lateral ANT (Figures 5.3 and 5.4), which we consider is most likely caused by passing through the medullary lamina of the thalamus. Using five transventricular and five extraventricular trajectories from five subjects, the same group investigated the firing characteristics of different nuclear groups of the thalamus.[30] They found that the spike frequency in recordings most likely taken from the ANT were significantly lower than the recordings taken from the ventral anterior nucleus (VA) with morphologically similar bursts. In contrast, we found a peak in mean firing rate at −2 mm to target from the recordings most likely taken from the ANT. Although we did not compare the firing rate between the ANT and VA, our findings suggest a higher firing rate in the ANT (within the approximate range of 10–60 Hz) than in other thalamic nuclei. While we cannot exclude that some of our recordings might have been taken from the VA, the aim of our study was not to distinguish between firing characteristics of different thalamic nuclei but to describe the change in firing characteristics when approaching and

transecting the ANT region using an extraventricular approach. The difference in firing rates found between Möttönen et al. and our study can possibly be explained by details of the surgical trajectory, analysis of spike rate, patient characteristics or used anesthesia. Although in the SANTE study a transventricular surgical trajectory was chosen[7] and is commonly advised for targeting the ANT[31] we have, like others,[32] consistently planned an extraventricular trajectory, avoiding the choroid plexus, thalamostriatal veins and branches of the internal cerebral veins to reduce the risk for intracranial hemorrhage associated with passing through the lateral ventricle.

Limitations

This study is, inherent to a microelectrode recording study, limited by the anatomical verification of recordings. Due to the small size and low intrinsic MR signal and contrast of the intrathalamic nuclei, it is challenging to distinguish between the different thalamic subnuclei with conventional MRI sequences. We therefore used well-described borders of the ANT (such as the mammillothalamic tract and external medullary lamina) to reach consensus between two observers if a patient-specific recording was taken from the IC, thalamus or ANT. Future studies projecting microelectrode recording data onto images obtained via ultra-high-field MR in conjunction with optimized sequences for visualization of the intrathalamic nuclei[33] will likely advance classification of the firing characteristics of different nuclear groups within the thalamus and possibly even within the ANT.

While we present the largest set of single-cell recordings from epilepsy patients who were treated with DBS to date, this study remains underpowered by the low number of subjects and group sizes. We found no difference in neuronal firing properties between responders and nonresponders to DBS and between patients with temporal and nontemporal lobe localizations of seizures. While analysis of larger cohorts is required, comparable neuronal firing properties of the ANT under general anesthesia in a heterogeneous epilepsy patient population might be an advantage for reliably targeting the ANT during DBS surgery using microelectrode recordings.

Conclusion

Single-cell firing rate acquired by microelectrode recordings under general anesthesia can aid targeting of the ANT during surgery, but is not related to clinical outcome in DBS for patients with refractory epilepsy.

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Chapter 6

Deep brain stimulation in epilepsy: a role for modulation of the mammillothalamic tract in seizure control?

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ABSTRACT

Background
Deep brain stimulation of the anterior nucleus of the thalamus (ANT-DBS) can improve seizure control for patientswith drug-resistant epilepsy (DRE). Yet, one cannot overlook the high discrepancy in efficacy among patients, possibly resulting from differences in stimulation site.

Objective
To test the hypothesis that stimulation at the junction of the ANT and mammillothalamic tract (ANT-MTT junction) increases seizure control.

Methods
The relationship between seizure control and the location of the active contacts to the ANT-MTT junction was investigated in 20 patients treated with ANT-DBS for DRE. Coordinates and Euclidean distance of the active contacts relative to the ANT-MTT junction were calculated and related to seizure control. Stimulation sites weremapped by modelling the volume of tissue activation (VTA) and generating stimulation heat maps.

Results
After 1 yr of stimulation, patients had a median 46% reduction in total seizure frequency, 50% were responders, and 20% of patients were seizure-free. The Euclidean distance of the active contacts to the ANT-MTT junction correlates to change in seizure frequency (r^2=0.24, p=.01) and is ~30% smaller (p=.015) in responders than in nonresponders. VTA models and stimulation heat maps indicate a hot-spot at the ANT-MTT junction for responders, whereas non-responders had no evident hot-spot.

Conclusion
Stimulation at the ANT-MTT junction correlates to increased seizure control. Our findings suggest a relationship between the stimulation site and therapy response in ANT-DBS for epilepsy with a potential role for the MTT. DBS directed at white matter merits further exploration for the treatment of epilepsy.

INTRODUCTION

DEEP BRAIN STIMULATION OF THE ANTERIOR NUCLEUS OF THE THALAMUS (ANT-DBS) has recently been approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with drug-resistant epilepsy (DRE) when resective procedures or less invasive neuromodulation therapies are not possible or have failed. The stimulation of the anterior nucleus of the thalamus for epilepsy (SANTE) trial demonstrated that bilateral thalamic stimulation in drug-resistant focal epilepsy is a safe procedure that reduces short and long-term seizure frequency and significantly improves well-being.[1,2] While several cohorts following the SANTE trial confirm these findings with mean reported responder rates across studies approximating 50% after 1 yr of ANT-DBS,[3-6] the degree of seizure control can vary highly between patients.[7] Knowledge obtained from DBS in movement disorders suggests that patient selection[8] and electrode placement[9] are important factors for predicting clinical outcome. As such, suggested denominators for seizure control by DBS are patient characteristics, such as the location of seizure onset, and the stimulation site.[4,6] Furthermore, data from the SANTE trial indicate that DBS leads were not consistently placed within the ANT,[10] yet effective stimulation with contacts outside the ANT has been reported.[11] Hence, the optimal stimulation site is debated.

DBS lead placement within the ANT is currently performed by direct neurosurgical targeting,[12,13] in which the mammillothalamic tract (MTT) functions as a key anatomical landmark.[14,15] The MTT is a prominent white matter bundle that arises from the mammillary bodies and ends in the medio-ventral part of the ANT, where it joins the internal and external lamina of the thalamus, also known as the ANT-MTT junction. Within the circuit of Papez, the ANT receives major afferent input from the hippocampal formation through the MTT next to its reciprocal cortical connections through thalamic radiations[16] and thalamocingulate fibers.[17] While the mechanism of action still remains elusive and it is unclear to what degree different brain networks and fiber tracts are stimulated, ANT-DBS is speculated to halt seizure propagation and/or modulate epileptogenic foci through its connections to the circuit of Papez.[18] The significance of the circuit of Papez as a potential seizure circuit is exemplified by depth recordings in humans[11,19,20] and lesion studies in animals,[21,22] Accordingly, the varied effects of ANTDBS possibly relate to unsuccessful stimulation of the MTT[23] to achieve seizure circuit control.

In this study, we hypothesized that stimulation of the ANTMTT junction increases seizure control. We performed an independent, clinical-outcome blinded analysis of the

active contacts in our ANT-DBS patient cohort to investigate the relationship between stimulation site and seizure control in DRE.

Methods

Patients, surgery, and DBS

We included all patients who qualified for on-label DBS treatment for DRE.[1,13] Patients were assessed by an epilepsy expert panel, were not eligible for resective surgery or did not respond to previous resective procedures or vagal nerve stimulation. Details of our DBS surgery for epilepsy are described elsewhere.[13] In short, DBS surgery was performed under general anesthesia guided by microelectrode recordings along an extraventricular surgical trajectory (Figure 6.1) and 3389 leads (Medtronic, Dublin, Ireland) were bilaterally implanted at the ANT. Following evaluation of the DBS lead position for minor postsurgical movement by the neurosurgeon, the pulse generator was turned on 6 weeks after surgery with the following stimulation parameters: frequency of 145 Hz, intensity of 5 V, pulse-width of 90 μs,[1] and thereafter adjusted at the discretion of the epileptologist. Therapy response was assessed at 1-yr follow-up after start of stimulation. We considered patients with ≥50% reduction in seizure frequency compared to baseline as responders and patients with <50% reduction as non-responders.

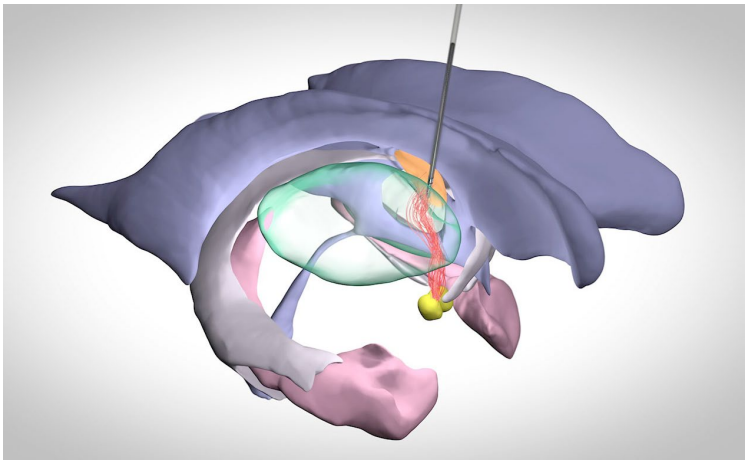

Figure 6.1 Schematic representation of the circuit of Papez and extraventricular DBS trajectory to the ANT-MTT junction. Orange, ANT; red, MTT; green, thalamus; yellow, mammillary bodies; pink, hippocampus; grey, fimbriae/fornix; purple, ventricle.

Ethical statement

The work described was conducted in accordance with the Declaration ofHelsinki. Approval by the institutional review board and patient consent were not required as the present study has no obligations to the Dutch Act of Scientific Research in Humans.

Imaging

All subjects had a preoperative 3T or 1.5T MRI (Philips, Eindhoven, The Netherlands) in case of an implanted vagal nerve stimulator. The sequences used were a 3D T1 with gadolinium (voxel sizes: 1 × 1 × 1 mm, TE/TR of 3.7/8.1 ms), axial T2 (voxel sizes: 0.45 × 0.45 × 2 mm, TE/TR of 80 ms/8264 ms), and a T1 inversion recovery (voxel sizes: 0.34 × 0.34 × 2 mm, TE/TR/TI of 10/7362/400 ms). Postoperative CT (Siemens, Erlangen, Germany) or 1.5T T1 (voxel sizes: 1 × 1 × 1 mm, TE/TR of 4.6/9.3 ms) MRI was performed for DBS lead localization in the week following DBS surgery.

ANT-MTT junction

Preoperative and postoperative images were fused in individual stereotactic space, also termed native space, on the Medtronic Stealthstation S7, and the midcommissural point (MCP) was identified by a neurosurgeon (YT). Coordinates relative to MCP were assessed for the ANT-MTT junction (Figure 6.2) for each hemisphere by 2 observers independently (FS and YT). Both observers were blinded to clinical outcome. Definite coordinates in lateral (*x*), anterior (*y*), and superior (*z*) directions were defined by the mean of the coordinates that were given by the two observers. In case the observers disagreed ≥1 mm in either the *x*, *y*, or *z* direction in the first observation, the final coordinates were based on their consensus in a second observation.

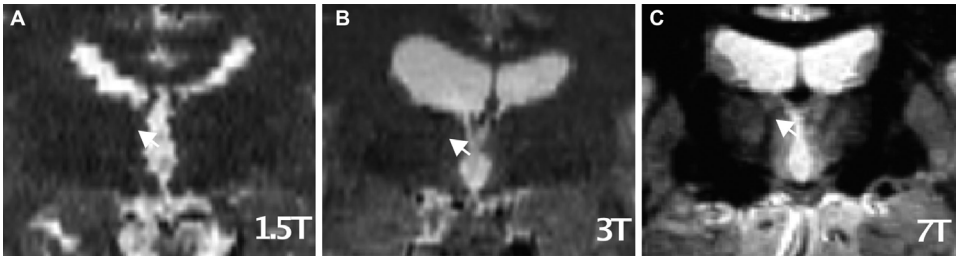

Figure 6.2 The ANT-MTT junction (arrow) at 1.5T **A**, 3T **B**, and 7T **C** MRI field strengths.

Active contact location

The locations of the active contacts were analyzed as previously published.[24,25] In short, the active contact coordinates were calculated from the coordinates of the lead tip as defined on fused pre- and postoperative images, a reference point within the trajectory and the interelectrode distance. For a bipolar contact configuration, the coordinates of the point halfway along the vector in between the cathode and anode were chosen.We analysed the distance of the active contact to the ANT-MTT junction in *x*, *y*, and *z* directions in native space and calculated the shortest distance, also known as the Euclidean distance. Using MATLAB (R2015a, MathWorks, Natick, Massachusetts), the locations of the active contacts were plotted in a common space, henceforth called ANT-MTT normalized space. The common origin in ANT-MTT normalized space [x=0, y=0, z=0] was set at the coordinates of the ANT-MTT junction in native space. Locations of the active contacts are presented as coordinates relative to this point of origin for each individual patient. The rate of the active contacts located within the ANT was assessed according to Lehtimäki et al 2018.[26]

Volume of tissue activation

Volume of tissue activation (VTA) for both monopolar and bipolar contact configurations was modelled according to the methods described by Chaturvedi et al 2013.[27] In short, the spatial extent of axonal activation was characterized by artificial neural networks based on finite element models of the electrical fields generated by the DBS lead with patient specific stimulation parameters. VTAs were subsequently plotted around the active contact coordinates in ANT-MTT normalized space. To visualize a common volume of tissue activated within each group, a stimulation heat map was generated for responders and non-responders using an activation score as described by Chueng et al.[28] These were subsequently superimposed on the Mai atlas 3rd edition[29] to visualize the hot-spot of stimulation (intersection of VTAs with the highest activation score) in anatomic space.

Statistical analysis

Intraclass correlation coefficients (ICC) were calculated to evaluate inter-observer reliability, and Pearson correlation was used to investigate the relationship between seizure control and the Euclidean distance of the active contact to the ANT-MTT junction. Coordinates relative to MCP or ANT-MTT junction and Euclidean distances were compared between groups by a Mann-Whitney U test. Patient characteristic were

compared between groups using a Mann-Whitney *U* or Chi-square test, as appropriate. *P*-values <.05 were considered statistically significant.

Results

Patients and seizure control

We included 20 patients with 1-yr follow-up of stimulation. We classified 10 patients as responders and 10 patients as non-responders, resulting in a responder rate of 50% with a median 46% reduction in total seizure frequency, and 20% of patients were seizure-free (Figure 6.3). Group characteristics and individual patient characteristics can be found in Tables, Supplemental Digital Contents 1 and 2. No significant differences were found between responders and non-responders for possible confounders such as age, epilepsy duration, the suspected seizure-onset zone or prior therapy. Of note, a seizure-onset zone in the temporal lobe was more prevalent in responders (4/10) compared to non-responders (2/10), but extratemporal seizure onset was similar between groups (5/10). Multifocal seizure onset wasmore prevalent in non-responders (3/10) than in responders (1/10).

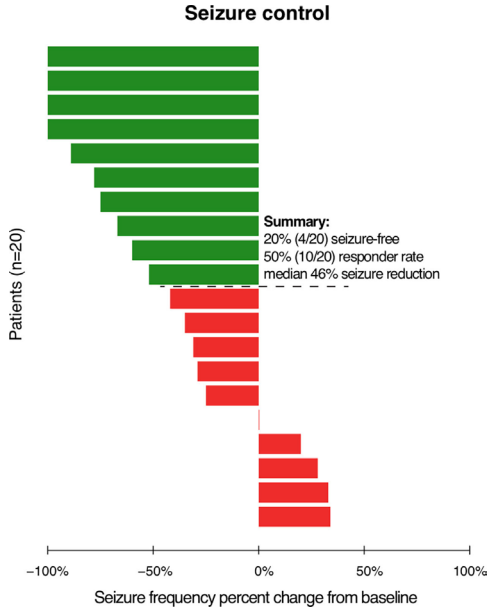

Figure 6.3 Seizure control by percentage of change from baseline in total seizure frequency at 1 yr after start of stimulation for responders (green) and non-responders (red).

ANT-MTT junction

For every individual patient, the ANT-MTT junction was identified on preoperative MR images by two independent observers. The inter-observer reliability was excellent (ICC=0.99, p<.001) for the first (independent) observations of the ANT-MTT junction. In 30/40 of these observations, the coordinates for the ANT-MTT junction by the two observers differed <1 mm in all directions and thus the mean coordinates of the first observation were used in further analysis. In 10/40 of first observations, the coordinates of the ANT-MTT junction by the 2 observers differed ≥1 mm in either the x, y, or z direction and, therefore, a consensus was reached in a second observation and used in the final analysis. The definite mean [SD] ANT-MTT junction coordinates relative to MCP for the total ANT-DBS population were x=6.2 mm [1.3], y=3.8 mm [1.7], and z=8.7 mm [1.6], which represent the stereotactic coordinates for indirect targeting of the ANT-MTT junction. These ANT-MTT junction coordinates considerably differ from the ANT coordinates (x=5-6 mm, y=0–2 mm, z=12 mm) commonly used for indirect targeting in ANT-DBS.

Active contact location

Active contact coordinates relative to MCP and the ANTMTT junction are presented in Table 6.1. The active contacts of responders were localized more medio-inferior towards the ANT-MTT junction compared to non-responders, which were localized more latero-superior and latero-inferior. The Euclidean distance of the active contact to the ANT-MTT junction is 29% smaller (p=.015) in responders (mean [SD]: 3.3 mm [1.0]) compared to non-responders (mean [SD]: 4.6 mm [1.25]). The Euclidean distance correlated with change in seizure frequency after 1 yr of DBS (r^2=0.24, p=.03), implicating active contacts located closer to the ANT-MTT junction are more likely to reduce seizure frequency (Figure 6.4). Of all 44 active contacts, 45% (20/44) were placed within the ANT and 55% outside the ANT (24/44). Contacts outside the ANT were situated in other thalamic subnuclei (eg, mediodorsal and ventral anterior nucleus) or white matter structures (eg, MTT and medullary lamina of the thalamus).Within the 2 groups, 36% (8/22) of active contacts of responders and 55% (12/22) of active contacts of non-responders were placed within the ANT.

VTA

Stimulation parameters at 1-yr follow-up entailed a mean amplitude [SD] of 5.6 V [0.4] with a pulse width of 90 μs, frequency of 145 Hz, and a stimulation-cycling mode of 1 min on and 5 min off in all subjects. Eighteen subjects received bilateral monopolar

stimulation and 2 subjects received bilateral bipolar stimulation considering side effects, namely irritability and sleep problems. Information on individual subjects' active contacts is included in Table, Supplemental Digital Content 2. VTA models were calculated from the patients' individual stimulation parameters, plotted in ANT-MTT normalized space, and superimposed on the Mai atlas[29] (Figure 6.5). The stimulation hot-spot of responders was at the medio-ventral ANT in close vicinity to the ANT-MTT junction. In non-responders, there was no evident stimulation hot-spot as the VTAs were heterogeneously distributed either at the dorsal ANT or ventral anterior nucleus (see Figure, Supplemental Digital Content 3 for heat maps in coronal and sagittal views).

Table 6.1 ANT-MTT junction and active contact locations.

	Responders, mean [SD] in mm	Non-responders, mean [SD] in mm	*p*-value, t-test
ANT-MTT junction coordinates relative to MCP			
x, lateral	6.6 [1.3]	5.9 [1.3]	0.076
y, anterior	3.7 [1.4]	3.9 [1.9]	0.741
z, superior	8.6 [1.0]	8.8 [2.1]	0.072
Active contact coordinates relative to MCP			
x, lateral	6.1 [2.0]	6.5 [1.9]	0.503
y, anterior	1.9 [1.4]	1.9 [2.8]	0.988
z, superior	8.6 [1.5]	10.2 [3.0]	0.050
Active contact coordinates relative to the ANT-MTT junction			
x, lateral	-0.5 [2.0]	0.6 [1.9]	0.067
y, anterior	-1.8 [1.6]	-2.0 [1.7]	0.698
z, superior	0.1 [1.6]	1.5 [3.4]	0.112
Euclidean distance of active contacts to the ANT-MTT junction	3.3 [1.0]	4.6 [1.25]	0.015

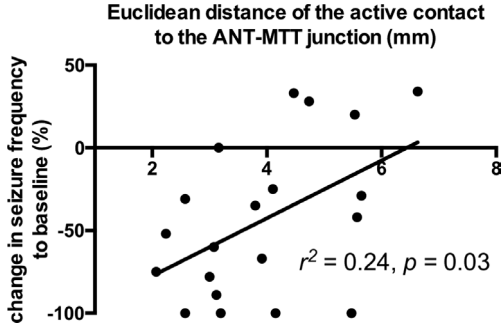

Table 6.4 Euclidean distance of the active contact to the ANT-MTT junction correlates to seizure control.

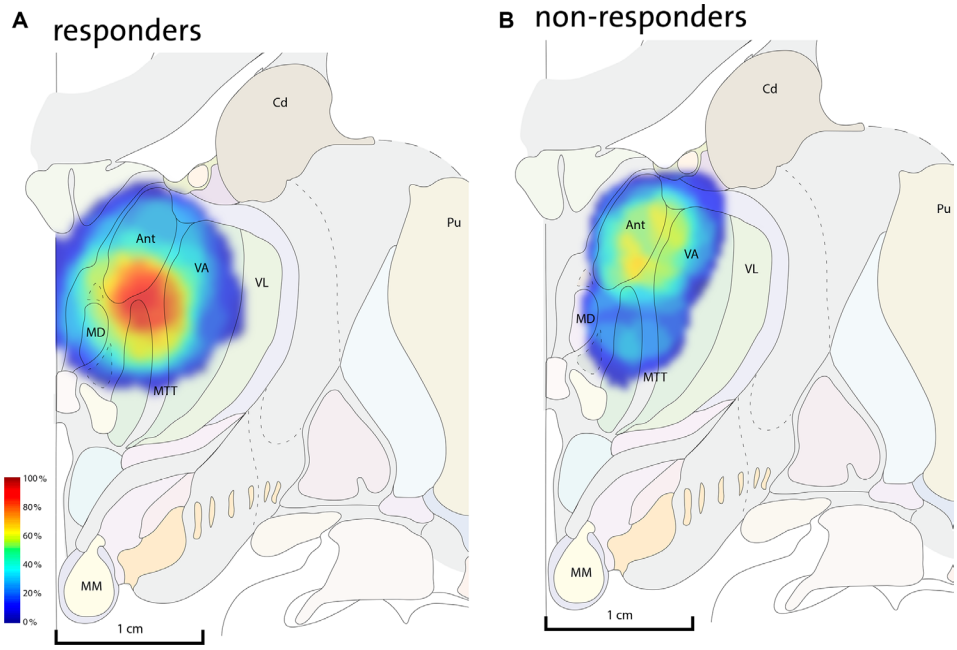

Figure 6.5 Stimulation heat maps superimposed on an adaptation of the Mai atlas 3rd edition (12,0 mm - coronal plate 31) to visualize the activation scores (range of 0%-100%) in anatomic space. The hot-spot (intersection of VTAs with the highest activation score) of responders **A** is located at the medio-ventral ANT in close vicinity to the ANT-MTT junction in contrast to no evident hot-spot in non-responders **B**.

Discussion

In this study, we investigated the relationship between stimulation site and therapy response to ANT-DBS in 20 patients with DRE by analyzing the locations of the active contacts and VTA in respect to the ANT-MTT junction. Our results indicate that the ANT-MTT junction can be used as an anatomical landmark for neurosurgical targeting in ANT-DBS and is identified with excellent interobserver reliability. Active contacts more closely located to the ANT-MTT junction were associated with increased seizure control. The stimulation hot-spot of responders was at the medio-ventral ANT in high vicinity to the ANT-MTT junction in contrast to no evident hot-spot in non-responders. Accordingly, the ANT-MTT junction is not only an anatomical landmark for direct neurosurgical targeting, but also a potential stimulation site for increased seizure control. Although not investigated in this study, neurosurgeons could revise the lead locations and neurologists could reprogram the pulse generator of nonresponders to

include stimulation of the ANT-MTT junction and optimize seizure control in patients with previously implanted DBS leads. Co-stimulation of white matter tracts may play a fundamental role in the mechanism of action of seizure control by ANT-DBS and warrants further study.

Historically, DBS is directed at grey matter and is thought to mimic a reversible, local lesioning effect. Recently, experience in movement disorders and psychiatric conditions has shown that DBS can have distant network effects, can modulate neurotransmitter release, induces neuroplasticity, and might even have permanent structural effects leading to disease course modification.[30] These global effects cannot be explained by a mere local lesioning effect and, consequently, researchers have concentrated on effects on brain states, modulated in part by white matter tracts. A recent promising example of neuromodulation specifically directed at white matter is the investigation of DBS of the fornix for dementia-related disorders.[31] Yet, in the epilepsy field, targeting fiber tracts has long shown to have striking effects, as callosotomy and VNS are well-established therapies for selected patients with DRE. Interestingly, in the first fundamental experimental studies suggesting involvement of the circuit of Papez in seizure control, Mirski et al.[21] revealed that lesioning or electrical stimulation of the MTT can protect against chemically induced seizures in guinea pigs. Consequently, interruption of this key connection by high frequency stimulation of the ANT had similar results in rats.[32,33] These experimental animal studies, the pioneering human pilots of Cooper and Upton[34] in the 1980 s, along with several case series[35,36] culminated in to the well-known investigation of ANT-DBS for the treatment of DRE by the SANTE study group.[7]

Effective stimulation sites in ANT-DBS for epilepsy

The SANTE study reported a median 56% reduction in seizure frequency compared to baseline after 2 yr with a 54% responder rate and a 69% seizure reduction with a 68% responder rate after 5 yr.[1,2] A post hoc analysis revealed that DBS lead placements were not always within the ANT. Henceforth, there is only scarce data available on the relation between the location of the active contacts and clinical outcome in ANT-DBS. A study by Lehtimäki et al.[4] made an in-depth analysis of lead placement and location of the active contacts in their ANT-DBS cohort of 15 patients. Similar to the approach used here, they manually defined the borders of the ANT in native space and constructed an ANT-normalized coordinate system. Coupling the individual active contacts to therapy response, they found that responding contacts were located at the anterior aspect of the ANT, anterodorsal to the ANT-MTT junction, compared to a slightly more postero-ventral localization in our study. Krishna et al.[6] report on the locations of the active contacts and VTA of 7 responders to ANT-DBS in Montreal

Neurological Institute (MNI) space. In line with our results, the stimulation hot-spot of responders was at the ANT-MTT junction. Contrary to our study, non-responders were not included in this analysis and a link between therapy response and stimulation site could therefore not be made. Our study reports on the clinical outcome of an extraventricular neurosurgical approach to the ANT and contributes to the current definition of effective stimulation sites in ANTDBS for epilepsy. In summary, we found that an extraventricular trajectory to the ANT results in similar (short-term) clinical outcome as reported in the SANTE trial, that the ANT-MTT junction can be identified with excellent inter-observer reliability, the active contact locations and stimulation hot-spots differ between responders and non-responders and that stimulation of the ANT-MTT junction correlates to increased seizure control.

Future perspectives in ANT-DBS targeting and stimulation

Neurosurgeons commonly use frontal transventricular and extraventricular approaches in ANT-DBS targeting, which are both safe and well tolerated. The current study suggests that the location of the effective stimulation site is similar for the transventricular and extraventricular neurosurgical approach (Figure 6.6). Although a transventricular trajectory is more likely to place the contacts within the ANT due to its perpendicular approach and additionally allows for more superior stimulation in the ANT, the ANT-MTT junction can be stimulated by both trajectories.[26] A novel posterior parietal extraventricular trajectory[37] has even been proposed recently, which is conventionally used for shunt surgeries. High accuracy (90%) for placing contacts into the ANT was found with this approach leveraging the MTT junction as an anatomical landmark.[38] Future studies comparing lead placement, stimulation sites, and clinical outcome of patients with different surgical trajectories will shed more light on the optimal surgical approach and stimulation site in ANT-DBS for epilepsy.

Considering the current available Level 3 evidence on stimulation sites in ANT-DBS, we advocate planning a neurosurgical trajectory to target the ANT-MTT junction and programming the pulse generator to stimulate this region. Given fibers can also be stimulated by lower frequencies,[39] low-frequency stimulation could be an alternative effective stimulation paradigm in ANT-DBS for epilepsy, as supported by experimental animal studies.[37,40] DBS directed at fiber tracts additionally vows to elongate battery life and decrease stimulation-induced side effects. Stimulation of a small population of axons could modulate a large population of distant (epileptic) neurons,[41] thus supporting the clinical use of lower stimulation intensities. Ultra-high-field MRI[42] and tractography based neurosurgical targeting methods[43] could facilitate distinct stimulation of the MTT (see Figure, Supplemental Digital Content 4 for tractography

of the MTT) or other fiber tracts to achieve seizure control for diverse forms of epilepsy. Potential white matter targets derived from the literature include the MTT,[44] thalamocingulate tract,[45] corpus callosum,[46] fornix,[47] cerebellothalamic,[48] and pallidothalamic[49] tracts. We anticipate renewed scientific interest and clinical exploration of white matter tract stimulation in DBS for epilepsy.

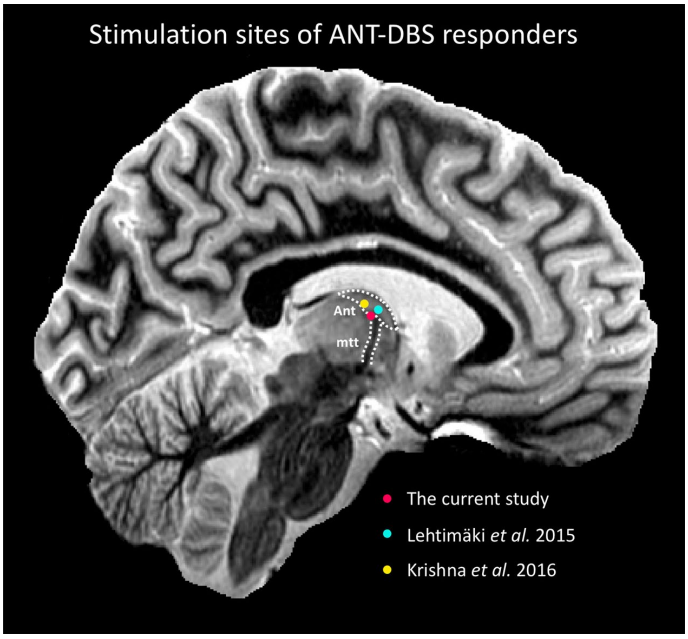

Figure 6.6 Visual representation of the stimulation sites of ANT-DBS responders in the current study and published studies, overlaid on a sagittal section of a 7T MR image. The sequence used was a T1 white-matternulled MPRAGE51 (voxel size: 0.8 × 0.8 × 0.8 mm, TE/TR/TI of 3.3/4.5/617 ms) obtained from a healthy control using a 7T magnet (Siemens, Erlangen, Germany) and a 32-channel head coil (NovaMedical,Wilmington, Massachusetts) at the Maastricht Brain Imaging Centre. Abbreviations: ANT, anterior nucleus of the thalamus; MTT, mammillothalamic tract.

Limitations

The main limitations of the present study are fourfold. First, the number of patients included is low, resulting in a limited power to detect significant changes. Yet, similar studies on anatomical localization of DBS electrodes have used comparable sample sizes.[4,28] The sample size here is the largest described yet for ANT-DBS and is thus representative, considering the currently scarce available evidence. Second, we did not

use Medtronic Suretune, the commercial software tool that is aimed at localizing DBS leads in respect to an atlas or manual segmentations of grey matter nuclei. Due to the high interindividual and interhemispheric variability in (mammillo)thalamic anatomy[10,14,50] and low MR contrast between the thalamic subnuclei[51], we instead chose to localize the leads to a patientspecific anatomical landmark (the ANT-MTT junction) using the Medtronic Stealthstation surgical navigation system. Subsequently, a normalized space for group analysis was constructed similarly to published studies.[4,25] Inherently to a study on DBS lead localizations, there are possible minimal inaccuracies of image registration and active contact localization. Third, considering the VTA model is designed to estimate the activation of largediameter axons (5.7 μm), the current predictions represent an overestimation of the spatial extent of stimulation and thus a "worst-case" scenario.[27] Fourth, although not statistically significant in our cohort, confounding by patient demographics, location of the suspected seizure-onset zone, prior epilepsy surgery, or VNS cannot be excluded due to the retrospective nature of the study. The results of our study are correlative, and the importance of these potential confounding variables is still unknown. Hence, our results should be interpreted with caution. Due to the inclusion criteria of the current CE mark of ANT-DBS, the patient population of our cohort is typically heterogeneous. Future studies with larger and more homogeneous patient populations should replicate our results and investigate stimulation hot-spots for different seizure types and seizure-onset zones to move towards seizure circuit and patient-tailored DBS in epilepsy.

Conclusion

Stimulation of the ANT-MTT junction correlates to increased seizure control. Our findings suggest a relationship between stimulation site and therapy response in ANT-DBS for DRE with a potential role for modulation of the MTT. DBS directed at white matter merits further exploration for the treatment of epilepsy.

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SUPPLEMENTARY MATERIALS

Table S6.1 Group characteristics.

	Responders	**Non-responders**	***p*-value**
Age at surgery, median in years [range]	44.5 [27-64]	37 [22-46]	0.09
Gender, number			0.53
Male / Female	8 / 2	9 / 1	
Epilepsy duration, median in years [range]	29 [12-48]	18.5 [9-30]	0.06
Suspected seizure-onset zone, number			0.43
Temporal lobe	4	2	
Extratemporal	5	5	
Frontal	3	3	
Parietal	2	2	
Multifocal	1	3	
Prior therapy, number			0.16
Vagal nerve stimulation	7	9	
Epilepsy surgery	4	1	

Table S6.2 Patient characteristics.

Subject	Group	Gender	Age at surgery (years)	Epilepsy duration (years)	Suspected seizure-onset zone	Previous therapy		Polarity of DBS	Active contacts at 1 year		Amplitude of DBS (V)		Seizures/month at baseline	Δ seizure frequency at 1 year DBS
						Resection	*VNS*		*Left*	*Right*	*Left*	*Right*		
1	R	Male	41	20	Temporal	Left temporal	No	Monopolar	1	1	5.5	6	3	Seizure free
2	R	Male	65	51	Left frontal	Left temporal	Removed	Bipolar	0-1	0-1	6	6	23	-52%
3	NR	Male	46	23	Right Parietal	No	Removed	Monopolar	1	1	6	6	5.3	+28%
4	R	Male	48	39	Left temporal	No	No	Monopolar	1	1	5.5	5.5	6	Seizure free
5	NR	Male	36	13	Bilareral temporal	No	ON	Monopolar	1	1	6	6	7.7	-35%
6	R	Female	30	12	Bilateral temporal	Left temporal	ON	Monopolar	1	1	5.5	5.5	11.3	Seizure free
7	NR	Male	40	30	Frontal	No	No	Monopolar	1	1	5.5	5.5	8.5	-42%
8	NR	Male	38	29	Left multifocal	No	ON	Monopolar	1	1	5.5	5.5	7.5	+20%
9	NR	Male	40	29	Bilateral parietal	No	Removed	Monopolar	1	1	5.5	5.5	269.8	+34%
10	NR	Female	22	19	Left frontal	No	OFF	Monopolar	1	1	5	5	12	No change
11	R	Male	36	32	Multifocal	No	OFF	Monopolar	1	1	5.5	5.5	6	Seizure free
12	NR	Male	23	14	Multifocal	No	OFF	Monopolar	1	1	6	6	36	-25%
13	R	Male	53	48	Left frontal	No	ON	Monopolar	1	1	6	6	392	-60%
14	NR	Male	45	18	Left frontal	Left frontal	ON	Bipolar	1-2	1-2	6	6	414.7	-29%
15	R	Male	27	20	Parietal	Left parietal	No	Monopolar	1	1	5.5	5.5	12	-67%
16	R	Male	55	37	Left temporal	No	Removed	Monopolar	1	1	5	5	122	-78%
17	R	Male	61	46	Right frontal	No	ON	Monopolar	1	1	5	5	8	-75%
18	R	Female	35	26	Parietal	No	ON	Monopolar	1	0	6	6	65.5	-89%
19	NR	Male	34	18	Right multifocal	No	OFF	Monopolar	1	1	5.5	5.5	10.5	+33%
20	NR	Male	26	9	Temporal	No	OFF	Monopolar	1	1	5	5	118	-31%

Abbreviations: R; responder, NR; non-responder, VNS; vagal nerve stimulation.

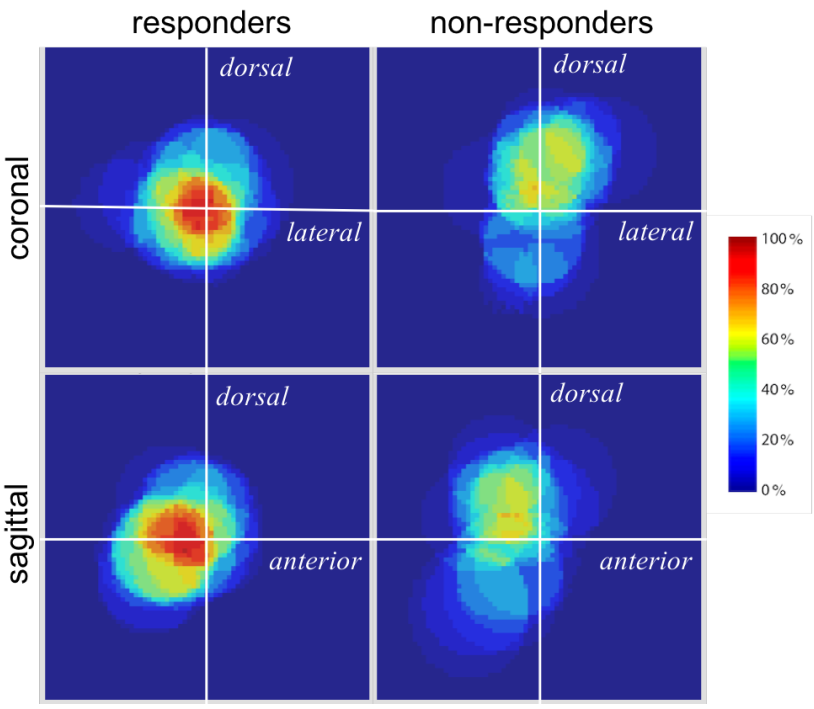

Figure S6.1 Stimulation heat-maps in coronal and sagittal view with the ANT-MTT junction as the origin [x = 0, y = 0, z = 0].

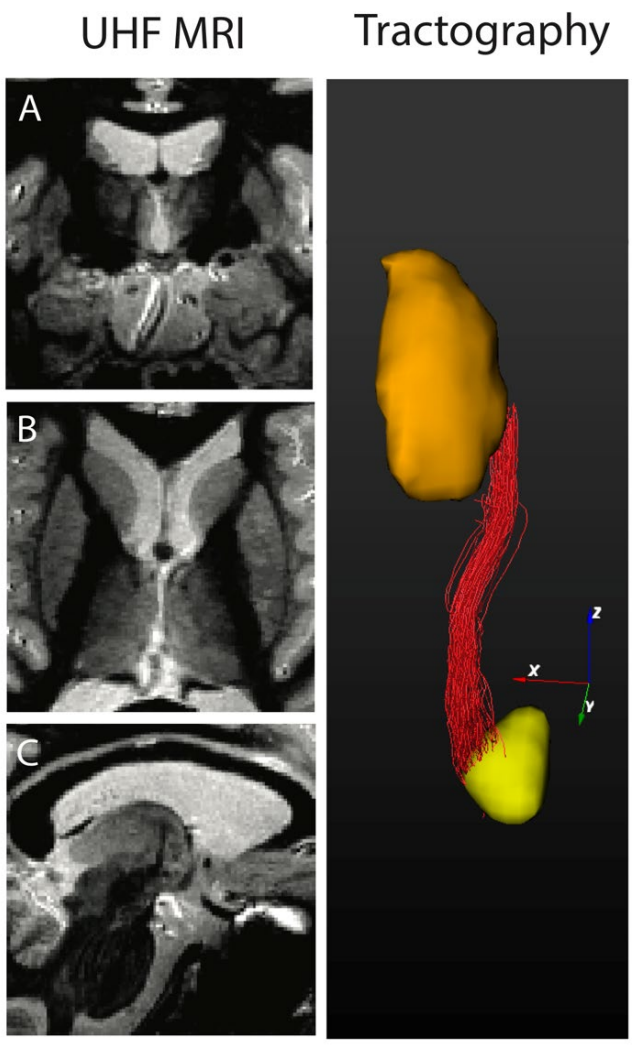

Figure S6.2 The mammillothalamic tract (white arrow and red tracts) visualized in coronal (A), axial (B) and sagittal (C) directions by ultra-high field (UHF) MRI and diffusion weighted imaging based tractography (D). The sequence used was a T1 white-matter-nulled MPRAGE (voxel size: 0.8x0.8x0.8mm, TE/TR/TI of 3.3/4.5/617ms) obtained from a healthy control using a 7T magnet (Siemens, Erlangen, Germany) and a 32-channel head coil (Nova Medical, Wilmington, MA, USA) at the Maastricht Brain Imaging Centre. The right ANT (orange) and mammillary body (yellow) were manually segmented on 7T MR images. Subsequently, probabilistic fiber tracking with FSL's probtrackx2 was performed starting in the mammilary body using the ANT as inclusion mask and manually excluding fibers leaking into the fornix.

PART III

ON COMPUTATIONAL STUDIES

"The least questioned assumptions are often the most questionable."

Paul Broca

Chapter 7

Brain stimulation and brain lesions converge on common causal circuits in neuropsychiatric disease

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Abstract

Damage to specific brain circuits can cause specific neuropsychiatric symptoms. Therapeutic stimulation to these same circuits may modulate these symptoms. To determine whether these circuits converge, we studied depression severity after brain lesions (n=461, five datasets), transcranial magnetic stimulation (n=151, four datasets) and deep brain stimulation (n=101, five datasets). Lesions and stimulation sites most associated with depression severity were connected to a similar brain circuit across all 14 datasets (p<0.001). Circuits derived from lesions, deep brain stimulation and transcranial magnetic stimulation were similar (p<0.0005), as were circuits derived from patients with major depression versus other diagnoses (p<0.001). Connectivity to this circuit predicted out-of-sample antidepressant efficacy of transcranial magnetic stimulation and deep brain stimulation sites (p<0.0001). In an independent analysis, 29 lesions and 95 stimulation sites converged on a distinct circuit for motor symptoms of Parkinson's disease (p<0.05). We conclude that lesions, transcranial magnetic stimulation and DBS converge on common brain circuitry that may represent improved neurostimulation targets for depression and other disorders.

INTRODUCTION

CAUSAL NEUROANATOMY CAN BE MAPPED IN ANIMAL MODELS BY PRECISELY modulating different brain circuits in well-controlled experiments.[1,2] However, it can be challenging to translate these findings into human therapeutics.[3,4] In humans, mapping of psychiatric symptoms is based primarily on correlation, resulting in a 'causality' gap when attempting to translate this information into effective treatments. Causality may be inferred in humans based on the clinical effects of focal brain lesions, transcranial magnetic stimulation (TMS) and deep brain stimulation (DBS).[2] These modalities have each been used to link depression symptoms to specific brain circuits based on the location of lesions or stimulation sites that affect depression severity.[2,5-9] Each result has been proposed as a potential solution to the causality gap between neuroimaging correlates and effective treatments.[2,10]

It remains unclear whether these three causal sources of information converge on the same circuit or therapeutic target.[2,11,12] Heterogeneity in lesion location, stimulation site location, neuromodulation modality, patient population, depression symptoms, depression subtypes and numerous other factors argue against a common neuroanatomical substrate. If these causal sources of information converge on a similar brain circuit despite this heterogeneity, this would have implications for localization and treatment of depression and for bridging the causality gap more generally.[2] For example, it has been proposed that TMS and DBS sites connected to similar circuits may modulate similar symptoms[13], lesions causing a symptom may be connected to the same circuit as brain stimulation targets that relieve that symptom5 and similar symptoms map to similar circuits across different diagnoses.[6,14] Confirmation of these hypotheses may lead to a transformative framework for targeting brain stimulation treatments.[2,12]

To address this, we analysed 14 independent datasets of patients with brain lesions, TMS or DBS. Each dataset included variability in the lesion or stimulation locations and variability in depression symptoms, measured after the lesion or before and after therapeutic brain stimulation. We also extended this approach to three additional datasets of patients with brain lesions or DBS sites associated with motor symptoms of Parkinson's disease (PD). The brain regions functionally connected to each location were identified using a normative connectome database. This method identifies a polysynaptic brain circuit underlying each location, allowing one to test whether lesions or stimulation sites in different brain regions intersect the same population-derived circuit.[5] We test whether TMS and DBS sites that affect depression are connected to the same brain circuit, whether lesion locations associated with depression and stimulation sites that affect depression are connected to the same brain circuit, whether this circuit is

associated with depression severity associated with depression severity or is relevant beyond depression.

Results

Characteristics of included datasets

We identified 14 datasets including 461 lesions (Figure 7.1a)[15], 151 TMS sites (Figure 7.1b)[8,16-18] and 101 DBS sites (Figure 7.1c)[9,19-23] (Supplementary Table S7.1). Five datasets included patients who were evaluated for depression severity after penetrating brain injury, ischaemic stroke or haemorrhagic stroke. Seven datasets included patients who were treated for primary major depressive disorder (MDD) with either TMS (four datasets) or DBS (three datasets). Finally, two datasets included patients receiving DBS for other disorders (PD or epilepsy), but which measured change in depressive symptoms as a potential side effect.

Similar 'depression circuits' across 14 independent datasets

The location of each lesion or brain stimulation site (Figure 7.2a-c, top panels) was mapped to an underlying brain circuit using a large normative connectome database (n=1,000) and previously validated methods (Figure 7.2a-c, bottom panels).[5] The normative connectome was used to estimate connectivity of each lesion or stimulation site to every voxel in the brain. At each voxel, a Pearson r value was computed for the correlation between depression score and lesion or stimulation site connectivity to that voxel (Figure 7.2a-c, right panels), yielding a population-derived 'circuit map' for each of the 14 datasets (Supplementary Figure S7.1).

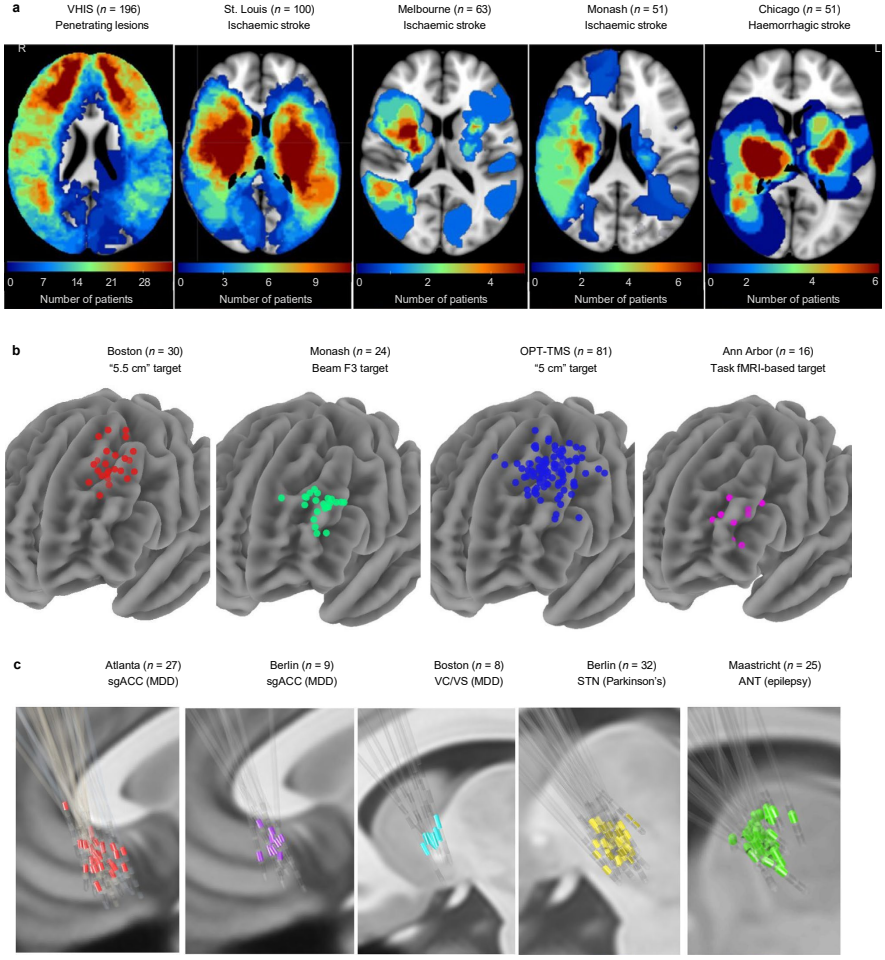

Figure 7.1 Lesion locations and brain stimulation sites across 14 datasets. **a–c**, The analysis included 461 brain lesions across five datasets and three different diagnoses (**a**); 151 TMS sites across four datasets, one diagnosis (major depressive disorder) and four different TMS targets (**b**); and 101 DBS sites across five datasets, three different diagnoses and four different DBS targets (**c**). OPT-TMS, Optimizing TMS for the Treatment of Depression Study; sgACC, subgenual anterior cingulate cortex; VC/VS, ventral capsule/ventral striatum; STN, subthalamic nucleus; ANT, anterior nucleus of the thalamus.

Cross-dataset similarity was assessed by computing the spatial correlation between each pair of circuit maps (for example, dataset 1 versus dataset 2) and by comparing each circuit map with a combined map from the other 13 datasets. Significance was assessed using permutation testing, in which the spatial correlation was re-computed after randomly pairing each patient's lesion or stimulation site with a different patient's depression score within the same dataset.[6] The average pairwise similarity between circuit maps, weighted by sample size, was higher than expected by chance (mean spatial r=0.24, 95% CI 0.19 to 0.29, p<0.001) (Figure 7.3a and Supplementary Figure S7.2a) and similar to a weighted mean map generated from the other 13 datasets (mean spatial r=0.45, 95% CI 0.33 to 0.57, p<0.001). Results were unchanged when using Kendall tau (p<0.001) or Euclidean distance (p=0.0013) instead of Pearson correlation or when including lesion size as a covariate.

To rule out methodological bias, we conducted a control analysis using patient age instead of depression scores. Age is presumably unrelated to stimulation or lesion location, so we hypothesized that this analysis would yield significantly weaker cross-dataset similarity. Indeed, the 14 control maps did not match one another (mean spatial r=−0.02, 95% CI −0.09 to 0.05, p=0.86, Bayes factor $(BF)_{01}$=1.01) and did not match a map generated from the other 13 datasets (mean spatial r=−0.01, 95% CI −0.14 to 0.11, BF_{01}=1.001). The control maps did not match the depression circuit maps (mean spatial r=−0.05, 95% CI −0.12 to 0.02, p=0.93, BF_{01}=1.003). Similarity between control maps was significantly weaker than similarity between depression circuit maps (p=0.0023).

Convergence across brain lesions, TMS and DBS

To determine whether lesions, TMS and DBS converge on the same circuit, we grouped the different datasets according to modality. Depression circuit maps derived from brain lesion datasets were similar to circuit maps derived from TMS datasets (mean spatial r=0.28, 95% CI 0.17 to 0.39, p=0.0025), DBS datasets (mean spatial r=0.19, 95% CI 0.10 to 0.28, p=0.0037) or both neuromodulation modalities combined (mean spatial r=0.25, 95% CI 0.18 to 0.32, p<0.001) (Figure 7.3 and Supplementary Figure 7.2a). Depression circuit maps derived from TMS were similar to those derived from DBS (mean spatial r=0.25, 95% CI 0.11 to 0.39, p<0.001) (Figure 7.3 and Supplementary Figure S7.2a).

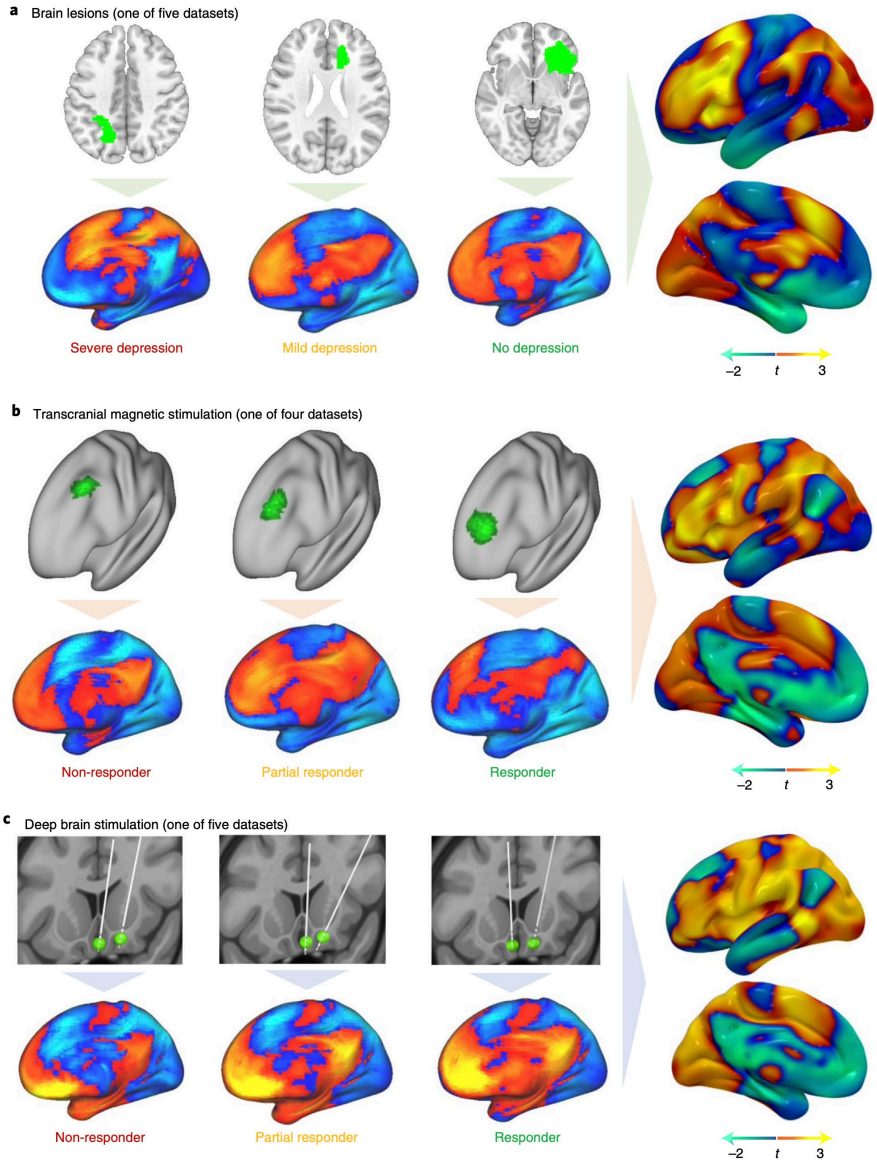

Figure 7.2 Identifying depression circuit maps for each cohort. **a–c**, Brain lesions (**a**), TMS sites (**b**) and DBS sites (**c**) were all mapped to a common brain atlas (top row of each panel). Functional connectivity of each lesion location or stimulation site was computed using a normative connectome database (bottom row of each panel). Positive functional connectivity is shown in warm colours (red, orange, yellow), and negative functional connectivity in cool colours (blue, teal, green). Connections most associated with depression score (lesion datasets) or change in depression score (brain stimulation datasets) were identified for each dataset (right column). The colour scale was inverted for TMS datasets because TMS sites that improve depression are thought to be anti-correlated to DBS sites that improve depression or lesion sites associated with lower risk of depression.

As a control, this analysis was also repeated using patient age instead of depression score. We hypothesized that this analysis would yield significantly weaker cross-dataset spatial correlation. Age-based circuit maps derived from brain lesions were not similar to those derived from TMS (mean spatial r=−0.04, 95% CI −0.17 to 0.09, p=0.70, BF_{01}=1.07), DBS (mean spatial r=−0.14, 95% CI −0.26 to −0.02, p=0.97, BF_{01}=6.8) or both neuromodulation modalities combined (mean spatial r=−0.07, 95% CI −0.17 to 0.02, BF_{01}=3.4). Control maps derived from TMS were not similar to those derived from DBS (mean spatial r=0.01, 95% CI −0.14 to 0.16, p=0.43, BF_{01}=0.99). In all cases, similarity between control maps was significantly weaker than similarity between depression circuit maps (p=0.0038). Control maps from neuromodulation datasets did not match depression circuit maps from lesion datasets (mean spatial r=−0.11, 95% CI −0.19 to −0.03, BF_{01}=16.9). Control maps from lesion datasets also did not significantly match depression circuit maps from neuromodulation datasets (mean spatial r=0.07, 95% CI −0.02 to 0.17), although Bayesian analysis indicates moderate evidence for a correlation (BF_{01}=0.29) (Figure 7.4a).

Finally, we assessed whether within-modality similarity of our depression circuit maps was stronger than between-modality similarity. We compared each depression circuit map with a combined map generated from the remaining datasets within a modality (for example, TMS dataset 1 versus three other TMS datasets) or between different modalities (for example, TMS dataset 1 versus nine DBS/lesion datasets). Within-modality similarity (spatial r=0.46) was identical to between-modality similarity (spatial r=0.46). We also repeated this analysis using pairwise comparisons between circuit maps, which yielded a similar result (spatial r=0.24 versus r=0.25, respectively).

The circuit is transdiagnostic but specific to depression

We compared depression circuit maps derived from datasets of patients with MDD (seven datasets, n=199) with those derived from datasets of patients with other diagnoses such as stroke, penetrating head trauma, PD and epilepsy (seven datasets, n=518).

Depression circuit maps derived from MDD datasets were similar to depression circuit maps derived from patients without MDD (mean spatial r=0.26, 95% CI 0.19 to 0.33, p<0.001) (Figure 7.4b and Supplementary Figure S7.2).

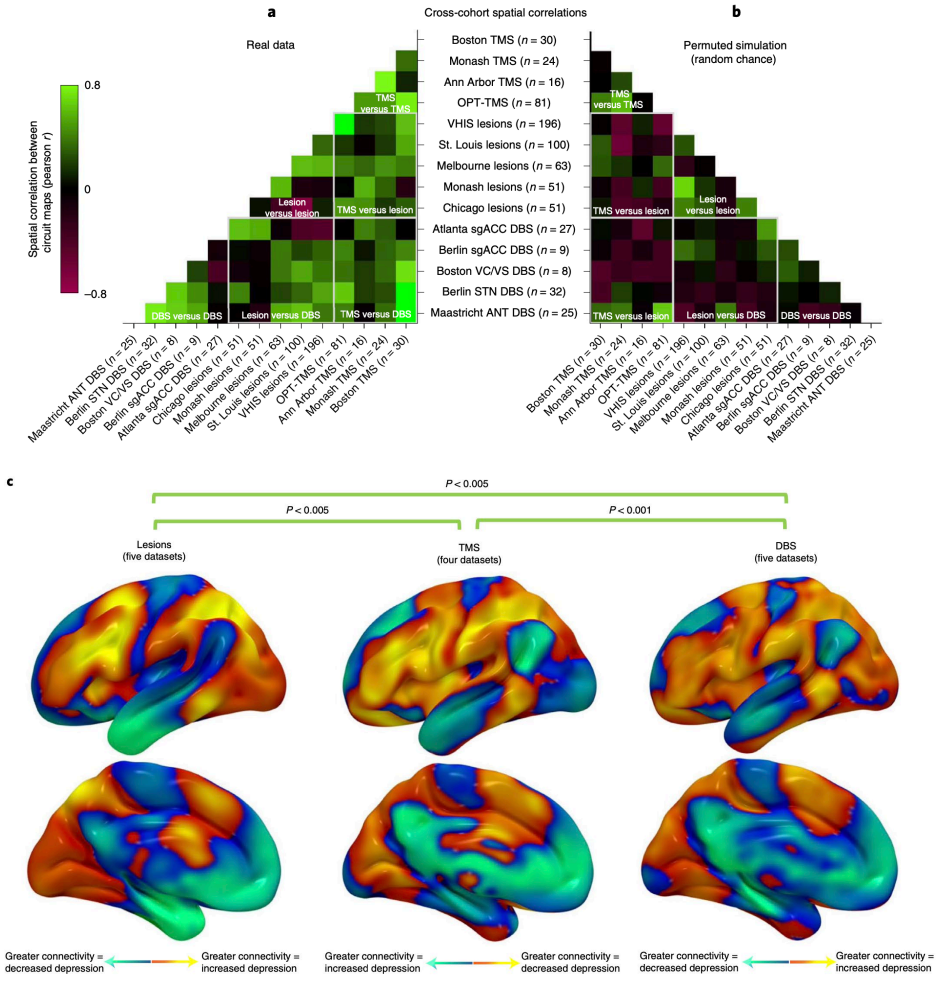

Figure 7.3 Depression circuit maps are similar across 14 datasets (n=713). **a**, The 14 circuit maps were consistently similar to one another (mean r=0.24, 95% CI 0.19 to 0.29), as depicted in this cross-correlogram comparing different datasets. Permutation testing confirmed that the weighted mean cross-correlation was significantly stronger than expected by chance (p<0.001, 10,000 permutations). Green colours represent high spatial correlation between circuit maps, black boxes represent neutral correlation and red boxes represent negative correlation. **b**, Representative example of correlation between circuit maps generated from randomly permuted data. This analysis confirmed that no overall cross-correlation is expected by chance (mean r=0.00, 95% CI -0.01 to 0.01). **c**, Depression circuit maps were similar between lesion datasets (n=461), TMS datasets (n=151) and DBS datasets (n=101). Permutation testing confirmed that each comparison was significantly stronger than expected by chance (p<0.005, 10,000 permutations). For display purposes, depression circuit maps were averaged (weighted mean) across datasets within each modality. The colour scale on TMS circuit maps is inverted to facilitate visual comparison with lesion and DBS circuit maps.

To assess whether this result was driven by overall clinical severity/disability rather than depression, this analysis was repeated using the severity of the primary presenting symptom in non-MDD datasets. This control analysis included stroke severity, PD motor improvement or seizure frequency improvement. Control circuit maps from non-MDD datasets failed to match depression circuit maps from MDD datasets (mean spatial r=−0.03, 95% CI −0.09 to 0.03, BF_{01}=1.04), and this spatial cross-correlation was significantly weaker than the cross-correlation between the depression circuit maps used in our primary analysis (p<0.001) (Figure 7.4b).

To assess specificity to depression versus other cognitive or emotional symptoms, we generated control circuit maps using 34 other cognitive/emotional scores, which were available in our two largest datasets (Vietnam Head Injury Study (VHIS) and St. Louis). Our leave-one-dataset-out depression circuit map (generated from the other 13 datasets) was more similar to the VHIS depression circuit map than to the 28 control circuit maps (r=0.54 versus r<0.35) (Supplementary Figure S7.3a). Our leave-one-dataset-out depression circuit map was also more similar to the St. Louis depression circuit map than to the six control circuit maps (r=0.39 versus r<0.23) (Supplementary Figure 7.3b). Across both datasets, the leave-one-dataset-out maps were significantly more similar to the depression circuit maps than to the other circuit maps (p=0.0032).

Combining all datasets and explaining clinical variance

We generated a combined depression circuit map by taking the mean of all 14 circuit maps, weighted by the sample size of each dataset (Figure 7.5a). Peak regions in this combined map include the intraparietal sulcus, dorsolateral prefrontal cortex, inferior frontal gyrus, ventromedial prefrontal cortex and subgenual cingulate cortex (Supplementary Table S7.2). Compared with a consensus brain network parcellation[24], our circuit was most similar to the dorsal attention network and frontoparietal control network, and was most anti-correlated to the default mode network and limbic network (Supplementary Figure S7.4).

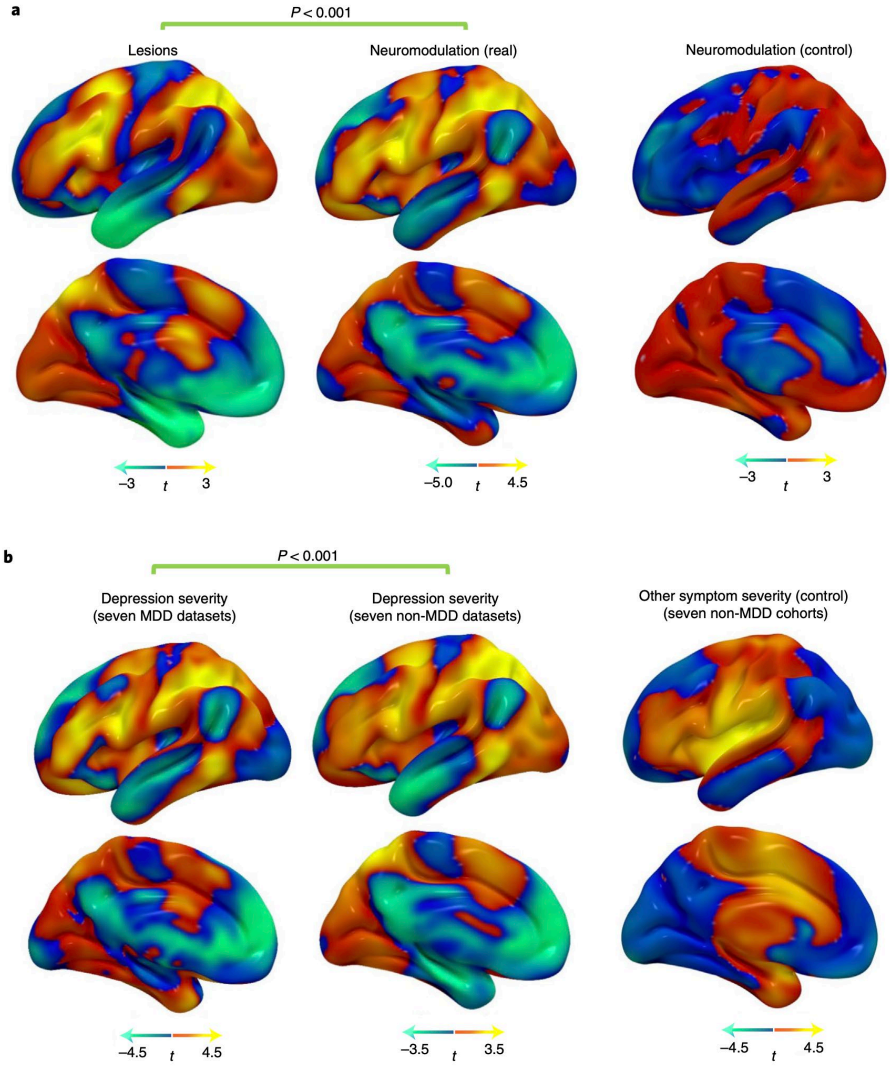

Figure 7.4 Depression circuit maps are similar across lesions, neuromodulation and diagnoses. a, Depression circuit maps were similar between lesion datasets and neuromodulation datasets (mean r=0.25, 95% CI 0.16 to 0.34). Permutation testing confirmed that this similarity was stronger than expected by chance (p<0.001, 10,000 permutations). In a control analysis, there was no similarity between depression circuit maps from lesion datasets and age-based circuit maps from neuromodulation datasets (r=–0.11, 95% CI -0.21 to -0.01, p=0.93). b, Depression circuit maps were similar between MDD patients and non-MDD patients (mean r=0.26, 95% CI 0.16 to 0.36, p<0.001). Permutation testing confirmed that this similarity was stronger than expected by chance (p<0.001, 10,000 permutations). In a control analysis, there was no similarity between depression circuit maps from MDD datasets and 'other symptom severity' circuit maps in non-MDD datasets (r=–0.03, 95% CI -0.12 to 0.06, p=0.77).

In a leave-one-dataset-out analysis, we assessed whether connectivity of the stimulation site to our depression circuit could predict depression outcomes after TMS and DBS. In each neuromodulation dataset, each patient's stimulation site connectivity profile was compared with a circuit map generated from the remaining 13 datasets using spatial correlations. Across all neuromodulation datasets, connectivity to our circuit predicted the efficacy of treatment targets (weighted mean r=0.22, 95% CI 0.11 to 0.33 p<0.001) (Figure 7.5b). The leave-one-dataset-out circuit independently predicted clinical variance in TMS datasets (weighted mean r=0.24, p=0.0034) and DBS datasets (weighted mean r=0.21, p=0.033).

Comparison with prior established methods

We hypothesized that our mapping and targeting approach would outperform established methods for both causal brain mapping and neuromodulation targeting. First, we repeated the primary analysis using voxel-lesion symptom mapping (VLSM), a tool that is widely used to localize behaviours using lesions.[25] Similar approaches have also been applied to TMS[16] and DBS.[26] VLSM failed to detect significant similarity across all 14 datasets (mean spatial r=−0.03, p=0.91, BF_{01}=1.001).

Next, we compared our approach with existing approaches for connectivity-based neuromodulation targeting. For each TMS and DBS site, we computed connectivity to the subgenual cingulate cortex, which has been shown to predict TMS response[8,18] and has been used as a DBS target.[27] Indeed, antidepressant efficacy of each stimulation site was correlated with its connectivity to the subgenual cingulate (weighted mean r=−0.13, 95% CI −0.24 to −0.02, p=0.039). Connectivity to our leave-one-dataset-out depression circuit predicted outcomes (weighted mean r=0.22, 95% CI 0.11 to 0.33, p<0.001) significantly better than connectivity to the subgenual cingulate (p=0.012).

Generalizability of the method beyond depression

To demonstrate that this approach can generalize to other neuropsychiatric disorders, we also repeated the analysis using previously published data on motor symptoms of PD, the most common clinical indication for DBS. This included 29 case reports of lesion-induced parkinsonism[28], 95 patients (two datasets) who received DBS for PD[28] and one TMS site (primary motor cortex, hand knob) which demonstrated efficacy for PD in a meta-analysis of ten randomized trials.[29]

The PD circuit derived from lesions was similar to the PD circuit derived from DBS (p=0.01) (Supplementary Figure S7.5). Connectivity to the motor cortex TMS target predicted change in PD motor symptoms with DBS (p=0.02) and risk of parkinsonism

after a brain lesion (p=0.0005) (Supplementary Figure S7.5). In a leave-one-dataset-out analysis, the PD circuit predicted motor improvement with DBS (r=0.26, p=0.01).
To confirm specificity, we used the PD circuit as a control for depression and vice versa. Connectivity to the PD circuit was independently predictive of motor improvement (p=0.0003) after controlling for connectivity to the depression circuit. Connectivity to the depression circuit was independently predictive of mood improvement (p=0.02) after controlling for connectivity to the PD circuit. By itself, the depression circuit did not significantly predict motor improvement with DBS (r=–0.06, p=0.58, BF_{01}=3.4). The PD circuit also did not significantly predict depression improvement with TMS and DBS (r=0.06, p=0.32), although Bayesian analysis indicates moderate evidence for a correlation (BF_{01}=0.29).

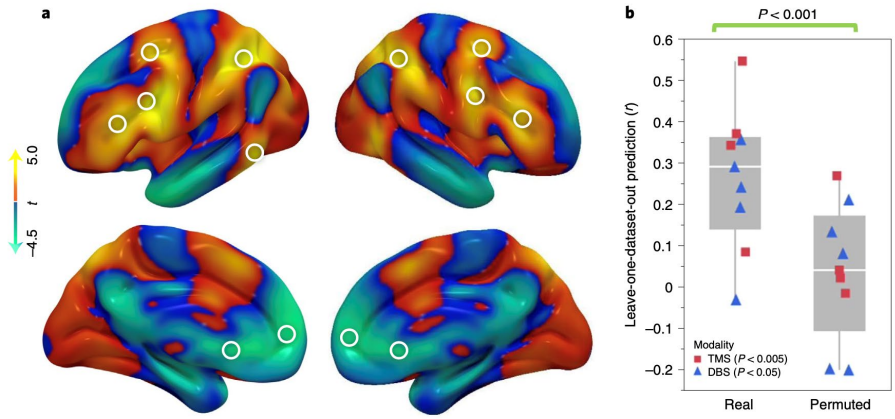

Figure 7.5 Combining all circuit maps and predicting clinical variance. **a**, A combined 'depression circuit' was generated from all 14 datasets. Peaks in this circuit are depicted by white circles. Positive peaks included the dorsolateral prefrontal cortex, frontal eye fields, inferior frontal gyrus, intraparietal sulcus and extrastriate visual cortex. Negative peaks included the subgenual cingulate cortex and ventromedial prefrontal cortex. Peaks are listed in Supplementary Table S7.2. **b**, Across the 9 neuromodulation cohorts (n=252), antidepressant efficacy was predicted by stimulation site connectivity to a circuit generated from the remaining 13 cohorts (mean r=0.22), shown as the median (line), interquartile range (box limits), outliers (whiskers) and the individual correlation value for each neuromodulation (points). Permutation testing confirmed that this similarity was stronger than expected by chance (p<0.001, 10,000 permutations). This was true for both TMS (n=151, r=0.24, p=0.0034 with 10,000 permutations) and DBS (n=101, r=0.21, p=0.033 with 10,000 permutations).

Discussion

Across 14 independent datasets, we found that mapping depression based on brain lesions, TMS sites and DBS sites converged on a common neuroanatomical substrate. This convergence was robust despite many sources of heterogeneity that should bias us against a common substrate, including different lesion distributions, lesion aetiologies, stimulation targets, stimulation modalities and neuropsychiatric diagnoses. Our convergent circuit includes regions previously implicated in depression such as the subgenual cingulate, ventromedial prefrontal cortex and dorsolateral prefrontal cortex.[30-34] However, our different datasets converged on a common brain circuit or brain network, not an individual brain region. The circuit was consistent with prior work on large-scale brain networks in depression, as it is similar to the dorsal attention network and the frontoparietal control network and anti-correlated with the default mode network and limbic network.[35] This neuroanatomical convergence has several important implications.

First, TMS sites and DBS sites that modulate depression were connected to a similar circuit. To our knowledge, this is the strongest evidence to date that invasive and non-invasive brain stimulation are targeting the same circuit to treat the same symptom.[12,13] Given recent negative trials of DBS[20,36] and TMS[37] for depression, our circuit may serve as a refined therapeutic target to improve neuromodulation outcomes in future trials. More broadly, this finding supports the use of circuit mapping to define neuromodulation targets[6,8,9] and translate therapy between stimulation modalities for various neuropsychiatric disorders.[13] Furthermore, our findings support the notion that high-frequency TMS and high-frequency DBS modulate brain circuits in opposite directions[13], as the TMS and DBS maps were inverted with respect to each other.

Second, lesion locations associated with depression and stimulation sites that modulate depression were connected to a similar circuit. This finding generalized to Parkinson's disease as lesion locations associated with parkinsonism and stimulation sites that modulate parkinsonism were connected to a similar circuit, which was distinct from our depression circuit. To our knowledge, this is the strongest evidence to date showing that lesions causing a symptom can identify therapeutic targets for symptom relief. Given that lesion network mapping has been used to map a broad range of neuropsychiatric symptoms, from amnesia to criminality[5], our approach may have therapeutic implications well beyond depression and Parkinson's disease.

Third, we identified similar depression circuits in patients with MDD, penetrating brain injury, stroke, epilepsy and PD. This suggests that depression symptoms map to a similar neuroanatomical substrate independent of whether the symptoms are caused by a

primary psychiatric disorder, a structural brain lesion or a side effect of DBS. This finding is consistent with the recent Research Domain Criteria initiative, which seeks to establish transdiagnostic constructs for psychiatric symptom severity.[38] Our findings were also specific to depression relative to other neuropsychiatric symptoms, but further work is needed to conclusively confirm specificity.

Fourth, our findings were consistent across 14 independent datasets. Most prior studies in depression have focused on a single dataset[30–34], although larger studies are beginning to appear.[14] Meta-analyses often find poor consistency in neuroimaging correlates of depression.[33,34] To our knowledge, our consistency across 14 datasets, including a leave-one-dataset-out analysis, is one of the strongest demonstrations of result consistency for a psychiatric condition. Furthermore, the results survived rigorous permutation-based statistical testing, a highly conservative approach that prevents type I error due to multiple comparisons or a biased analysis.

Fifth, it is worth highlighting our focus on 'causal' sources of information such as lesions and brain stimulation. This resolves some of the interpretive ambiguity associated with neuroimaging correlates of depressive symptoms or antidepressant efficacy of non-anatomically targeted treatments.[39] By combining brain lesions and brain stimulation, this study moves us towards the goal of "mapping causal circuitry in human depression"[2], potentially facilitating more direct translation to targeted therapeutics.

Finally, our parsimonious mapping and targeting model outperformed established approaches for both lesion-based brain mapping and connectivity-based neuromodulation targeting. Our approach identified relationships that were not apparent using VLSM, illustrating the potential of brain connectivity to detect trends beyond what is possible using anatomical location alone. Our approach also explained more clinical variance than subgenual connectivity, which is widely used to target neuromodulation.[40-44]

Our analysis may seem circular or biased given that the TMS and DBS sites for MDD were chosen because they were already known to be part of a 'depression circuit'. However, our depression circuit was derived from the variance across stimulation sites within each target, not simply the location of the intended target. For example, the left prefrontal cortex appears as part of our depression circuit not because it was targeted with TMS but because different TMS sites across the left prefrontal cortex produced different effects on depression, different DBS sites produced different effects on depression symptoms depending on their connectivity to the left prefrontal cortex and different lesion locations were associated with different amounts of depression depending on their connectivity to left prefrontal cortex. It is also worth noting that this concern is not relevant for lesions, which were randomly distributed throughout the

brain yet identified a depression circuit that was very similar to the circuit identified from TMS or DBS sites.

There are several limitations. First, this analysis was retrospective, taking advantage of existing datasets with heterogeneous populations and outcome metrics, limiting the amount of variance that can be explained. Prospective validation is required to confirm whether targeting our circuit results in improved antidepressant response. Second, most datasets only included a single depression score without subscales, which may also limit the amount of variance that can be explained. Given that different symptom clusters respond to stimulation of different circuits with TMS[6], future work with more detailed phenotyping may enable further subclassification. Third, we used a normative functional connectome for all circuit mapping, as prior work suggests that using a disease-matched connectome makes little difference for either depression or Parkinson's disease.[6,8] However, this analysis could be repeated using connectomes that are age, gender and disease matched to each dataset. Similarly, this analysis could be repeated using measures of structural white matter connectivity or individualized functional connectivity.[9,18,45] Individualized connectivity may explain additional variance, but adds additional noise to the analysis.[46] Individualized neurostimulation-induced electric field modelling may also be valuable, but prior work has shown it to yield similar functional connectivity estimates to our simplified model.[47]

In conclusion, these results support the existence of at least one neuroanatomical substrate for depression symptoms. More broadly, by combining lesion locations, non-invasive stimulation sites and invasive stimulation sites, we introduce a method for identifying a convergent neuroanatomical substrate for neurological and psychiatric symptoms. Future work should seek to prospectively determine whether this convergent substrate provides an improved target for neuromodulation therapies.

Methods

Characteristics of included datasets

We sought out multiple datasets that included magnetic resonance imaging or computed tomography of focal brain lesions and stimulation sites. Lesions and stimulation sites showed incidentally variable locations in different patients. Localization methods are described in the Supplementary Information. All depression datasets included continuous scores on a validated depression metric. All PD datasets included either a clear case description of lesion-induced parkinsonism or continuous scores on the

Unified Parkinson's Disease Rating Scale (UPDRS). In each dataset, participants provided informed consent to data collection or the institutional review board approved retrospective analysis of symptom and imaging data.

Patients with missing data were excluded from the analysis. To avoid bias due to unequal variances, unequal sample sizes or inconsistent severity cut-offs for different datasets, each dataset was analysed independently. Study characteristics are summarized in Supplementary Table S7.1.

No statistical methods were used to pre-determine sample size, but our sample sizes are larger than the largest prior studies of lesions[7], TMS sites6 or DBS sites[21] in depression.

Generation of circuit maps

A normative human connectome database was used to compute mean resting-state functional connectivity of each patient's lesion or stimulation site based on 1,000 healthy subjects, as previously described.[5-7] This yielded a whole-brain connectivity map of each patient's lesion or stimulation site (Figure 7.2).

In the TMS and DBS datasets with depression outcomes, these connectivity maps were compared with change in depression score using partial Pearson correlation at each voxel, controlling for pre-treatment depression severity. In the lesion datasets with depression outcomes, connectivity maps were compared with overall depression scores using Pearson correlation at each voxel. For each dataset, this analysis yielded a whole-brain 'circuit map' of connections correlated with antidepressant efficacy (for TMS and DBS) or depression severity (for lesions). TMS-based circuit maps were multiplied by −1 because TMS sites that improve depression are thought to be anti-correlated to DBS sites that improve depression[13] or lesion sites associated with lower risk of depression.[5,7] Inverting the circuit maps for TMS also facilitates visual comparison across all three modalities (Figure 7.2).

In the PD DBS datasets, patient-specific connectivity maps were compared with change in UPDRS score. The connectivity of lesions causing parkinsonism was estimated using a one-sample *t* test at each voxel. For each dataset, this yielded a whole-brain circuit map of connections associated with parkinsonism. In the absence of individualized TMS sites, we generated a group-mean region of interest at the M1 hand knob (MNI coordinates [−40, −20, 62]), which has been shown to be the most effective TMS target for Parkinson's disease.[29]

We generated control circuit maps using two different approaches. For all datasets, control maps were generated using patient age, which is presumably unrelated to stimulation site or lesion location, rather than depression scores. For all non-MDD datasets, additional control maps were generated using severity of the primary presenting

symptom, including National Institutes of Health Stroke Scale (stroke patients), Neurobehavioral Rating Scale (penetrating brain injury patients), UPDRS (Parkinson's disease patients) and seizure frequency (epilepsy patients).

Computational and statistical methods

All computational/statistical analyses were conducted using customized MATLAB scripts, except as otherwise specified. All correlation coefficients were Fisher-transformed before further analysis. To facilitate comparison across datasets with different sample sizes, voxel-wise Fisher z values were converted to t values. All parametric p values were computed using a two-tailed hypothesis test. Similarity between different maps was assessed using spatial correlations.

To confirm similarity across different datasets, we computed the mean spatial cross-correlation between the circuit maps in each analysis. Because the datasets were collected in highly heterogeneous settings, they could not be assumed to have identical distributions. To address this, statistical significance was addressed using a non-parametric multi-level block permutation testing approach. In this permutation test, the mean spatial correlation was re-computed 25,000 times in simulated data. The null distribution of this permutation test was defined by randomly re-assigning each patient's connectivity map with a different patient's clinical variables within the same dataset. A p value was defined as the percentage of randomly permuted results that were stronger than the real result, as in prior work.[6]

For null findings, the resulting t values were used to compute BFs, which were used to compare likelihood of the null hypothesis with the likelihood of the alternative hypothesis.[48] In the case of spatial correlations, the null hypothesis was that there is no similarity between the two maps in question. Thus, for the purpose of calculating BFs, stronger positive correlations were considered to support the alternative hypothesis, while weaker positive correlations and negative correlations were considered to support the null hypothesis.[49]

Combining and comparing circuit maps

The 14 circuit maps were then categorized to assess for similarity between different modalities or diagnoses. Categories included TMS, DBS, neuromodulation (TMS and DBS combined), lesions, MDD (all modalities) and non-MDD (all modalities). MDD and non-MDD datasets were defined according to the inclusion criteria of the original study. We hypothesized that (1) TMS, DBS and lesion datasets would yield similar circuits, (2) lesions and neuromodulation would yield similar circuits and (3) MDD and non-MDD patients would yield similar circuits. To statistically compare different

categories, we computed the mean spatial cross-correlation of all circuit maps in one category with all circuit maps in the other category. Significance was assessed using permutation testing as above.

To visualize the map for each category, circuit maps from different datasets were combined into a mean circuit map across all voxels, weighted by the sample size of each dataset. This weighted mean approach was chosen over a combined linear model because it maintains independence between datasets, thus reducing the statistical penalty associated with combining heterogeneous datasets.[50]

Each dataset's circuit map was also compared with a leave-one-dataset-out circuit map generated by taking the weighted mean of the other 13 circuit maps. This yielded a leave-one-dataset-out spatial correlation for each dataset. The weighted mean of these spatial correlations was considered to represent the overall similarity between each circuit map and the remaining circuit maps. This value was assessed for significance using permutation testing as above.

Assessing specificity to depression

To confirm that the results were not driven by overall clinical severity, we repeated the analysis using the control circuit maps generated from severity of non-depressive symptoms in non-MDD datasets. Using the same statistical methods described above, we hypothesized that (1) the control circuit maps would not be significantly similar between different datasets, modalities or diagnoses and (2) the control circuit maps would not significantly match the depression circuit maps. We also hypothesized that the spatial cross-correlation between depression circuit maps would be significantly stronger than the spatial cross-correlation between control circuit maps using a paired t test.

To assess specificity to depression, we then generated symptom-specific circuit maps based on other cognitive/emotional scales, which were available in our two largest datasets. In the VHIS dataset (n=196), we generated 28 circuit maps based on the Mini Mental State Examination and each of the 27 symptoms measured by the Neurobehavioral Rating Scale. In the St. Louis dataset (n=100), we generated six circuit maps based on the Boston Naming Test, animal naming test (verbal fluency), Hopkins Verbal Learning Test (learning/memory), Brief Visuospatial Memory Test (visual memory), clock draw test (visuospatial skills) and spatial span test (attention). In each dataset, we used spatial correlations to compare the symptom-specific maps with a leave-one-dataset-out depression map generated from the other 13 datasets. We hypothesized that the leave-one-dataset-out depression maps would be more similar to each dataset's depression map than to its other symptom-specific maps.

To test for significance, we regenerated these cognitive/emotional circuit maps 25,000 times after randomly permuting each patient's clinical outcomes with a different patient's neuroimaging results. We again used spatial correlation to compare each of these maps with a leave-one-dataset-out depression map. We averaged the resulting Fisher-transformed spatial correlations, yielding a null distribution of 25,000 spatial correlation values expected by random chance. We computed a p value as the percentage of these values that exceeded the weighted mean correlation between the leave-one-dataset-out map and each dataset's depression circuit map.

Explaining clinical variance

For each neuromodulation dataset, treatment-induced change in depression score was predicted using a leave-one-dataset-out map constructed from the other 13 datasets. Within each dataset, spatial correlations were computed between each patient's stimulation site connectivity profile and the leave-one-dataset-out map. This yielded a metric representing the similarity between the patient's stimulation site connectivity and the 'ideal' stimulation site connectivity. In each dataset, this similarity metric was compared with improvement in depression score using partial Pearson correlation, controlling for baseline depression severity. Across all datasets, these correlations were combined into a single weighted mean value representing the degree to which our circuit predicted neuromodulation outcomes across all datasets. Significance was assessed using permutation testing as above.

Finally, a combined depression circuit map was generated based on the weighted mean of all 14 datasets. Peaks in this circuit map were identified using the functional MRI (fMRI) of the brain software library (FSL) 'cluster' algorithm with a detection threshold of $p<0.00005$ and minimum cluster extent of 100 mm3, consistent with conservative statistical guidelines.[51]

Comparison with prior established methods

We hypothesized that our model would be superior to existing methods for both causal brain mapping and neuromodulation targeting. First, we compared our causal mapping approach with VLSM, a tool that can identify lesion locations or stimulation sites associated with a particular behavioural outcome (without considering connectivity).[25] Next, we compared our connectivity-based targeting approach with the current consensus approach, which identifies optimal TMS targets based on subgenual cingulate connectivity.[8,18]

Using VLSM, we assessed whether particular lesion locations and stimulation sites were associated with depression, irrespective of their connectivity. At each voxel, we used a

t test to compare depression severity between patients whose lesions or stimulation sites overlapped with that voxel versus patients whose lesions or stimulation sites did not overlap with that voxel. This yielded a whole-brain map of lesion locations or stimulation sites associated with depression severity.

We then attempted to explain clinical variance using stimulation site connectivity to the subgenual cingulate. Within each dataset, we computed the mean connectivity of each patient's stimulation site to the subgenual cingulate, following the methods described in ref.[8] In each dataset, subgenual connectivity was compared with improvement in depression score using partial Pearson correlation, controlling for baseline depression severity. Across all datasets, these correlations were combined into a single weighted mean value representing the degree to which our circuit predicted neuromodulation outcomes across all datasets. The predictive value of subgenual connectivity was compared with the predictive value of our depression circuit using a *Z* test for dependent correlations within each dataset.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY STATEMENT

This paper used de-identified data from 14 different datasets collected by 14 different teams of investigators at various institutions across four different countries. Each dataset is available upon reasonable request from each respective team of investigators. Data sharing will be subject to the policies and procedures of the institution where each dataset was collected as well as the laws of the country where each dataset was collected.

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SUPPLEMENTARY INFORMATION

Supplementary methods

Localization of lesions and stimulation sites

Localization was conducted using the following approaches:

1. Lesions were localized using head CT or brain MRI. Each lesion was manually traced and registered to common atlas space as described in prior lesion network mapping work.[1]
2. DBS sites were localized on post-operative CT scans. The DBS-induced electric field was modelled using LEAD-DBS as described in prior work.[2]
3. TMS sites were localized using three different approaches as described in prior work using the same datasets. In the Boston[3] and Monash[4] cohorts, patients received traditional clinical targeting using scalp landmarks, and the incidental stimulation sites were localized retrospectively using neuronavigation. In the Ann Arbor cohort[5], stimulation sites were identified using task fMRI and treatment was delivered prospectively with neuronavigation. In the OPT-TMS cohort[6], patients received scalp landmark-based targeting and the incidental stimulation sites were recorded using fiducial markers during an MRI scan. The TMS-induced electric field was modelled using a previously-validated conical model of spatial field decay.[7]

Statistical methods

Unless otherwise specified, statistical analyses were conducted using permutation testing. The parameter of interest was re-computed 25,000 times after randomly re-assigning each subject's neuroimaging data to a different subject's clinical data. The resulting *p*-value was defined as the percentage of randomly-permuted results that were stronger than the real result. If the real result was stronger than 95% of permuted results, the result was considered significant ($p<0.05$).

Pearson's *r* was used as the primary outcome for all regression analyses. Prior to further analysis, all r values were transformed using Fisher's r-to-z transform.

Except as otherwise specified, all quantitative analyses were conducted using MATLAB R2018b (Mathworks, Natick, MA). Additional tools were also used for data visualization. Heatmaps were constructed using Graphpad Prism 8.2.1. Box plots and scatter plots were constructed in JMP Pro 14.

Brain images were visualized using Surfice (average surface space) or Connectome Workbench (subject specific surface space).

Table S7.1 14 datasets were included in this analysis across various stimulation sites, study settings, sample sizes, outcome metrics, patient populations, and localization approaches. To address heterogeneity in depression scales and other methods, each dataset was analyzed independently. The modalities were classified into three categories, including DBS, TMS, and lesions. DBS targets included anterior nucleus of the thalamus (ANT), subthalamic nucleus (STN), ventral capsule/ventral striatum (VC/VS), and subgenual anterior cingulate cortex (sgACC). TMS targets were within different parts of the dorsolateral prefrontal cortex, including 5 cm or 5.5 cm anterior to the motor cortex ("5 cm rule" or "5.5 cm rule"), a scalp measurement technique that estimates the location of the EEG F3 electrode (Beam F3), and a task fMRI-based target. Lesions included penetrating head trauma, ischemic stroke, and hemorrhagic stroke.

Modality	Dataset identifier	Institution of data collection and ethics approval	Setting	*n*	Primary Outcome	Patient population	Localization approach	Age, mean (SD)	Sex
DBS (ANT)	Maastricht	Maastricht University Medical Center	Naturalistic	25	BDI	Epilepsy	Post-op CT	39 (12)	76% M, 24% F
DBS (STN)	Berlin	Charite University Medicine Berlin	Naturalistic	32	BDI	Parkinson disease	Post-op CT	61 (10)	69% M, 31% F
DBS (VC/VS)	Boston	Massachusetts General Hospital	Clinical trial	8	MADRS	MDD	Post-op CT	40 (17)	62% M, 38% F
DBS (sgACC)	Berlin	Charite University Medicine Berlin	Clinical trial	9	HAMD	MDD	Post-op CT	45 (10)	33% M, 67% F
DBS (sgACC)	Atlanta	Emory University Hospital	Clinical trial	27	HAMD	MDD	Post-op CT	50 (13)	56% M, 44% F
TMS ("5.5 cm rule")	Boston	Beth Israel Deaconess Medical Center	Naturalistic	30	BDI	MDD	Retrospective Neuronavigation	53 (10)	33% M, 67% F
TMS (Beam F3)	Monash	Epworth Healthcare, Monash University	Naturalistic	24	BDI	MDD	Retrospective Neuronavigation	44 (13)	50% M, 50% F
TMS (Task fMRI)	Ann Arbor	University of Michigan Hospital	Clinical trial	16	MADRS	MDD	Prospective Neuronavigation	47 (11)	69% F, 31% M
TMS ("5 cm rule")	OPT-TMS	MUSC, NYPH, Emory, UW*	Multi-center trial	81	HAMD	MDD	MRI fiducial marker	47 (11)	57% F, 43% M
Lesions (penetrating)	VHIS	US Army Medical Research Command	Observational	196	BDI	Penetrating TBI	CT (chronic)	58 (3)	100% M
Lesions (stroke)	St. Louis	Barnes-Jewish Hospital, Washington Unviersity	Observational	100	GDS	Ischemic stroke	MRI	54 (11)	51% M, 49% F
Lesions (stroke)	Melbourne	Austin Hospital, Box Hill Hospital, Royal Melbourne Hospital	Observational	63	PHQ9	Ischemic stroke	MRI	67 (13)	65% M, 35% F
Lesions (stroke)	Monash	Southern Health, Monash University	Observational	55	HADS	Ischemic stroke	MRI	63 (14)	61% M, 39% F
Lesions (stroke)	Chicago	Northwestern Memorial Hospital	Observational	51	SF36	Hemorrhagic stroke	CT (acute)	61 (14)	55% M, 45% F

*Medical Univeristy of South Carolina, New York Presbyterian Hospital (Columbia University), Emory University Hospital, and University of Washington Medical Center.

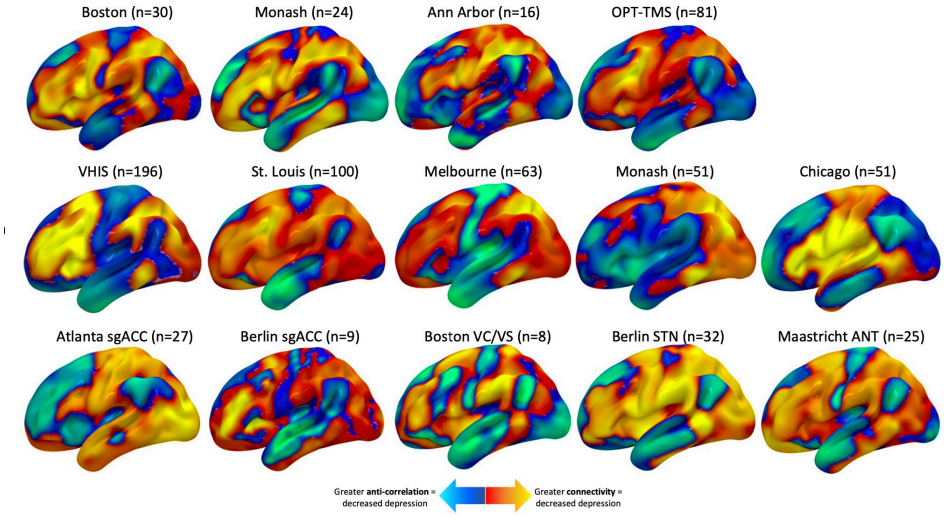

Figure S7.1 Circuit maps generated from each of the 14 datasets.

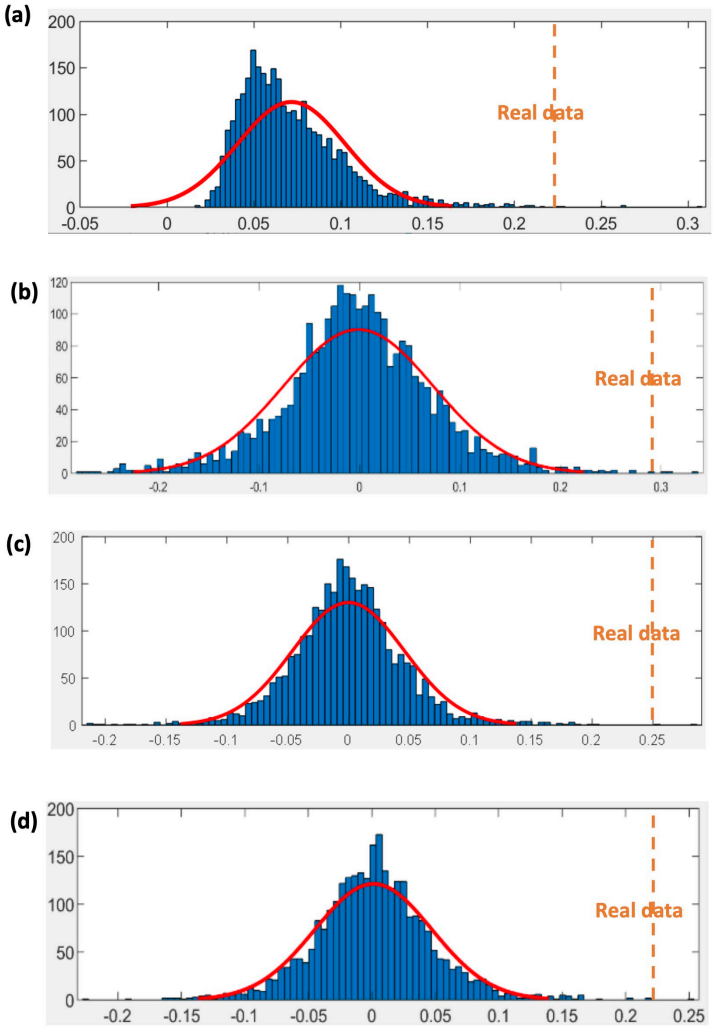

Figure S7.2 Permutation test confirmed that the cross-cohort spatial correlations were stronger than expected by the chance distribution. **(a)** Mean spatial correlation between all fourteen datasets in comparison with the chance distribution. **(b)** Mean spatial correlation between TMS, DBS, and lesion datasets (categorized by modality) in comparison with the chance distribution. **(c)** Mean spatial correlation between MDD and non-MDD datasets in comparison with the chance distribution. **(d)** Mean spatial correlation between lesion and neuromodulation datasets in comparison.

Table S7.2 Positive and negative peaks in the combined circuit map.

Positive peaks:

Region	Coordinates	Cluster size (mm^3)	t-value
Left dorsolateral prefrontal cortex	(-53, 41, 15)	152	4.29
Right dorsolateral prefrontal cortex	(48, 38, 23)	1128	4.79
Left inferior frontal gyrus	(-46, 9, 31)	6024	4.93
Right inferior frontal gyrus	(46, 4, 35)	2112	4.74
Left intraparietal sulcus	(-33, -53, 46)	8384	5.00
Right intraparietal sulcus	(34, -51, 46)	8152	4.91
Left extrastriate visual cortex	(-57, -50, -8)	264	4.52

Negative peaks:

Region	Coordinates	Cluster size (mm^3)	t-value
Subgenual cingulate cortex	(8, 24, -4)	1136	-4.66
Ventromedial prefrontal cortex	(-6, 58, 10)	1144	-4.46

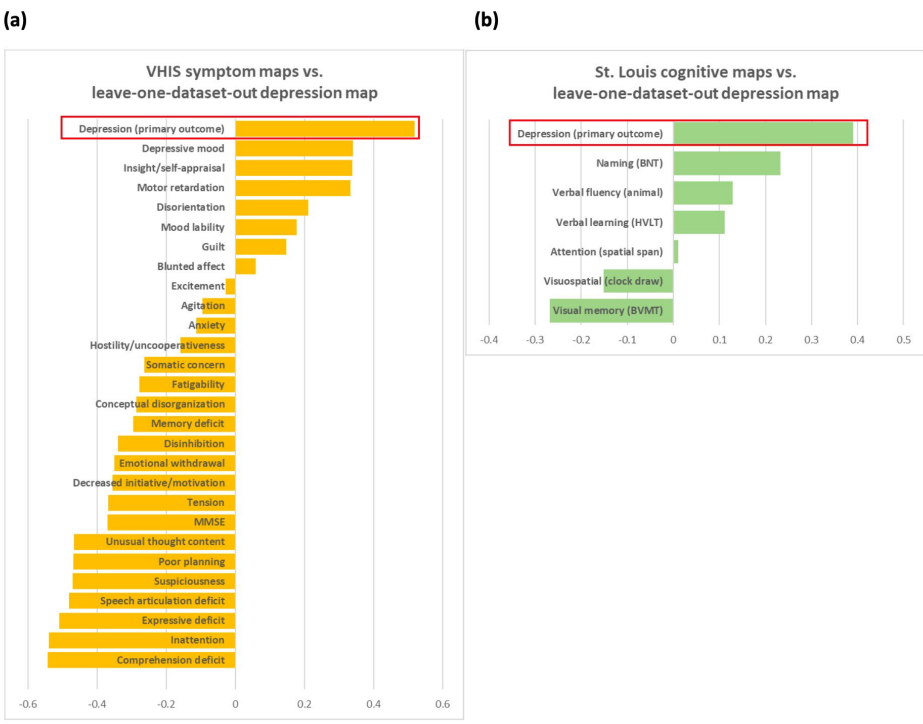

Figure S7.3 Specificity to depression versus other emotional/cognitive symptoms. **(a)** Our leave-one-dataset-out depression circuit (n=517) was more correlated with the VHIS depression circuit (n=196) than any other VHIS symptom circuit. **(b)** Our leave-one-dataset-out depression circuit (n=613) was more correlated with the St. Louis depression circuit (n=100) than any other St. Louis cognitive circuit.

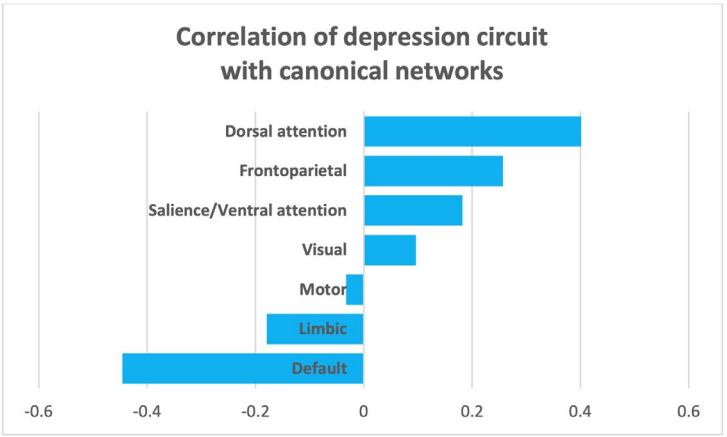

Figure S7.4 In comparison with the canonical 7-network parcellation by Yeo et al.[8], our depression circuit was most similar to the dorsal attention and frontoparietal control networks, and was most anti-correlated with the default mode and limbic networks.

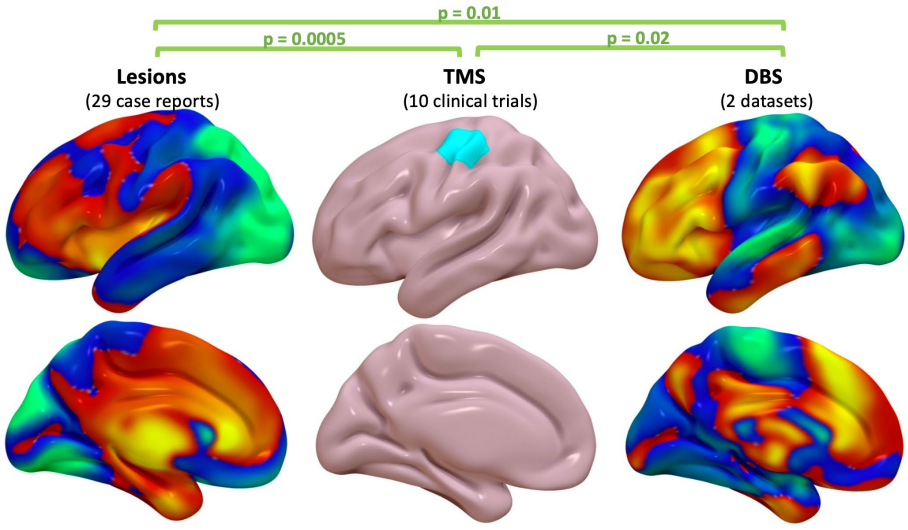

Figure S7.5 Lesion/stimulation site network mapping also predicts optimal treatment targets for Parkinson disease (PD). PD circuit maps were significantly similar between lesion datasets and DBS datasets. Both of these maps predicted that primary motor cortex would be an effective TMS site, consistent with a recent meta-analysis of 10 clinical trials9. For display purposes, PD circuit maps were averaged (weighted mean) across datasets within each modality.

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Chapter 8

Lesion-related epilepsy maps to a common brain network

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Under review

ABSTRACT

Background
Focal epilepsy is increasingly conceptualized as a brain network disease, but the location of this network remains unknown. Lesion locations related to epilepsy may help identify a common brain network and lead to new treatment targets.

Methods
We investigated lesion locations from patients with stroke-related epilepsy (n=76) and control lesions (n=625). Lesion locations were mapped to a common brain atlas and the brain network functionally connected to each lesion location was computed using human connectome data (n=1000). Functional connections associated with stroke-related epilepsy were identified. Generalizability was assessed using four datasets with different lesion types (n=772). Finally, therapeutic relevance of these connections was assessed using outcome data from patients who received thalamic deep brain stimulation for drug resistant focal epilepsy (n=30).

Results
Lesion locations of stroke-related epilepsy map to a specific brain network defined by functional connectivity to nodes in the basal ganglia and cerebellum. Functional connectivity to these same nodes was associated with the risk of epilepsy across different lesion types and with therapeutic response to thalamic deep brain stimulation.

Conclusions
Lesion-related epilepsy maps to a common brain network with therapeutic potential for neuromodulation.

Introduction

Focal epilepsy affects over 30 million patients worldwide, of which 30-40% are estimated to be drug resistant.[1,2] Treatment of these patients focuses on resection or ablation of the seizure-onset zone, which can be curative.[3,4] However, we often fail to identify a clear seizure-onset zone and seizures frequently recur after surgery, highlighting the need for new therapeutic approaches.[5]

One approach with therapeutic potential is neuromodulation of brain networks.[6–8] This network framework has motivated treatments such as vagal nerve stimulation[9] (VNS), deep brain stimulation[10] (DBS), and responsive neurostimulation[11] (RNS). The prevailing dogma is that epilepsy surgery or neuromodulation should be tailored to each patient's individual epilepsy network. However, animal studies[12,13] and some experimental human work,[14-16] have suggested there are intrinsic brain networks related to epilepsy that are common among patients.[8] While this concept is controversial and the location of such a network remains unknown,[15–17] it bears the potential to lead to new treatment targets.

Brain lesions, such as stroke, are a common cause of new onset epilepsy in adults[18] and may provide unique insights into brain regions or networks involved in epilepsy.[19] However, studies examining damage to specific brain regions have generated mixed results.[20–25] We have recently developed a technique, termed lesion network mapping, that can map neuropsychiatric symptoms to brain networks based on the lesion locations associated with the symptom and a wiring diagram of the human brain (i.e. the human connectome).[26] Brain networks identified using this technique align with effective neuromodulation targets.[27–29] Here, we use this approach to test whether lesion locations related to epilepsy map to a common brain network.

Methods

This study was carried out in accordance with the Declaration of Helsinki and approved by the institutional review board of the Brigham and Women's Hospital, Boston, USA (Protocol no. 2020P002987). For full details on each analysis, please see supplementary methods.

Stroke patients and brain lesions

We studied 76 patients with new onset ischemic stroke-related epilepsy.[30] Lesion locations were manually segmented on high-resolution patient-specific MRI scans and then spatially normalized to a common atlas (Montreal Neurological Institute (MNI) space, Figure 8.1A). Two independent datasets of consecutive stroke patients were used as controls (n=135[31], n=490[32]) as in prior work from our group.[29,33,34] These control datasets were not explicitly tested for epilepsy, but the presence of any patients with epilepsy in these cohorts should bias us against identifying group differences. Patient demographics are presented in Supplementary Table S8.1.

Lesion location mapping

To test whether lesions related to epilepsy map to a particular brain region, we calculated the lesion overlap (damage) to the cortex, subcortex, cortical lobes (including mesial temporal lobe), and vascular territories. Association between damage to these each of these regions and epilepsy was analyzed with an Aspin-Welch test, assessed using permutations, while controlling for lesion volume as a covariate and correcting for multiple comparisons. To identify any lesioned brain voxels associated with epilepsy, we used univariate voxel-based lesion-symptom mapping (VLSM) in NiiStat (https://github.com/neurolabusc/NiiStat)[35,36] and multivariate VLSM[37,38] in the SVR-LSM toolbox (https://github.com/atdemarco/svrlsmgui)[39]. We limited the voxel-based permutation tests to voxels occurring in at least 5% of lesions, while controlling for lesion volume as a covariate and correcting for multiple comparisons, in line with best-practice recommendations.[35,36,40]

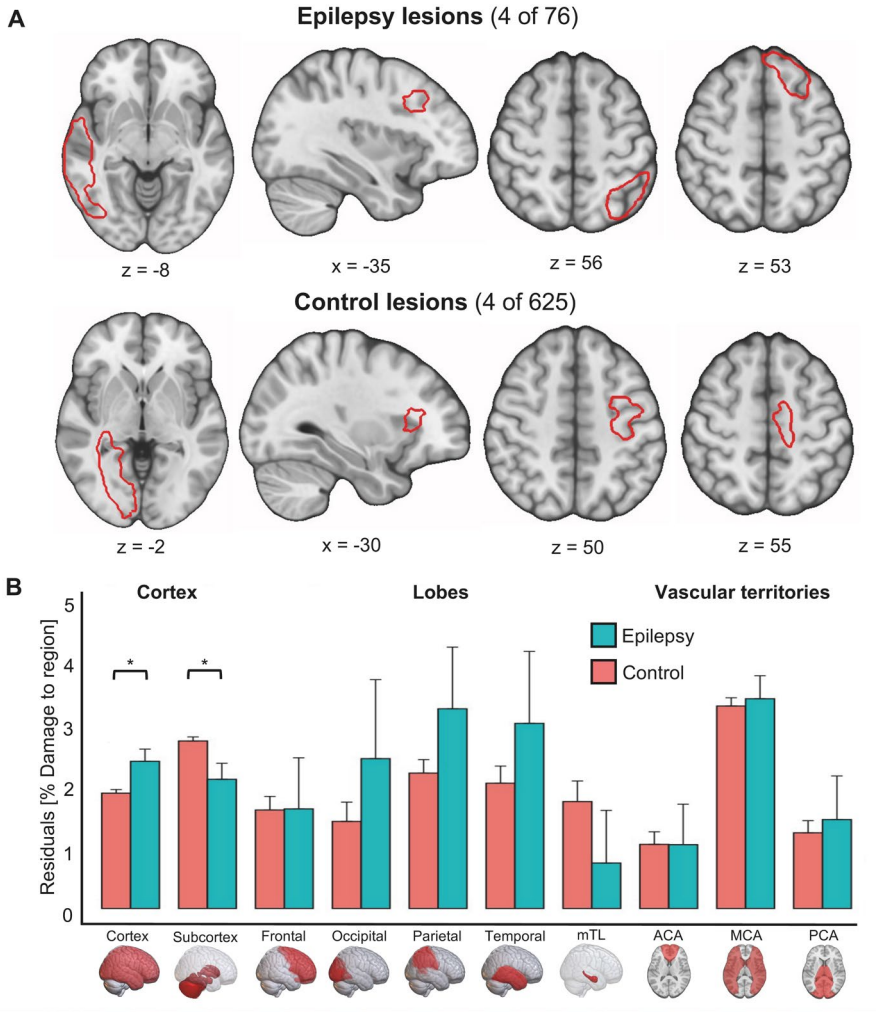

Figure 8.1 Lesion location mapping. Lesion locations (red outline) related to epilepsy (A) and control lesions (B) are both heterogeneously distributed across the brain. More damage to the cortex and less damage to the subcortex, but not a particular lobe or vascular territory, is associated with epilepsy (C). Bars and error bars represent means and 95% confidence intervals. Damage scores are plotted after correction for lesion volume (uncorrected damage scores can be found in Supplementary Table 4 and Supplementary Figure 4). *P<0.001 after family wise error rate correction for multiple comparisons. *Abbreviations: mTL, mesial temporal lobe; ACA, anterior cerebral artery; MCA. middle cerebral artery, PCA; posterior cerebral artery.*

Lesion network mapping

To test whether lesions associated with ischemic stroke-related epilepsy map to a brain network, we performed lesion network mapping using our previously validated method.[27,33,41] We computed the functional connections between each lesion location and all other brain voxels using the resting state functional connectivity data (2 x 2 x 2 mm resolution) from 1000 healthy participants (https://dataverse.harvard.edu/dataverse/GSP),[42,43] resulting in a lesion-network map. To identify the connections associated with epilepsy, we performed a voxel-based permutation test using the software Permutation Analysis of Linear Models (PALM) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM), while controlling for lesion volume as a covariate and correcting for multiple comparisons, in line with previous lesion-network mapping studies.[44,45] The resulting output is a spatial map of voxels more positively or negatively connected ("anticorrelated")[46] to lesion locations related to epilepsy versus control lesions.

To assess the consistency of our findings, we tested whether lesion-network mapping results were independent of the control dataset, connectome preprocessing (with and without global signal regression), age and sex, known epilepsy risk factors (damage to the cortex, subcortex and MCA territory), seizure type (focal only or focal to bilateral tonic clonic), delay to first seizure after stroke (within or after 6 months), and EEG abnormalities. We also tested whether our results were similar using subgroups matched for lesion volume and cortical/subcortical involvement of the lesion (propensity score matching, https://github.com/kosukeimai/MatchIt)).[4748–50] To assess the relationship between the independent and dependent variables, we performed statistical mediation analyses using the *lavaan* R package (https://github.com/yrosseel/lavaan.git)[51], with the recommended 5000 bootstrap samples to calculate significance of the indirect pathway via confidence intervals.

Generalizability across different lesion types

To test for generalizability, we studied four datasets of other lesion etiologies: brain hematoma locations in patients with hemorrhagic stroke (n=320, 7% with epilepsy),[52] brain injury locations in Vietnam war veterans with penetrating head trauma in (n=197, 44% with epilepsy)[21], brain tumor locations in patients with glioblastoma multiforme (n=132, 46% with epilepsy)[24], and cortical tuber locations in children with Tuberous Sclerosis Complex (n=123, 81% with epilepsy).[53] We used the lesion locations that had been previously outlined[23,26,46] or outlined using a validated segmentation algorithm (https://github.com/msharrock/deepbleed)[54], avoiding any potential risk for bias. Patient demographics are presented in Supplementary Table S8.2.

Using the connections derived from ischemic stroke lesions as an a priori region of interest (ROI, Figure 8.2A-B), we tested the hypothesis that each of the other lesion types would show similar connectivity differences between epilepsy and control lesions. We repeated the same voxel-based permutation test in PALM that we used in our primary analysis but limited our search space to this a priori ROI. Note that we have previously reported on a subset of these connections in tubers associated with infantile spasms[54] (a specific infantile epilepsy syndrome), which is different from the current analyses focused on epilepsy diagnosis and consistency across different lesion types.

Next, we combined these four datasets, and identified the connections significantly associated with epilepsy across lesion types (leaving out ischemic stroke lesions). This whole-brain analysis was identical to our primary lesion network mapping analysis in ischemic stroke but used a random-effects model and Aspin-Welch test, assessed with permutations, to accommodate multiple datasets. Next, we computed the functional connectivity between each ischemic stroke lesion (left out dataset) to the map generated from the other four lesion types. To explore prognostic relevance, association between ischemic stroke-related epilepsy and this out-of-sample lesion connectivity value were tested using logistic regression, controlling for lesion volume and known epilepsy risk factors (damage to the cortex, subcortex, and MCA territory). This leave-one-lesion-type-out process was then repeated five times, each time leaving out a different dataset / lesion type.

These lesion connectivity values were then used to stratify patients into three risk categories similar to previous work[27]: high-risk (functional connectivity one SD above the mean), low-risk (functional connectivity one SD below the mean) and moderate-risk (patients in between the high and low risk groups). A Chi-squared test was performed to compare the proportion of epilepsy across the different risk groups. To ensure results were independent of our risk group cutoffs, we repeated this analysis using receiver operating characteristics (ROC) and computed the area under the curve (AUC).

Therapeutic relevance for deep brain stimulation

We analyzed data from 30 patients who received anterior thalamic DBS for drug-resistant focal epilepsy.[55] Patient demographics are presented in Supplementary Table S8.3. Clinical outcome was measured by the percentage of change in seizure frequency, obtained from standard seizure diaries. DBS electrodes were localized in MNI space using Lead-DBS (https://www.lead-dbs.org), similar to previous studies.[56,57] Each patient's stimulation site was modelled using patient specific stimulation settings and the connectivity of each stimulation site to the map derived from the ischemic stroke data (Figure 8.2A-B) was calculated. We then tested for correlation between this connectivity

value and clinical outcome with a Pearson correlation (R) and permutation testing. Next, we performed a voxel-based analysis using PALM to identify connections significantly associated with DBS response. This analysis was performed both within the a priori ROI derived from the ischemic stroke data (Figure 8.2A-B) and using a whole-brain analysis.

RESULTS

Lesion location mapping

Lesion locations associated with ischemic-stroke related epilepsy were heterogeneously distributed across the brain (Figure 8.1A) with a maximum lesion overlap of only 24% (18 of 76) (Supplementary Figure S8.1). As expected, control lesions were also heterogenous, with a maximum overlap of 16% (98 of 625). Lesions related to epilepsy were larger than control lesions (P_{corr}=0.023, Supplementary Table S8.4). After controlling for lesion volume, more damage to the cortex (P_{corr}<0.001) and less damage to the subcortex (P_{corr}<0.001) was associated with epilepsy, but there were no significant associations with a specific lobe or vascular territory (Figure 8.1B and Supplementary Figure S8.2). Similarly, damage to no single brain voxel was statistically associated with epilepsy (VLSM, not significant).

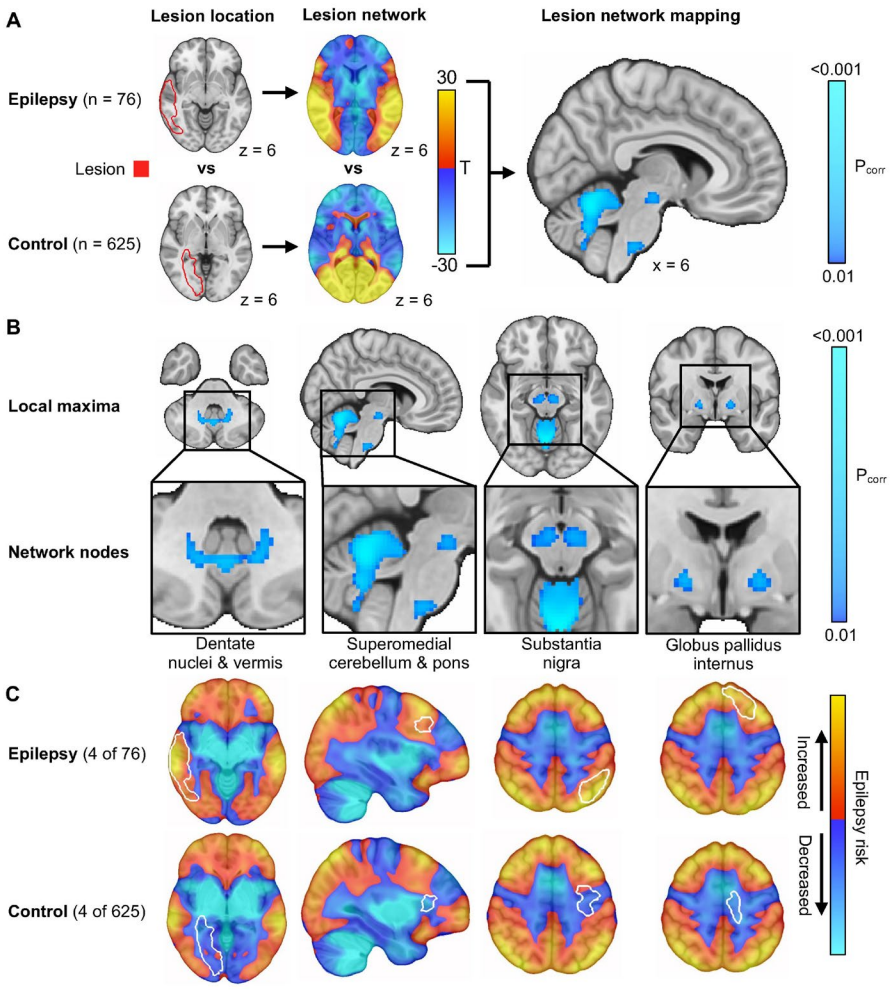

Figure 8.2 Lesion network mapping. Functional connectivity between each lesion location (red outlines) and all other brain voxels was computed using resting state functional connectivity data from 1000 healthy participants (i.e. the human connectome) (**A**). Positive correlations with the lesion location are shown in warm colors and negative correlations are shown in cool colors. Peak network nodes specifically associated with epilepsy versus control lesions were identified in the basal ganglia and cerebellum (**B**). By definition, connectivity with these network nodes defines a distributed brain network that identifies lesion locations (white outlines) at increased risk of epilepsy (warm colors) and decreased risk of epilepsy (cool colors) (**C**). P-values are shown after family wise error rate correction for multiple comparisons.

Lesion network mapping

Functional connectivity between lesion locations and the basal ganglia and cerebellum was strongly associated with ischemic stroke-related epilepsy, controlling for lesion

volume (peak P_{corr}<0.001, Figure 8.2A-B). Lesion locations related to epilepsy were more negatively connected ("anticorrelated") to nodes in the substantia nigra, globus pallidus internus (GPi), and cerebellum (superomedial cerebellum, dentate nuclei, vermis) compared to control lesions. Results were independent of the control dataset, connectome preprocessing method, age and sex, known epilepsy risk factors, seizure type, delay to first seizure after stroke, EEG abnormalities (Supplementary Figure S8.3) or subgroups matched for lesion volume or cortical/subcortical damage (Supplementary Figure S8.4). The relationship between epilepsy and lesion connectivity was not mediated by cortical/subcortical damage, but the relationship between epilepsy and cortical/subcortical damage was fully mediated by lesion connectivity (Supplementary Figure S8.5). By definition, functional connectivity with these network nodes defines a distributed brain network that best differentiates lesion locations related to epilepsy from control lesions (Figure 8.2C). VLSM results, using liberal statistical cutoffs, were consistent with lesion network mapping results but only identified part of the network (Supplementary Figure S8.5).

Generalizability across different lesion types

In each of the four other lesion types (hematomas, traumas, tumors, and tubers), functional connectivity between lesion locations and voxels in the substantia nigra, GPi, and cerebellum was associated with epilepsy (P_{corr} <0.05, Figure 8.3A-B). Combining these four datasets and performing an unbiased whole-brain analysis (leaving out ischemic stroke lesions), we identified connections significantly associated with epilepsy that were nearly identical to the initial results from our ischemic stroke dataset (peak P_{corr}<0.001, Figure 8.4A). Functional connectivity between lesion locations from the ischemic stroke dataset to the nodes derived from the other lesion types (Figure 8.4A) was significantly associated with ischemic stroke-related epilepsy (OR=2.82, 95% CI=2.02 to 4.10, p<0.001). This result remained significant after controlling for lesion volume (OR=2.72, 95% CI=1.93 to 4.00, p<0.001) or known epilepsy risk factors (damage to the cortex, subcortex, and MCA territory) (OR=2.27, 95% CI=1.58 to 3.39, p<0.001). Risk categories defined solely by functional connectivity between ischemic stroke locations and the nodes (derived from the other lesion types) showed a significant difference in the proportion of ischemic stroke-related epilepsy (p<0.001, Figure 8.4A) with a relative risk ratio of 25.92 [95% CI=3.54 to 189.70] in the high-risk group compared to the low-risk group (18.9% vs. 0.01% proportion of epilepsy). Results were similar using an ROC analysis that is independent of risk group cutoffs (AUC=0.72, 95% CI=0.67 to 0.77, p<0.001, Supplementary Figure S8.6).

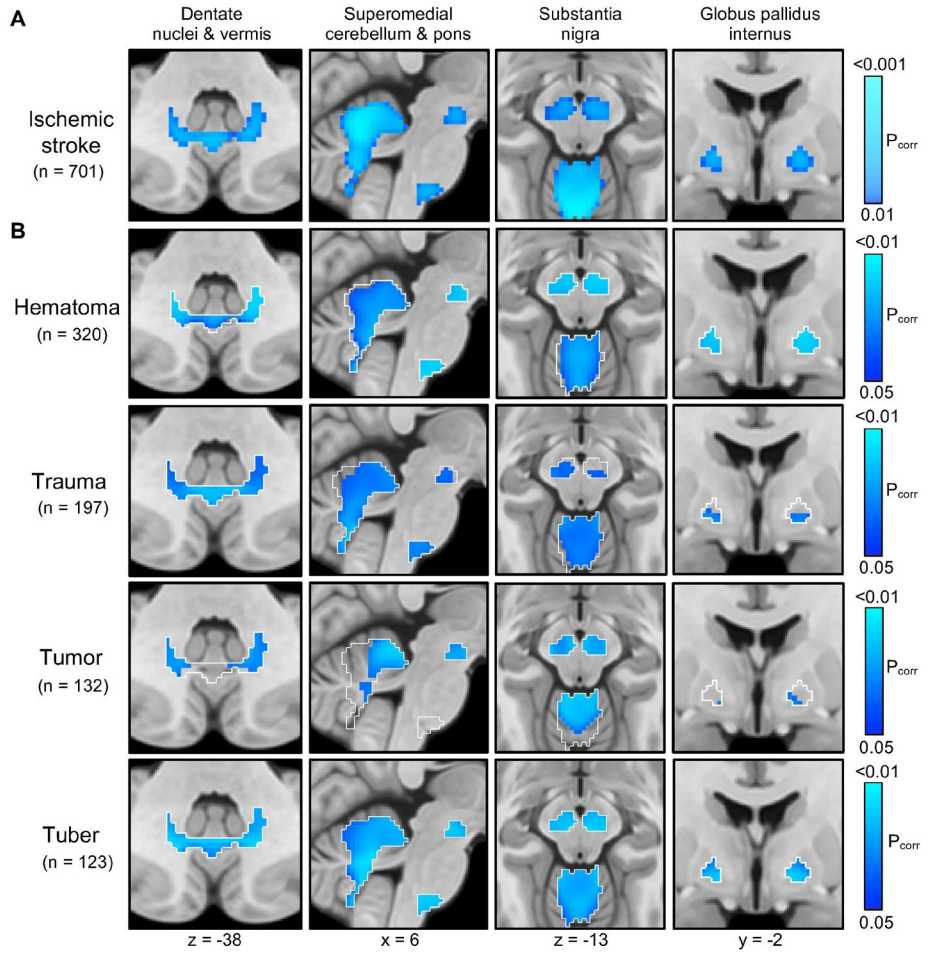

Figure 8.3 Generalizability across different lesion types. Network nodes derived from ischemic stroke lesions (A) were used as an a priori search space (white outlines) to test for similar findings in four datasets with different lesion etiologies (B). Connectivity to voxels in the cerebellum and basal ganglia was significantly associated with epilepsy in hematomas, traumas, tumors, and tubers. P-values are shown after false discovery rate correction for multiple comparisons.

We repeated this leave-one-lesion-type-out analysis five times and found that functional connectivity between lesion locations (from the left-out dataset) and the nodes (derived from the other four datasets) was associated with the proportion of epilepsy across risk groups (p<0.001, Figure 8.4B). This result was similar whether we stratified patients into risk groups within each lesion type or across all lesion types (Supplementary Figure S8.7) and without using risk group cutoffs (AUC=0.77, 95% CI=0.74 to 0.79, p<0.001, Supplementary Figure S8.8).

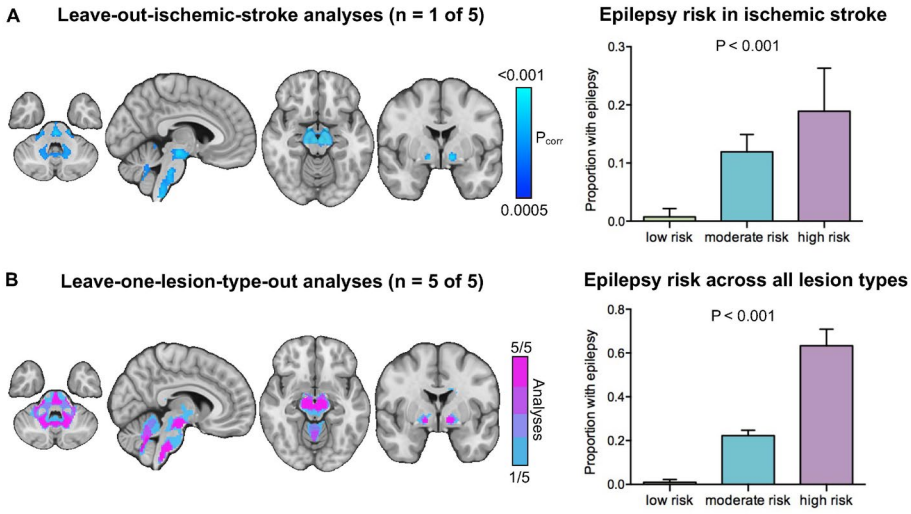

Figure 8.4 Cross-validation across different lesion types. Connections significantly associated with epilepsy were identified after combining the hematoma, trauma, tumor, and tuber lesion datasets, but leaving the ischemic stroke lesions out (A, left). Connectivity of ischemic stroke lesion locations (n = 701) to network nodes derived from these four other lesion types (n = 772) was associated with epilepsy risk (A, right). We repeated this leave-one-lesion-type-out analysis five times, each time identifying voxels significantly associated with epilepsy across four lesion types and datasets (B, left). Connectivity between lesion locations from the left-out dataset to network nodes derived from the other four datasets was associated with epilepsy risk across all lesion types (B, right). P-values are shown after family wise error rate correction for multiple comparisons.

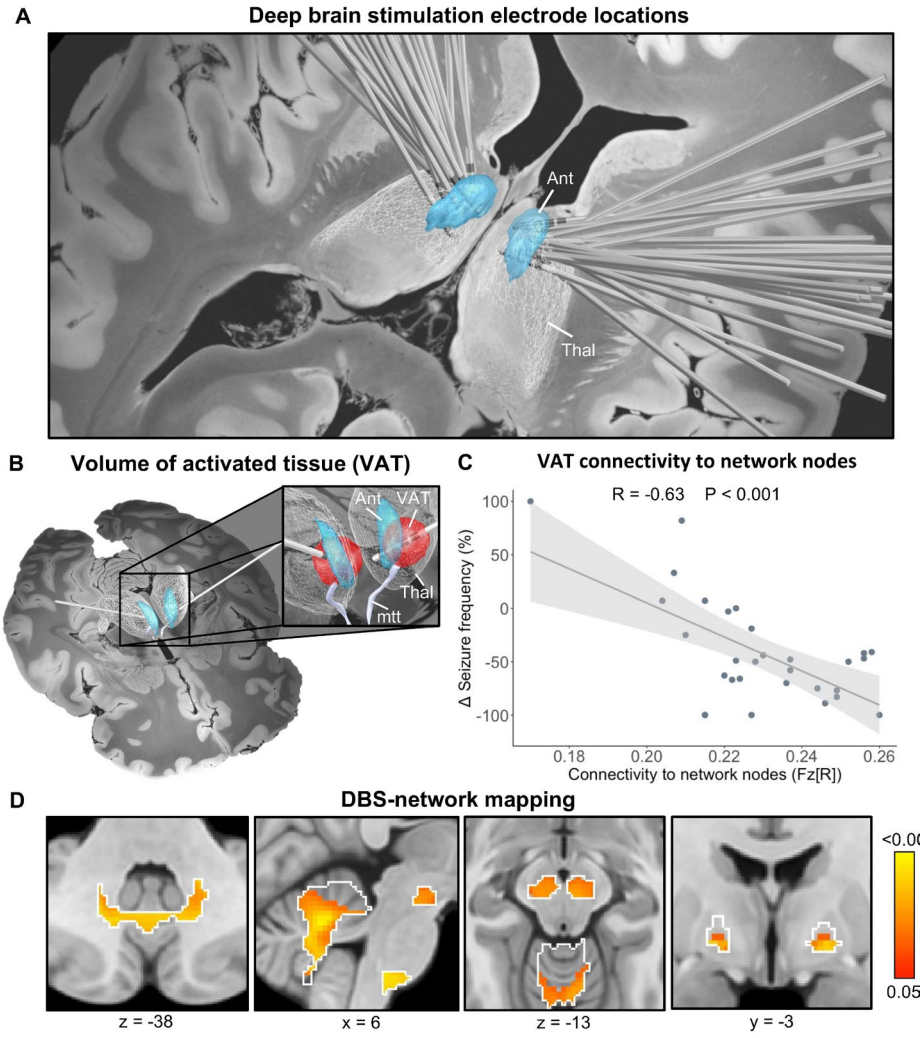

Figure 8.5 Therapeutic relevance for deep brain stimulation. Deep brain stimulation electrodes from 30 patients with drug resistant focal epilepsy show slight variability in electrode location within the anterior thalamus[112] (A). The stimulation site for each patient was identified by computing the volume of activated tissue (VAT) based on individualized stimulation settings (B). Functional connectivity between patient-specific stimulation sites and the network nodes derived from stroke lesions was associated with better seizure outcome (C). Positive functional connectivity between patient-specific stimulation sites and multiple voxels within an a priori search space defined by ischemic stroke lesions (white outlines) was significantly associated with therapeutic response after DBS (D). P-values are shown after false discovery rate correction for multiple comparisons. *Abbreviations: DBS, deep brain stimulation; Ant, anterior nucleus of the thalamus; Thal, thalamus; mtt, mammillothalamic tract.*

Therapeutic relevance for deep brain stimulation

To test whether this same network may have therapeutic relevance, we analyzed data from patients who received anterior thalamic DBS for drug resistant focal epilepsy (Figure 8.5A). Functional connectivity of each patient's stimulation site (Figure 8.5B) to the nodes derived from the ischemic stroke lesions (see Figure 8.2A-B) correlated with an improvement in seizure frequency after anterior thalamic DBS (R=-0.63, p<0.001, Figure 8.5C). Results were similar after controlling for stimulation amplitude (R=-0.54, p<0.001), stimulation volume (R=-0.51, p=0.002), or excluding an outlier with worse seizure control after DBS (R=-0.48, p<0.01, Supplementary Figure S8.9). Neither DBS amplitude (R=-0.22, p=0.24) nor VAT volume (R=-0.31, p=0.11) were significantly correlated with seizure outcome. When we performed a voxel-based analysis, we found that better outcome was associated with more positive functional connectivity of the patient's stimulation site to voxels in the substantia nigra, GPi, and cerebellum (peak P_{corr}<0.005, Figure 8.5D). These same clusters remained significant using an unbiased whole-brain analysis (Supplementary Figure S8.10).

Discussion

Brain lesions related to epilepsy map to a common brain network defined by functional connectivity to nodes in the basal ganglia and cerebellum. This network generalizes across different lesion types and connectivity to this network is associated with therapeutic response to DBS. Collectively, these results support a network framework for understanding focal epilepsy with potential prognostic and therapeutic implications.

Focal epilepsy as a disease of brain networks

Consistent with previous studies in stroke-related epilepsy, we found that larger lesions and more damage to the cortex was associated with an increased risk of epilepsy[18,20,22] while damage to subcortex with a decreased risk of epilepsy.[19,58] However, we found no relationship with damage to any particular lobe or brain region, which may explain the inconsistent results across prior studies.[20–24] In contrast, functional connectivity between these same lesion locations to remote nodes in the basal ganglia and cerebellum was strongly associated with epilepsy.

This result was not specific to stroke but generalized across five different lesion etiologies and five lesion datasets. This convergence is important as prior work on lesion locations

related to epilepsy, including work from our group[53], focused on just a single lesion type.[20–24] These studies have implicated different brain regions across different lesion types.[20–24] Our results suggest that despite many differences, different lesion types and locations related to epilepsy share connectivity to a common brain network.

It is important to highlight that these findings do not contradict the prevailing dogma of individual epilepsy networks, but rather suggest the co-existence of an intrinsic brain network related to epilepsy that is common among patients. The finding that brain lesions related to epilepsy better map to a brain network than individual brain regions is consistent with the network hypothesis of epilepsy[8] and lesion network mapping studies across multiple different neuropsychiatric symptoms.[26] For example, lesions associated with amnesia are connected to the subiculum in the hippocampus,[59] lesions associated with depression are connected to the left dorsolateral prefrontal cortex[27], and tubers associated with infantile spasms (a specific infantile epilepsy syndrome) are connected to the GPi and vermis.[53] Here, we found that lesions associated with epilepsy diagnosis are connected to multiple regions in the basal ganglia (substantia nigra and GPi) and cerebellum (vermis, dentate, and superomedial cerebellum), across 5 different lesion etiologies. As such, a brain network connected to these regions represents a plausible neuroanatomical substrate for lesion-related epilepsy. While epilepsy is often considered a cortical disease, these subcortical regions have previously been implicated in the modulation of seizures in animals and, in some cases, in humans (for reviews see[60–63]).

The basal ganglia and cerebellum in epilepsy

Prior hypotheses suggest the basal ganglia and cerebellum may act like "choke points"[64] in a universal "gating system",[60] and inhibition of these regions may prevent or stop seizures.[6566] However, there is also evidence suggesting the basal ganglia and cerebellum may act like a "brake",[67] and activation of these regions may aid seizure termination.[6869] In fact, both lesions and stimulation of the basal ganglia and cerebellum can reduce seizures, but results vary depending on the target, animal model, and patient population studied.[14,60,62,70–78]

Of the regions identified in our lesion network mapping analysis, the substantia nigra (SN) is supported by the most animal data implicating this region in seizure modulation. Lesions, high-frequency electrical stimulation, and optogenetic inhibition of the SN consistently reduces seizures across multiple different animal models of epilepsy.[13,79–81] While the SN has not been directly targeted for seizure treatment in humans, the anatomically adjacent STN has,[82] and improved outcomes appear to be associated with DBS contacts at the STN/SN transition zone[83,84] or even the SN itself.[85] Clinical observations that onset of Parkinson's Disease in patients with epilepsy can be associated

with sudden seizure freedom supports the hypothesis that the SN may modulate seizures in humans.[86,87] Similar to the SN, the GPi has also been reported to modulate seizures in animal models, however results are more dependent on the specific model.[60] In humans, there are case reports of seizure reductions after lesioning[71] or DBS[88] to the GPi, or lesioning the adjacent field of Forel.[72,73]

Finally, the cerebellum has been implicated in seizure modulation in both animals[89,90-91] and humans.[14,74–77] Both lesions and stimulation of the cerebellum can reduce seizures in animals, depending on the specific cerebellar region or cell type studied. In fact, the cerebellum was one of the first human brain regions to be targeted for neuromodulation of seizures,[14,75] and a recent double-blind, randomized trial reported some evidence of efficacy.[76] The stimulation site used in this study (and prior uncontrolled studies[14,92,93]) was in the superomedial cerebellum,[12] aligning surprisingly well with the network result presented here. The deep cerebellar nuclei (dentate nuclei and vermis), have also shown some promise as a lesion or neuromodulation target for epilepsy, consistent with our results.[62,63,77,74,94]

Potential network mechanisms

As noted above, there is an extensive literature implicating the basal ganglia and cerebellum in the modulation of seizures. However, why is functional connectivity between lesion locations and the basal ganglia and cerebellum associated with epilepsy? We found that lesions related to epilepsy are more negatively connected ("anticorrelated")[46] to the basal ganglia and cerebellum, which means that when the fMRI signal at the lesion location goes up, the fMRI signal in the basal ganglia and cerebellum goes down, and vice versa.[26,95] One possibility is that lesions may have a "diaschisis-like"[96] effect on the basal ganglia and cerebellum,[97] modulating activity in these remote regions and predisposing the brain to epilepsy. For example, lesions may act as an irritative zone, increasing activity at the lesion location and suppressing activity in the basal ganglia and cerebellum.[98] Conversely, lesions may also act like a lesion, decreasing activity at the lesion location and increasing activity in the basal ganglia and cerebellum.[99,100] Diaschisis could also result in more complex modulatory effects that go beyond simple increases or decreases in activity of remote brain regions.[101] A different possibility is that functional connectivity to the basal ganglia and cerebellum may define the topography of brain regions with more intrinsic susceptibility to epilepsy, which may have emerged through evolution or other yet unknown mechanisms.[102,103] Although these mechanistic questions cannot be answered by the current study, the network topography presented here may serve as a useful guide for where to intervene to

investigate these questions. This network topography could also have potential clinical implications for prognosis and treatment.

Potential clinical implications

The ability to better predict which stroke patients are at highest risk of epilepsy could help guide inclusion criteria for future antiepileptogenic trials, antiseizure treatment decisions, or patient counselling. The current model for predicting post-stroke epilepsy risk includes stroke severity, large-artery atherosclerosis, early seizures (≤7 days) and involvement of the cortex and MCA territory.[18] Our results suggest that lesion connectivity may be another valuable factor and may generalize to other lesion types beyond stroke. Whether including lesion connectivity in predictive models improves accuracy requires prospective testing.

Our results may also have implications for guiding therapeutic neuromodulation such as DBS, as the optimal DBS target for epilepsy remains unclear. Consistent with recent results in movement disorders[57] and psychiatric disorders,[104] our results suggest that the antiseizure effects of DBS may depend on connectivity between the stimulation site and other brain regions.[105,106] Connectivity to the basal ganglia and cerebellum might be used to guide DBS (re)programming or eventually to refine neurosurgical targeting.

Limitations

There are several limitations. First, the brain network identified here was derived from focal brain lesions. Although our findings generalized across five different lesion etiologies, it remains unknown whether our results are relevant for other etiologies of focal epilepsy (such as mesial temporal sclerosis), or for generalized epilepsy. Second, the use of a normative connectome derived from functional connectivity data of a large population of healthy individuals (n=1000) provides information on intrinsic brain connectivity in the average human brain, but does not account for individual differences. However, prior studies using an age-matched, disease-matched or patient-specific connectome lead to similar lesion- and DBS-network mapping results.[57,107–110] Third, our analysis focused on functional connectivity, which is sensitive to polysynaptic connections between lesion locations and distant brain regions, but complimentary findings may be apparent using structural connectivity, which may be more sensitive to monosynaptic connections.[111] Likewise, complimentary findings may be found using neuroimaging connectomes with a higher resolution, or neurophysiological connectomes derived from (stereo)-EEG or magnetoencephalography. Fourth, due to the retrospective design and data availability, we could not control for variables such as physical disability after stroke, seizure frequency, subtle structural abnormalities, blood-

brain barrier dysfunction, predisposing genetic factors, and use of antiepileptic drugs. Similarly, small errors in lesion tracing and atlas registration are to be expected. However, these limitations should all introduce noise, biasing us against the present findings. Finally, any clinical implications should be interpreted with caution, as our study was based solely on retrospective analyses of existing datasets. Future prospective studies are needed to determine if this network can be used as a tool for prognosis of epilepsy risk or as a therapeutic target for neuromodulation.

Conclusion

Lesion-related epilepsy maps to a common brain network with therapeutic potential for neuromodulation.

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SUPPLEMENTARY METHODS

Stroke patients and brain lesions

Stroke patients

We studied 76 patients with new onset ischemic stroke-related epilepsy. A diagnosis of post-stroke epilepsy was made by retrospective review of diagnosis codes, clinical charts, semiology, EEG and neuroimaging. A description of this dataset has been posted on medRxiv,[1] but this paper does not include any of the analyses or results presented here. All included patients had: (i) a diagnosis of new onset epilepsy associated with ischemic stroke according to current ILAE criteria, including at least two unprovoked seizures occurring more than 24 hours apart, more than seven days after stroke onset (i.e. late seizures), (ii) brain MRI obtained three months prior or after epilepsy diagnosis (iii) one or more focal ischemic stroke lesions visible on MRI, (iv) no other brain lesions or structural abnormalities, and (v) no history of seizures prior to their stroke. Patients with a single seizure, evoked seizures, or only seizures within seven days after stroke onset (i.e., early seizures) were excluded. Patients with secondary brain lesions after their first stroke or other likely causes of epilepsy such as intracranial surgery or electrolyte disturbances were excluded.

Brain lesions

Lesion locations were manually segmented on high-resolution patient-specific MRI scans using FSLeyes[2] software (https://fsl.fmrib.ox.ac.uk/fsl/ fslwiki/FSLeyes). All slices in the coronal, sagittal and horizontal planes were examined and the lesion location was segmented in all three planes on T1 or T2 weighted sequences. Lesioned voxels were assigned a 1 and non-lesioned voxels were assigned a 0, resulting in a three-dimensional binary lesion mask. Each patient's lesion mask was subsequently spatially normalized to a common atlas (Montreal Neurological Institute (MNI) space, Figure 8.1A) using FMRIB's linear image registration tool (FLIRT) implemented in FSL. Linear registration was used as opposed to non-linear registration because structural brain lesions such as stroke may affect the gross brain anatomy leading to bias in lesion location.

In line with our prior work,[3–5] two independent datasets of consecutive stroke patients were used as controls (n=135[6], n=490[7]). The first control dataset included 135 lesion masks, part of the Washington University Stroke Project.[6] The second control dataset included 490 lesion masks, part of the Genes Associated with Stroke Risk and

Outcomes Study (GASROS) collected at Massachusetts General Hospital.[7] These control datasets were not explicitly tested for epilepsy diagnosis, but the presence of any possible patients with epilepsy in these cohorts should bias us against identifying group differences. See Supplementary Table S8.1 for patient demographics.

Lesion location mapping

A priori region of interest analysis

To test whether lesions associated with epilepsy map to a particular brain region, we calculated the lesion overlap (or damage) of each lesion location to masks of a priori regions of interest (ROIs): the cerebral cortex, subcortex, cortical lobes (including mesial temporal lobe), and vascular territories. The cerebral cortex mask was defined by combining all cortical lobe masks from the Harvard-Oxford Cortical Atlas[8] (masks were thresholded and binarized at 25% probability). The subcortex mask was defined by subtracting the cerebral cortex mask from the MNI brain mask. The lobar masks of the frontal, parietal, occipital, and temporal lobes (including mesial temporal structures) were defined by the MNI Structural Atlas[9], as this atlas includes the adjacent white matter in contrast to only the gray matter included in the Harvard Oxford Cortical Atlas.[8] The vascular territories masks were defined by the 'Vascular Territory template and atlas in MNI space'.[10] Damage to these a priori ROIs was quantified by calculating the number of lesioned voxels intersecting with each mask and dividing by the number of voxels in each brain region mask, resulting in the percentage of the brain region damaged by the lesion. Association between percentage damage to these regions, which are skewed (Supplementary Figure S8.2), and epilepsy diagnosis was analyzed with an Aspin-Welch test, assessed using permutations, while controlling for lesion volume as a covariate and correcting for multiple testing. The Aspin-Welch test, implemented in the tool PALM (Permutation Analysis of Linear Models, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM),[11] does not assume identical variances, thus accommodating different distributions between the groups.

Voxel-based lesion symptom mapping analysis

To identify any lesioned brain voxels associated with epilepsy, we used univariate voxel-based lesion-symptom mapping (VLSM) in NiiStat (https://github.com/neurolabusc/NiiStat)[12,13] and multivariate VLSM[14,15] in the SVR-LSM toolbox (https://github.com/atdemarco/svrlsmgui).[16]

NiiStat is a Matlab software package that performs univariate VLSM, with a general linear regression model and permutation test, controlling for covariates. We limited our

analysis to voxels occurring in at least 5% of lesions, assessed with Freedman-Lane permutations (the default setting of 2000 permutations was used), while controlling for lesion volume as a covariate and correcting for multiple testing. These parameters were chosen based on published best-practice recommendations.[12,13,17] A two-tailed family wise error corrected *p*-value <0.05 was considered significant. SVR-LSM is a Matlab software package that performs multivariate voxel- and cluster-based LSM with a machine learning regression, termed the support vector regression (SVR). In contrast to univariate VLSM, which considers neighboring voxels as independent, multivariate SVR-VLSM simultaneously considers many voxels at once when determining whether damaged brain regions contribute to behavioral deficits.[1516] These multivariate LSM approaches are capable of identifying complex dependences that traditional univariate VLSM approaches cannot. In line with the univariate VLSM analysis in NiiStat, we limited our analysis for multivariate VLSM to voxels occurring in at least 5% of lesions, assessed with permutations (default setting of 10,000 permutations was used), while controlling for lesion volume using the standard "direct total lesion volume control" (dTLVC) approach and correcting for multiple testing. A two-tailed family wise error corrected *p*-value <0.005 was considered significant. These parameters were chosen based on published best-practice recommendations.[14–16]
Additionally, we explored univariate and multivariate VLSM results using liberal statistical cutoffs and assessed their alignment with our findings from lesion-network mapping by overlapping statistically significant VLSM voxels with lesion-network mapping results.

Lesion network mapping

To test whether lesions associated with ischemic stroke-related epilepsy map to a brain network, we performed lesion network mapping using our previously validated method.[18] We computed the functional connections between each lesion location and all other brain voxels (2 x 2 x 2 mm resolution) using a resting state functional connectivity dataset from 1000 healthy participants, also termed the human connectome.

Computing lesion-network maps with the human connectome

Resting state functional connectivity was obtained from 1000 healthy participants, using a high-resolution 3T MRI scanner in the 'Open Access' Brain Genomics Superstruct Project (GSP) (https://dataverse.harvard.edu/ dataverse/GSP). Preprocessing of these scans has been fully described elsewhere,[19,20] and included regression of noise variables derived from motion, CSF, white matter, and the global signal. To compute a lesion-network map, each lesion location was used as a seed in resting state functional

connectivity analysis of the data collected from each of the 1000 participants included in the human connectome. The time series for voxels within the lesion location were correlated with the time series from all other brain voxels and results were statistically combined across the 1000 participants to create a voxel-based T-map, as described before.[21–23] The resulting T-map, also termed a lesion-network map, represents the strength and consistency of functional connectivity for each lesion location between all other brain voxels (Figure 8.2A-B). Positive functional connectivity (warm colors) refers to a positive correlation of blood-oxygen-level-dependent (BOLD) timeseries between the lesion location and all other brain voxels, while negative functional connectivity (cool colors) refers to a negative correlation (i.e. anticorrelation) of BOLD timeseries between lesion location and all other brain voxels. In other words, when the BOLD activity in the lesion location goes up, BOLD activity will also go up in the brain regions positively correlated to the lesion location, but will go down in the regions negatively correlated and vice versa.

Identifying the functional connections associated with epilepsy

To identify connections associated with epilepsy, we performed a whole-brain voxel-based permutation test using the software Permutation Analysis of Linear Models (PALM) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM), while controlling for lesion volume as a covariate and correcting for multiple testing, in line with previous lesion-network mapping studies.[11]We used 2000 permutation and the p-values were computed using a generalized Pareto distribution fitted to the tail of the permutation distribution.[11] The resulting output is a spatial map of voxels more positively or negatively connected ("anticorrelated)[24] to lesion locations associated with epilepsy. A two-tailed family wise error corrected p-value <0.05 was considered significant, however higher statistical thresholds were often used to highlight the most significant findings (see Figure legends).

Consistency of results with and without global signal regression

It is worth highlighting that our normative connectome was preprocessed using global signal regression, which greatly reduces the influence of non-specific variance, but may complicate interpretation of negative correlations.[25,26] To ensure our results were similar between connectome preprocessing methods, we repeated our lesion network mapping analysis using a different 100-subject functional connectivity dataset generated without using global signal regression, similar to prior studies.[21,27] Resting state functional MRI data were processed using the aCompCor strategy as implemented in the Conn Toolbox (www.nitrc.org/projects/conn),[28,29] which includes regression of noise variables derived

from motion, CSF, and white matter, but not the global signal. All settings for preprocessing and regression were kept as default/recommended.

Consistency of results after controlling for covariates

To test whether lesion network mapping results were similar after controlling for variables such as age and gender or known epilepsy risk factors (damage to the cortex, subcortex and MCA territory), we repeated our lesion network mapping analyses including these variables as a covariate in the PALM design matrix. To ensure our results were similar across multiple stroke lesion datasets, seizure type (focal only or focal to bilateral tonic clonic), delay to first seizure after stroke (within or after 6 months), or EEG abnormalities (epileptiform / slowing or normal), we repeated our lesion network mapping analysis in PALM for these subgroups of patients.

Consistency of results in a matched subgroup analysis

To test whether lesion network mapping results were similar comparing epilepsy lesions to a subgroup of controls that were matched for lesion volume and damage to the cortex and subcortex, we used propensity score matching (https://github.com/kosukeimai/MatchIt).[30–33] Propensity score matching is a validated method used to account for confounds in observational studies which allows you to precisely generate two matched groups that are equivalent across multiple confounds/covariates except for the independent variable of interest. Specifically, a propensity score was calculated for each subject by fitting a logistic regression model where the response variable is group membership (epilepsy vs. control) and the explanatory variables are the confounds (damage to the cortex and subcortex). A matched control subject (n=76 of 625 original controls) for each epilepsy subject (n=76) was selected, based on the closest propensity score. We performed this matched group analyses twice, first matching groups based on lesion volume (model: epilepsy ~ lesion volume), and second, matching groups on damage to the cortex and subcortex (model: epilepsy ~ percentage damage to the cortex + percentage damage to the subcortex). After matching groups, we performed a lesion-network mapping analysis in PALM on this subset. We tested whether the primary findings from our full ischemic stroke cohort persisted in this smaller matched subset (Figure 8.2B). We show that the same associations in the basal ganglia and cerebellum were identified after matching groups based on these potential confounders (Supplementary Figure S8.4).

Mediation analysis to assess the relationship between independent and dependent variables

To assess the relationship between lesion connectivity, lesion volume, damage to the cortex and subcortex, and epilepsy diagnosis, we performed statistical mediation analyses using the *lavaan* R package (https://github.com/ yrosseel/lavaan.git),[51] with the recommended 5000 bootstrap samples to calculate significance of the indirect pathway via confidence intervals.

Generalizability across different lesion types

Other lesion type datasets

To test for generalizability, we studied four datasets of other lesion etiologies: brain hematoma locations in patients with hemorrhagic stroke (n=320, 7% with epilepsy),[34] brain injury locations in Vietnam war veterans with penetrating head trauma in (n=197, 44% with epilepsy),[35] brain tumor locations in patients with glioblastoma multiforme (n=132, 46% with epilepsy),[36] and cortical tuber locations in children with Tuberous Sclerosis Complex (n=123, 81% with epilepsy).[5] These lesion datasets were selected for inclusion in the current study because 1) they were used in prior publications relating lesion locations to epilepsy, 2) lesion locations were made available to us for analyses, and 3) a control dataset of similar lesions not associated with epilepsy was also available. All datasets meeting these criteria were included in the current study. No datasets were included or excluded after analysis. For each dataset, we used either a validated segmentation algorithm (https://github.com/msharrock/deepbleed)[37] to outline the lesion locations (hemorrhagic stroke) or used the previously published lesion outlines (penetrating heat trauma, glioblastoma multiforme, tuberous sclerosis complex) to avoid any potential risk of bias. See Supplementary Table S8.2 for patient demographics and details on each dataset.

Generalizability of ischemic stroke network findings to other lesion types

Using the connections derived from ischemic stroke lesions as an a priori region of interest (ROI, Figure 8.2A-B), we tested the hypothesis that each of the other lesion types would show similar connectivity differences between epilepsy and control lesions. We repeated the same voxel-based permutation test in PALM that we used in our primary analysis, but limited our search space to our a priori ROI derived from the ischemic stroke data and used a more liberal statistical cutoff given the reduced sample size (one-tailed false discovery rate corrected p-value <0.05). Note that we have previously reported on a subset of these connections in tubers associated with infantile

spasms[54] (a specific infantile epilepsy syndrome) but are extending these results here to epilepsy diagnosis and other lesion types.

Leave-one-lesion-type-out analysis

Next, we combined these four datasets, and identified the connections significantly associated with epilepsy across lesion types (leaving out ischemic stroke lesions). This whole-brain PALM analysis was identical to our primary lesion network mapping analysis in ischemic stroke, but controlled for lesion type to identify the connections specific to epilepsy across different lesion types, while correcting for lesion volume as a covariate and correcting for multiple testing. We controlled for unequal variance across lesion types using a voxel-based Aspin-Welch test, assessed with permutations, and controlled for batch effects using exchangeability blocks to permute within each lesion type.[38] As in our primary analysis in ischemic stroke, a two-tailed family wise error corrected p-value <0.05 was considered significant. Next, we computed the functional connectivity between each ischemic stroke lesion (left out dataset) to the network nodes generated from the other four lesion types (Figure 8.3A). To explore prognostic relevance, association between ischemic stroke-related epilepsy and this out-of-sample lesion connectivity value was tested using logistic regression, controlling for lesion volume and known epilepsy risk factors (damage to the cortex, subcortex, and MCA territory). This process was then repeated five times, each time leaving out a different dataset / lesion type.

These lesion connectivity values were then used to stratify patients into three risk categories similar to previous work[39]: high-risk (functional connectivity one SD above the mean), low-risk (functional connectivity one SD below the mean) and moderate-risk (patients in between the high and low risk groups). A Chi-squared test was performed to compare the proportion of epilepsy across the different risk groups. To ensure our results were not biased by lesion type, we repeated this risk stratification by categorizing subjects on the basis of their connectivity value within each lesion type. To ensure results were independent of our risk group cutoffs, we repeated this analysis using receiver operating characteristics (ROC) and computed the area under the curve (AUC).

Therapeutic relevance for deep brain stimulation

DBS patients and stimulation sites

To test whether the functional connections derived from brain lesions have therapeutic relevance, we analyzed data from 30 patients who received anterior thalamic (ANT)-DBS for drug-resistant focal epilepsy.[40] See Supplementary Table S8.3 for patient

demographics. Clinical outcome was measured by the percentage of change in seizure frequency after DBS, obtained from standard seizure diaries. DBS electrodes were localized in MNI space using Lead-DBS (https://www.lead-dbs.org), similar to methods described in previous studies.[41,42] Briefly, pre-operative T1 / T2 MRI sequences and post-operative MRI / CT images were linearly co-registered using SPM (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/[43]). Co-registration was further refined using the 'brainshift correction[144]' option and images were normalized to MNI space using the Advanced Normalization Tool (http://stnava.github.io/ANTs/[45]). DBS electrode trajectories and contacts were automatically pre-localized and manually refined using Lead-DBS. Each patient's stimulation site (also termed volume of activated tissue or VAT) was modelled in MNI space using patient specific stimulation settings and a finite element approach in an adapted version of the Fieldtrip/Simbio pipeline included in Lead-DBS (http://www.fieldtriptoolbox.org/; https://www.mrt.unijena.de/simbio/[46]).

Functional connectivity of DBS sites to network nodes derived from brain lesions

Functional connectivity of each patient's stimulation site to the network nodes derived from the ischemic stroke data (Figure 8.2A-B) was calculated using the same functional connectivity dataset (n=1000) used in the lesion network mapping analysis described above. We then tested for correlation between connectivity of the stimulation site to the network nodes derived from the ischemic stroke data, and clinical outcome after DBS with a Pearson correlation (R) and permutation testing. To control for the effect of stimulation amplitude, we repeated this correlation using DBS amplitude (V) and VAT volume (voxels) as a covariate. To test whether these results were robust to outliers, we repeated this correlation excluding an outlier with worsened seizure control after DBS.

DBS-network mapping analysis

We performed a voxel-based DBS-network mapping analysis using PALM to identify connections significantly associated with DBS response within the a priori ROI of the network nodes derived from the stroke data (Figure 8.2A-B). A two-tailed false discovery rate corrected p-value <0.05 was considered significant, similar to the analyses for voxel-based testing of generalizability to other lesion types (Figure 8.3). Finally, clusters of connections significantly associated with DBS response outside this a priori ROI were defined using an whole-brain PALM analyses with threshold-free cluster enhancement. A two-tailed family wise error corrected p-value <0.05 was considered significant.

Table S8.1 Patient demographics of the stroke-related epilepsy lesion dataset and two independent consecutive stroke cohorts (control datasets 1 and 2). Values are presented as means and standard deviations [SD] or percentages as appropriate.

	Ischemic stroke-related epilepsy	**Control dataset 1**	**Control dataset 2**
Reference	**Nordberg *et al.* 2021[1]**	**Corbetta *et al.* 2015[6]**	**Wu *et al.* 2015[7]**
N	76	135	490
Sex (male/female)	39 / 37	63 / 72	303 / 187
Age at scan (y)	61 [14.6]	53.6 [10.8]	65 [14.9]
Time between stroke and first seizure (days)	978 [2162]	NA	NA
Seizure type, n (%)		NA	NA
Focal seizures only	31 (40.8%)		
Focal to bilateral tonic clonic seizures	45 (59.2%)		
EEG abnormalities, n (%)		NA	NA
Normal	23 (30.3%)		
Epileptiform	25 (32.9%)		
Focal slowing	20 (26.3%)		
Unknown	8 (10.5%)		
Brain scan	MRI	MRI	MRI

Note that while descriptions of these lesion datasets have been previously published, all analyses and results are unique to the present paper.

Table S8.2 Patient demographics of the other lesion type datasets. Values are presented as means and standard deviations [SD] or percentages, as appropriate.

	Hemorrhagic stroke	**Penetrating head trauma**	**Glioblastoma multiforme**	**Tuberous Sclerosis Complex**
Reference	**Greef *et al* 2014[34]**	**Raymont *et al.* 2010[35]**	**Cayuela *et al.* 2018[36]**	**Cohen *et al.* 2021[5]**
N total	320	197	132	123
N with epilepsy	23	87 (44%)	61 (46%)	100 (81%)
N without epilepsy	297	110 (56%)	71 (54%)	23 (19%)
Sex (male/female)	172 / 148	197 / 0	83 / 49	63 / 51
Age at scan (years)	71.1 [13.6]	58.3 [3.1]	60.7 [11.6]	2.66 [0.947]
Brain scan	CT	CT	MRI	MRI

Note that while descriptions of these lesion datasets have been previously published, all analyses and results are unique to the present paper.

Table S8.3 Patient demographics of the anterior thalamic deep brain stimulation dataset.

Subject	Gender	Age at surgery (years)	Epilepsy duration (years)	Seizure-onset zone	Previous resection or VNS	DBS Active contacts		DBS amplitude (V)		Δ Seizure frequency after DBS
						Left	*Right*	*Left*	*Right*	
1	Male	41	20	Temporal	Left temporal resection	1-C+	1-C+	6	6	-100
2	Female	35	33	Bilateral temporal	VNS	1-C+	1-C+	6	6	-48
3	Male	65	51	Left frontal	Left temporal resection and VNS	0-1+	0-1+	5	5	-42
4	Male	46	23	Right Parietal	VNS	2-C+	1-C+	6	6	-19
5	Male	48	39	Left temporal	None	1-C+	0-C+	5.5	5.5	-100
6	Male	36	13	Bilateral temporal	VNS	1-C+	1-C+	7	7	+82
7	Female	30	12	Bilateral temporal	Left temporal resection and VNS	1-C+	1-C+	6.2	6.2	-66
8	Male	40	30	Frontal	None	1-C+	1-C+	6	6	-47
9	Male	38	29	Multifocal	VNS	2-C+	1-C+	5	5	-49
10	Male	40	29	Bilateral parietal	VNS	1-C+	1-C+	5.4	5.4	-70
11	Female	22	19	Left frontal	VNS	1-C+	1-C+	2	2	-100
12	Male	36	32	Multifocal	VNS	1-C+	1-C+	5.5	5.5	-100
13	Male	23	14	Multifocal	VNS	1-2+	1-2+	3.5	3.5	+7
14	Male	53	48	Left frontal	VNS	2-C+	2-C+	6.15	6.15	-41
15	Male	45	18	Left frontal	Left frontal resection and VNS	2-C+	2-C+	3	3	-77
16	Male	27	20	Parietal	Left parietal resection	1-C+	1-C+	5.7	5.7	-83
17	Male	55	37	Left temporal	VNS	0-C+	1-C+	4	4	-63
18	Male	61	46	Right frontal	VNS	2-C+	1-C+	4.5	4.5	-44
19	Female	35	26	Parietal	VNS	1-C+	0-C+	5.8	5.8	-89
20	Male	34	18	Multifocal	VNS	0-1+	1-C+	3	5.5	+7
21	Male	26	9	Temporal	VNS	1-2+	1-2+	3.5	3.5	-3
22	Male	23	18	Bilateral temporal	None	2-C+	1-C+	5	5	-50
23	Female	53	31	Bilateral temporal	VNS	1-C+	1-C+	5.7	5.7	-58
24	Male	38	31	Bilateral parieto-occipital	None	0-1+	0-1+	3.5	3.5	+33
25	Female	34	18	Multifocal	VNS	1-C+	1-C+	3	3	-67
26	Female	20	11	Multifocal	None	1-C+	1-C+	2.5	2.5	+100
27	Male	59	40	Bilateral temporal	None	1-C+	2-C+	5.5	5.5	-50
28	Male	51	50	Multifocal	None	1-C+	1-C+	2.5	2.5	0
29	Female	46	39	Multifocal	None	1-C+	1-C+	5.9	5.9	-75
30	Female	48	22	Bilateral frontal	VNS	1-C+	1-C+	5	5	-25

Note that while a description of this DBS dataset (including 20 of these 30 patients) has been previously published[40], all analyses and results are unique to the present paper. *Abbreviations: VNS, vagal nerve stimulation.*

Table S8.4 Lesion distribution. Values are presented as means and standard deviations [SD] or percentages as appropriate.

	Epilepsy	Control	Total
Reference	**Nordberg *et al.* 2021[1]**	**Corbetta *et al.* 2015[7] and Wu *et al.* 2015[8]**	
Patients, n	76	625	701
% Damage to brain region			
Brain (i.e. lesion volume)	3.4% [4.3]	2.0% [3.5]	2.1% [3.6]
Cortex / Subcortex			
Cortex	3.8% [4.8]	1.7% [3.7]	2.0% [3.9]
Subcortex	3.5% [4.7]	2.6% [4.0]	2.7% [4.1]
Lobes			
Frontal lobe	3.0% [5.3]	1.5% [4.7]	1.6% [4.8]
Occipital lobe	3.1% [6.2]	1.4% [4.4]	1.6% [4.7]
Parietal lobe	4.7% [6.9]	2.1% [4.7]	2.4% [5.0]
Temporal lobe	4.6% [8.5]	1.9% [5.4]	2.2% [5.8]
Mesial temporal lobe	1.7% [4.7]	1.6% [5.2]	1.7% [5.1]
Vascular territories			
Anterior cerebral artery	1.8% [3.4]	1.0% [3.4]	1.1% [3.4]
Middle cerebral artery	5.6% [7.8]	3.1% [6.0]	3.4% [6.3]
Posterior cerebral artery	2.0% [3.7]	1.2% [2.9]	1.3% [3.0]
Frequency of involvement of brain region, n (%)			
Cortex / Subcortex			
Cortex	68 (89%)	447 (72%)	515 (74%)
Subcortex	76 (100%)	622 (99%)	698 (99%)
Lobes			
Frontal lobe	45 (59%)	305 (49%)	350 (50%)
Occipital lobe	35 (46%)	187 (30%)	222 (32%)
Parietal lobe	54 (71%)	349 (56%)	403 (57%)
Temporal lobe	46 (61%)	290 (46%)	336 (48%)
Mesial temporal lobe	17 (22%)	166 (27%)	183 (26%)
Vascular territories			
Anterior cerebral artery	54 (71%)	395 (63%)	449 (64%)
Middle cerebral artery	72 (95%)	522 (84%)	594 (85%)
Posterior cerebral artery	51 (67%)	417 (67%)	468 (67%)

Note that lesions with at least one voxel overlapping with the mask of an a priori brain region were considered to involve that region.

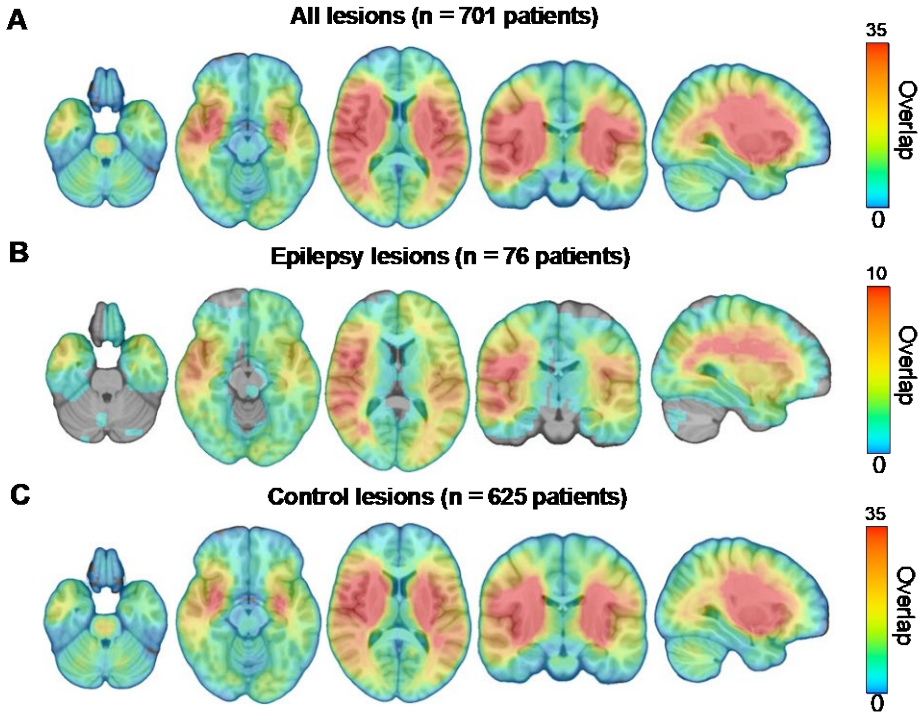

Figure S8.1 Lesion overlap of all lesions (**A**), ischemic stroke-related epilepsy lesions (**B**), and control lesions (**C**).

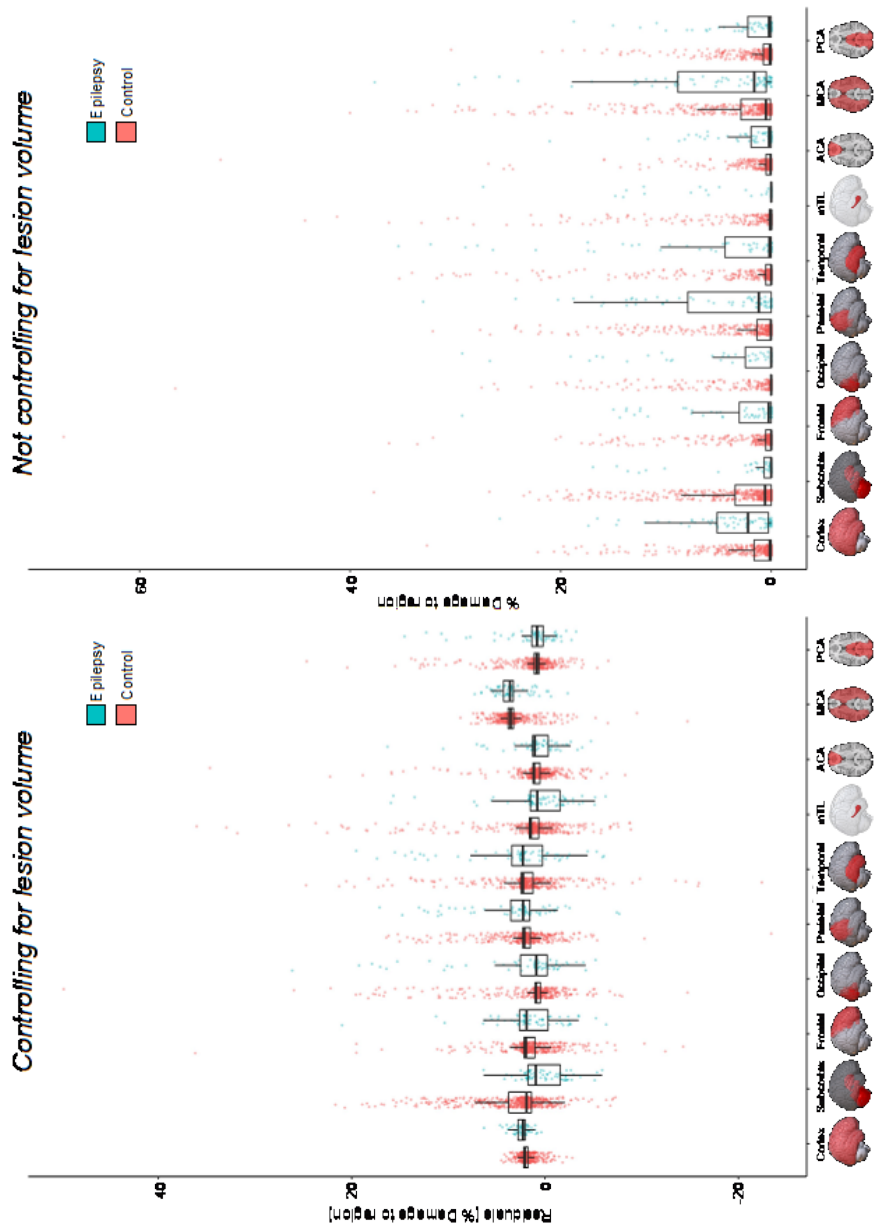

Figure S8.2. Distribution of lesion damage to each a priori brain region, with and without correcting for lesion volume.

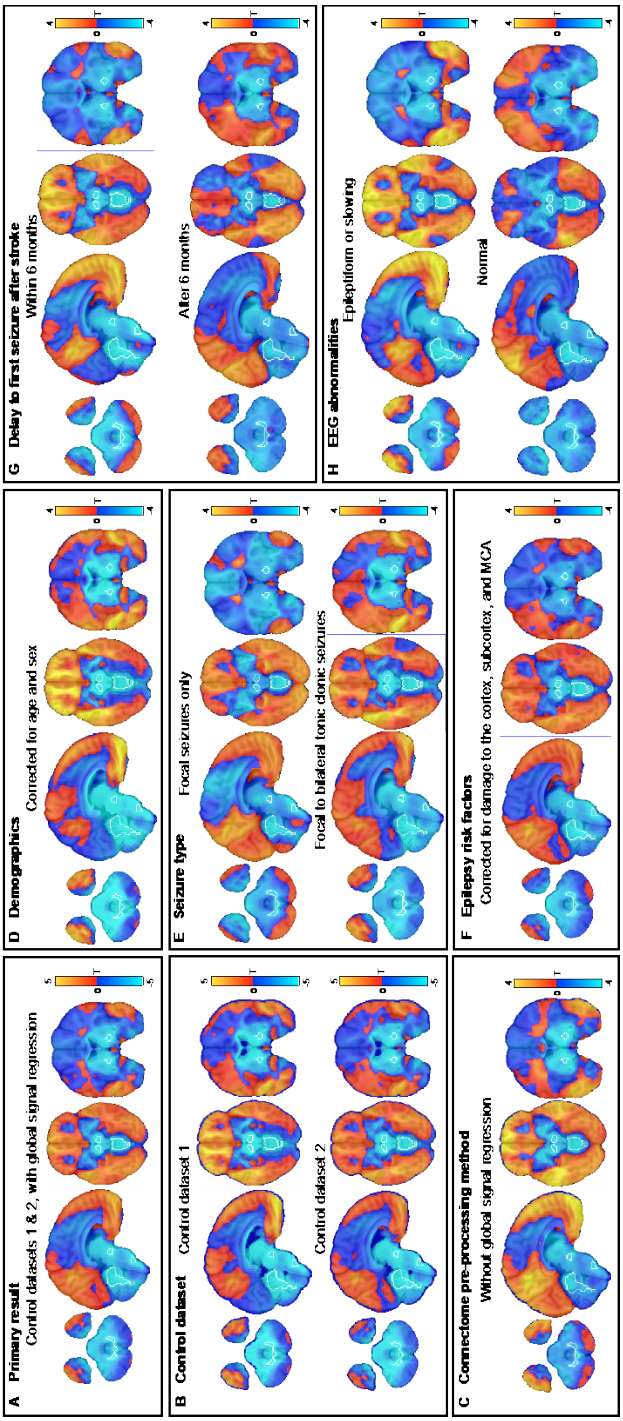

Figure S8.3. Our primary lesion network mapping result (**A**) implicating nodes in the cerebellum and basal ganglia (white outline) are similar with different control datasets (**B**), connectome pre-processing methods (**C**), after controlling for demographics (**D**), seizure type (**E**), known epilepsy risk factors (**F**), delay to first seizure after stroke (**G**), or presence of EEG abnormalities (**H**).

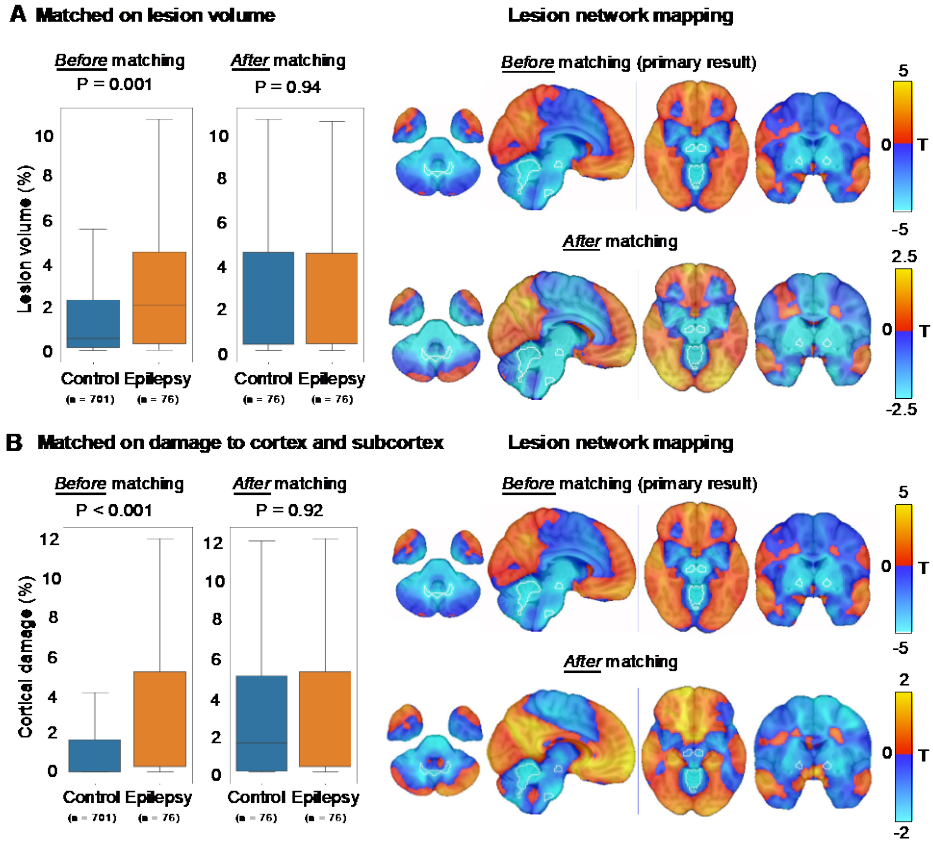

Figure S8.4 Lesion network mapping with matched subgroups. Subgroups were equally matched on lesion volume (**A**) and damage to the cortex and subcortex (**B**). Lesion network mapping results of matched subgroups were consistent with results identified in the total ischemic stroke dataset (white outline). Note that the strength of the relationship (T-values) between connectivity and epilepsy drops consistent with the smaller subgroup (n=76) compared to our original control (n=625), but the direction and topography remain the same.

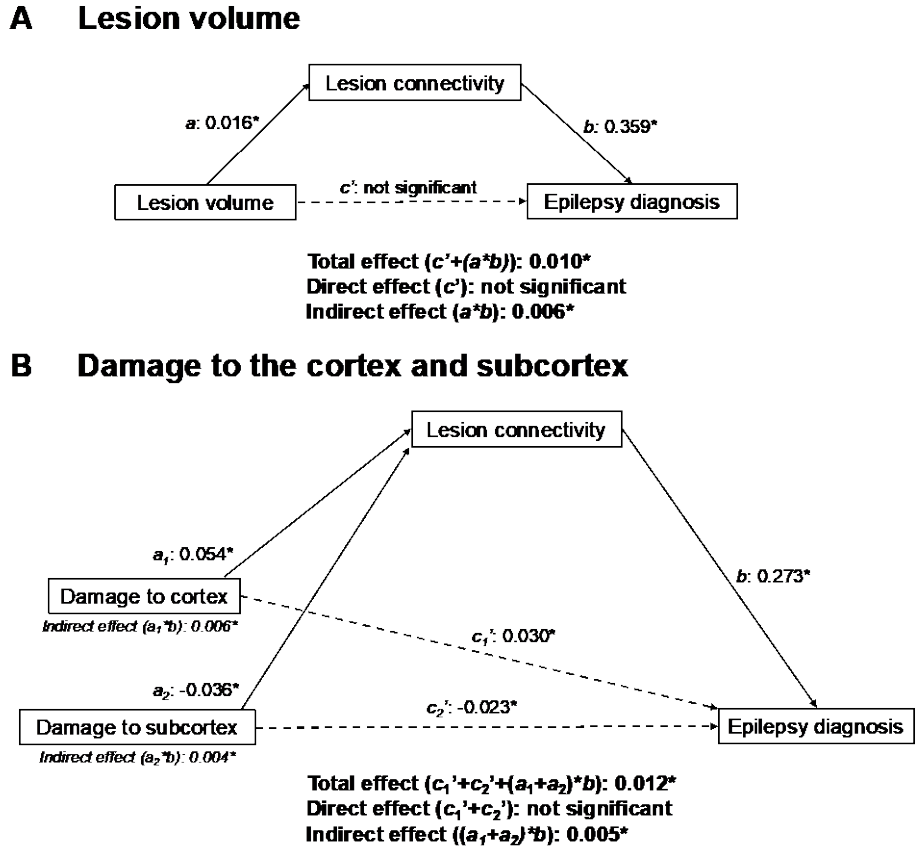

Figure S8.5 A statistical mediation analyses identified that the relationship between lesion volume and epilepsy diagnosis was fully mediated by lesion connectivity (indirect effect = 0.006, boot standard error [SE] = 0.001, 95% CI = 0.004 to 0.008, **A**). Similarly, the relationship between damage to the cortex and subcortex was also fully mediated by lesion connectivity (indirect effect = 0.005, boot standard error [SE] = 0.001, 95% CI = 0.003 to 0.007, **B**). *p<0.05.

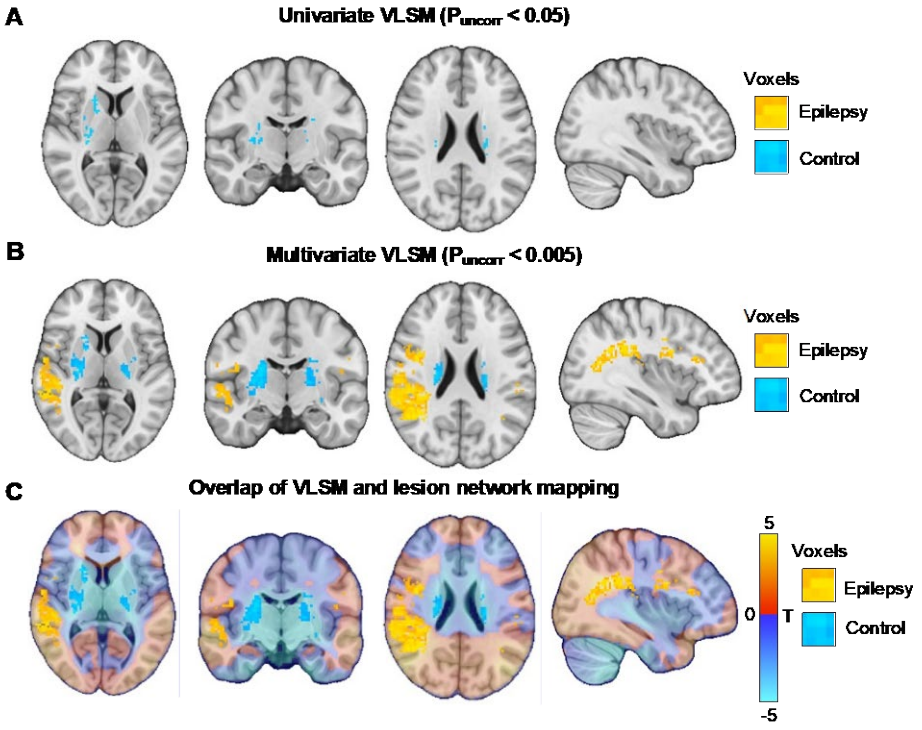

Figure S8.6 Voxel-based lesion sympom mapping (VLSM) results with more liberal statistical cutoffs (P_{uncorr}<0.05) using both univariate (**A**) and multivariate (**B**) methods. VLSM results aligned with lesion network mapping results (**C**) but only identified part of the network.

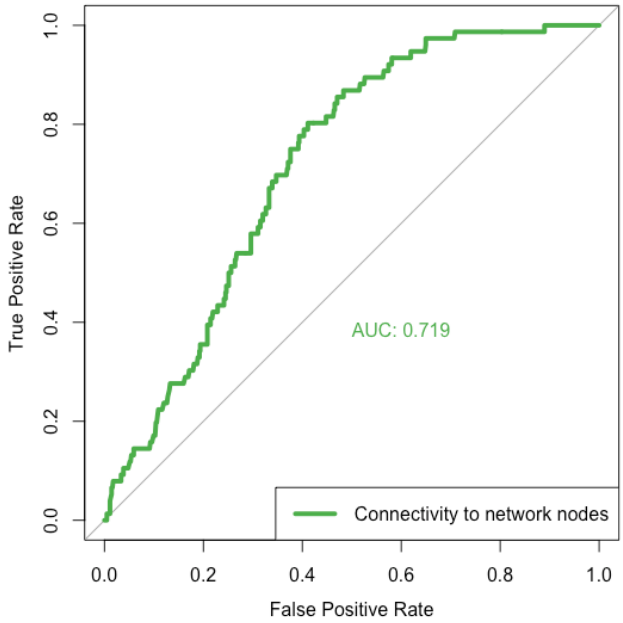

Figure S8.7 ROC curve showing prediction of ischemic stroke-related epilepsy based on lesion connectivity (p<0.001). To avoid circularity, connectivity was computed between each ischemic stroke lesion location and the network nodes derived from the four other lestion type datasets.

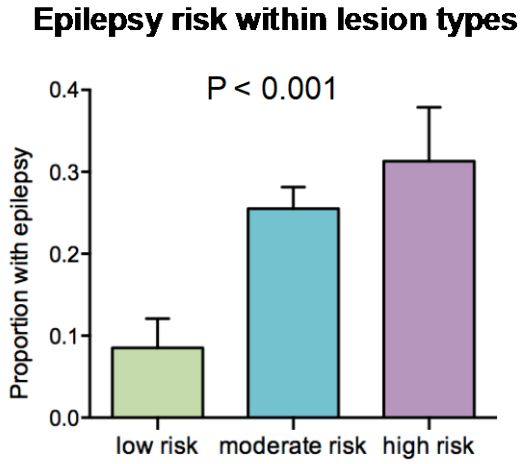

Figure S8.8 Risk groups based on lesion connectivity are associated with the proportion of epilepsy. Connectivity was computed from each lesion location to the nodes derived from the other four lesion type datasets (leave-one-lesion-type-out). Note that this analysis is similar to the analysis presented in main text (see Figure 8.4B), but risk was assessed within each lesion type rather than across all lesion types.

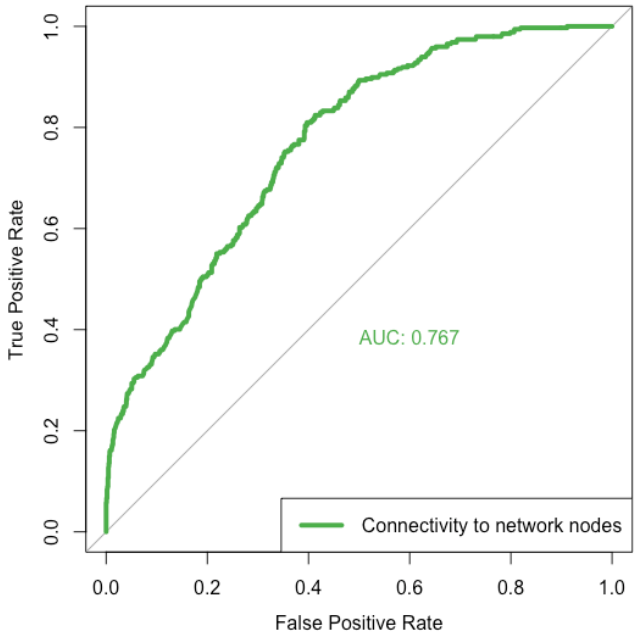

Figure S8.9 ROC curve showing prediction of epilepsy across all data based on lesion connectivity (p<0.001). To avoid circularity, connectivity was computed between each lesion location and the network nodes derived from the four other lesion type datasets.

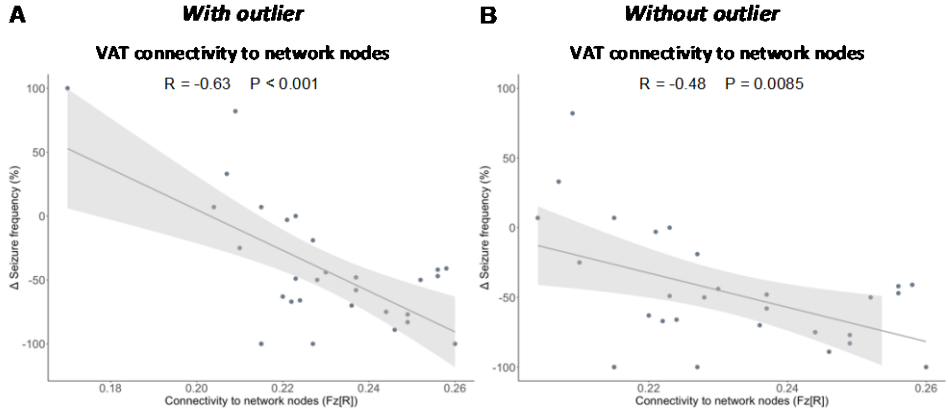

Figure S8.10 DBS correlation with and without an outlier that had worsened seizure control after DBS.

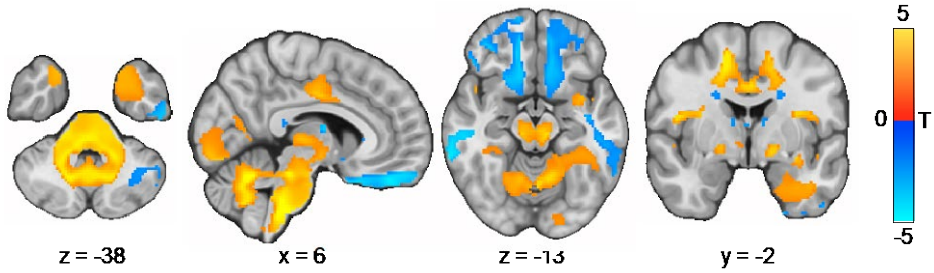

Figure S8.11 Whole-brain DBS-network mapping results implicating the same nodes in the cerebellum and basal ganglia (P_{corr}<0.05). Results are shown after family-wise error correction for multiple testing and threshold free cluster enhancement. This analysis is similar to that presented in the main text (see Figure 8.5D) but is a whole-brain analysis rather than restricted to our a priori ROI derived from lesions.

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"People don't have ideas. Ideas have people."

Carl Jung

Chapter 9

Discussion

Mapping and Zapping
Deep brain stimulation takes hold of epilepsy

HERE, WE CLOSE WITH A GENERAL DISCUSSION OF THE STUDIES PERFORMED IN THIS THESIS AND THE FUTURE PERSPECTIVES FOR THE FIELD.

PROBLEM STATEMENT

In Chapter 1, we provide a brief background on the current treatment of patients with drug resistant focal epilepsy and formulate the *problem statement* of this thesis. Patients with drug-resistant focal epilepsy are left with few therapy options if epilepsy surgery is not possible or fails. Anterior thalamic DBS (ANT-DBS) is a potentially effective neuromodulation therapy for these patients, but knowledge gaps on the predictors of response, optimal stimulation site and brain networks that are potentially crucial for achieving seizure control impede its widespread use as a standard neuromodulation therapy. In this thesis, we performed a series of animal, human and computational studies to identify the optimal stimulation site and brain networks that are crucial for achieving seizure control with ANT-DBS.

PART 1 – ON ANIMAL STUDIES

We first aimed to perform a series of animal studies and test whether DBS to different ANT subnuclei has differential effects on seizure control and side effects. Defining the optimal stimulation site within the ANT target area may eventually improve DBS surgery and programming for patients with epilepsy. We therefore started with establishing a translational rodent model and a stimulation paradigm that closely resembles the clinical use of ANT-DBS for patients with drug resistant epilepsy.

In Chapter 2, we first review the literature on the animal evidence for ANT-DBS. We found that most rodent studies were performed in acute seizure models and focused on modulation of the seizure threshold by ANT-DBS, not the modulation of spontaneous recurrent seizures. Only a few studies performed ANT-DBS in rodent models of chronic epilepsy, exhibiting spontaneous recurrent seizures, with mixed results between different chemically induced models of chronic epilepsy. Some studies also tested whether uni- or bilateral thalamic stimulation is more effective and found that, in line with common clinical practice, bilateral stimulation was associated with more seizure reduction. All

studies targeted the rodent homologue of the human ANT, the anteroventral nucleus of the anterior thalamic nucleus complex, which includes the anteroventral, anteromedial, and anterodorsal thalamic nuclei. Unfortunately, comparisons on the efficacy of DBS to different subnuclei within the ANT target area could therefore not be made. However, stimulation parameters were highly heterogenous between studies and were programmed with both high (100-150 Hz) and low frequency stimulation (1-30 Hz), with various amplitudes (100-800 μA), with short constant stimulation paradigms for 15 minutes, one or several hours per day. Surprisingly, none of these studies performed the clinical stimulation paradigm that was used in the human SANTE trial: a cycled stimulation mode of 1 minute ON and 5 minutes OFF for 24 hours a day. In conclusion, rodent studies support the use of bilateral over unilateral stimulation, and find that both low and high frequency stimulation can be effective in reducing seizures, but no study models the clinically used 24 hours a day cycled stimulation paradigm.

In Chapter 3, we report on a rat model of electrically induced epilepsy that allows for the assessment of chronic ANT-DBS under controlled experimental conditions. In this study, we addressed the hypothesis that cycled ANT-DBS (1 min ON and 5 min OFF) reduces seizure frequency and has behavioural side effects on short-term memory and anxiety, similar to the hypothesis of the human SANTE trial that resulted in FDA approval of ANT-DBS in patients.[1,2] To the best of our knowledge, this is the first rodent study that applied cycled ANT-DBS for 7 consecutive days (24 hours a day), using a clinically relevant stimulation paradigm to test therapeutic efficacy and behavioural side effects of ANT-DBS in rats. In the limited number of animals (n=5) that completed the study, we did not observe an effect of cycled ANT-DBS on seizure frequency. In this animal model of epilepsy, characterized by reduced spatial memory[3], cycled ANT-DBS did improve spatial memory, yet recognition memory, anxiety and locomotion remained unchanged. These results suggest cycled ANT-DBS may have a circuit wide neuromodulatory effect within the Circuit of Papez, resulting in improved spatial memory. Due to the unexpectedly large loss to follow-up, the power of the current study, and the conclusions that may be made from it, are limited. Further, we demonstrate that even in a controlled experiment, studies of chronic DBS with long-term EEG monitoring in epilepsy rodents show a substantial inter-and intra-individual variability in seizure frequency, consistent with other studies.[4] Some of these limitations may be overcome by designing future studies that use 'wireless' continuous long-term and daily EEG monitoring, in conjunction with continuous 24 hours a day DBS, and automated seizure detection for focal and generalized seizures. While the clinical stimulation paradigm can be modelled in the rodent, we learned that establishing an animal model that closely resembles the clinical situation remains challenging and may

not be the ideal method to define the optimal stimulation site for ANT-DBS in humans. We could therefore not proceed with our primary aim of testing different targets within ANT subnuclei in this rodent model and compare the efficacy of different stimulation sites. To this end, we turned to existing human data that was collected in patients receiving ANT-DBS to test whether neurophysiological or neuroanatomical characteristics of the ANT target area may identify an optimal DBS target.

PART 2 – ON HUMAN STUDIES

We investigated whether we could identify an optimal stimulation site using existing data from patients that received ANT-DBS for drug resistant epilepsy. We focused on two aspects of the ANT target area: its neurophysiological properties and its neuroanatomical surroundings.

In Chapter 4, we first review the rationale, safety, clinical efficacy, and the proposed mechanisms of action to inform our subsequent research questions. We focused on whether there are specific patient characteristics or other factors related to the treatment that could identify patients that respond or do not respond to ANT-DBS. We found that the clinical implementation of ANT-DBS in standard clinical practice still faces great challenges. While the SANTE trial and several open-label studies have shown safety and efficacy of ANT-DBS for patients with drug resistant focal epilepsy on a group level, for the individual patient, there is only a 50% chance of a 50% reduction in seizure frequency after 1 year of DBS treatment.[1] We identified multiple opportunities for improving the localization of the DBS target area, including its neurophysiological characteristics and neuroanatomical surroundings, to eventually improve seizure control.

In Chapter 5, we explore the neurophysiological characteristics of the ANT in humans using microelectrode recordings obtained during DBS surgery (n=10). Microelectrode recordings are regularly used during DBS surgery in patients with Parkinson's disease to identify the characteristic burst activity of the subthalamic nucleus (STN).[5] As such, this consistent physiomarker of the STN target are in Parkinson's disease, in conjunction with test stimulation and neurological examination, can inform the neurosurgical team to adjust the DBS lead placement intraoperatively. In DBS surgery for patient with epilepsy, there is no such validated physiomarker to identify the ANT target area and little is known about the neuronal firing properties of the ANT humans or their relation to lead placement and clinical outcome after DBS. The clinical utility of microelectrode

recordings during ANT-DBS surgery thus remains unclear. In this study, we hypothesized that single-cell recordings acquired by microelectrode recordings could aid targeting of the ANT during DBS surgery. We first identify a set of neurophysiological characteristics when entering and exiting the ANT, and second test whether there are differences in single-cell firing properties along the surgical trajectory between responders and non-responders to ANT-DBS. Using data from 10 patients and a total of 19 trajectories, we found an incremental increase in firing rate when entering the ANT and a decrease in firing rate and burst rate when exiting the ANT, which may hypothetically be associated with traversing the white matter of the medullary lamina of the thalamus. There were no changes in other firing characteristic such as, spike amplitude, spikes per burst, burst duration and inter-burst duration. We then compared the trajectories of responders to non-responders and found no differences in the neuronal firing properties per se nor their locations of peak firing/burst rate relative to the position of the active contact. We therefore conclude that single-cell firing rate detected by microelectrode recording under general anesthesia can aid DBS lead placement within the ANT during surgery, but is not related to therapy response. In conclusion, single-cell recordings of the ANT target area did not identify a reliable physiomarker that could optimize seizure control by informing DBS surgery or programming.

In Chapter 6, we explore a potential role of white matter in seizure control by ANT-DBS and investigate whether stimulation of the mammillothalamic tract (MTT) is associated with optimal seizure control. Within the circuit of Papez, the ANT receives major input from the MTT and its reciprocal cortical connections through thalamic radiations and thalamocingulate fibers.[6] This neuroanatomical location of passing fiber tracts is also called the ANT-MTT junction.[7] While the mechanism of action of ANT-DBS still remains elusive and it is unclear to what degree different brain networks and fiber tracts are stimulated, ANT-DBS is speculated to halt seizure propagation and/or modulate epileptogenic foci through its network connections within the circuit of Papez.[8] Accordingly, it is plausible that the varied effects of ANT-DBS on seizure control possibly relate to unsuccessful stimulation of the MTT and thus the Circuit of Papez.[9] In this study, we hypothesized that stimulation of the ANT-MTT junction is associated with increased seizure control. We first test whether the ANT-MTT junction can reliably be identified by two independent reviewers that were blinded for clinical outcome. Secondly, we test whether the shortest distance between the active contact to the ANT-MTT junction relates to seizure control. Third, we test whether the stimulation sites, also termed volume of activated tissue (VAT), are differently located between responders and non-responders. Using data from patients that received ANT-

DBS for drug resistant epilepsy (n=20), we assessed the locations of both the ANT-MTT junction and the active contacts in stereotactic (or native) space. We found that the ANT-MTT junction can reliably be identified by two independent reviewers and can serve as a reproducible anatomical landmark for neurosurgical targeting in ANT-DBS. Active contacts more closely located to the ANT-MTT junction were associated with increased seizure control. The stimulation 'hot-spot' of responders was at the medio-ventral ANT in high vicinity to the ANT-MTT junction in contrast to no evident hot-spot in non-responders. Accordingly, the ANT-MTT junction is not only a reliable neuroanatomical landmark for direct neurosurgical targeting, but also a potential optimal stimulation site for increased seizure control. In conclusion, we recommend planning a neurosurgical trajectory to target the ANT-MTT junction and programming the pulse generator to stimulate this region for optimal seizure control.

PART 3 – ON COMPUTATIONAL STUDIES

In Chapter 7, we introduce a novel technique, termed 'lesion network mapping' that combines a 'wiring diagram' of the human brain (the human connectome) with causal information from brain lesions and brain stimulation.[10] While this chapter is focused on neuropsychiatric diseases such as depression and Parkinson's disease, it serves as an introduction to the next chapter where we apply this same brain circuit mapping technique to epilepsy.

Brain lesions cause damage to specific brain circuits that may lead to specific neuropsychiatric symptoms. Brain stimulation of these same circuits could potentially modulate these same neuropsychiatric symptoms. To determine whether brain lesion locations and brain stimulation sites modulating the same neuropsychiatric symptom converge on common brain circuits, we studied depression severity after brain lesions (n=461, five datasets), transcranial magnetic stimulation (TMS) (n=151, four datasets) and DBS (n=101, five datasets). The DBS data in this study included 25 of our ANT-DBS patients that completed a Beck depression scale and observed changes in their depressive symptoms following ANT-DBS. We computed the functional connections between each lesion location and all other brain voxels using the resting state functional connectivity data from 1000 healthy participants (the human connectome)[11]. While this method using a normative connectome does not control for individual differences, it provides information on intrinsic brain connectivity in the average human brain. Lesion network mapping identifies a polysynaptic brain circuit connected to each lesion location

or stimulation site, allowing one to test whether lesions or stimulation sites in different brain regions intersect the same brain circuit. Using depression as an example, we found that lesions and brain stimulation sites most associated with depression severity were connected to a similar brain circuit. Circuits independently derived from lesions, deep brain stimulation and transcranial magnetic stimulation were also similar, as were circuits derived from patients with major depression versus other diagnoses. Peak regions of this functional network include the intraparietal sulcus, dorsolateral prefrontal cortex, inferior frontal gyrus, ventromedial prefrontal cortex and subgenual cingulate cortex. Compared with a consensus brain network parcellation, this depression circuit was most similar to the dorsal attention network and frontoparietal control network. This circuit was also therapeutically relevant, as connectivity to this circuit predicted out-of-sample antidepressant efficacy of TMS and DBS sites. Likewise, in an independent analysis in patients with Parkinson's disease, 29 lesions and 95 stimulation sites converged on a common brain circuit for motor symptoms of Parkinson's disease. We conclude that lesions, TMS and DBS map to common brain circuitry that may represent improved neurostimulation targets for depression or other neuropsychiatric diseases.

In Chapter 8, we apply lesion network mapping to epilepsy and test whether lesion-related epilepsy maps to a common brain network. We first studied lesion locations from patients with stroke-related epilepsy (n=76) and control lesions (n=625). Lesion locations were mapped to a common brain atlas and the brain network functionally connected to each lesion location was computed using the human connectome (n=1000), identical to the methods used in the previous chapter. The functional connections associated with stroke-related epilepsy were identified. Generalizability to other lesion etiologies was assessed using four datasets with different lesion types (n=772). We then tested the therapeutic relevance of these connections using the outcome data from patients who received ANT-DBS for drug resistant focal epilepsy at our center (n=30). We found that lesion locations of stroke-related epilepsy map to a specific brain network defined by functional connectivity to nodes in the basal ganglia and cerebellum. Functional connectivity to these same nodes was associated with the risk of epilepsy across different lesion types and with therapeutic response to thalamic deep brain stimulation. We conclude that lesion-related epilepsy maps to a common brain network with therapeutic potential for neuromodulation.

MAPPING BRAIN CIRCUITS: FROM ANIMAL, TO HUMAN, TO COMPUTATIONAL STUDIES

In this thesis, we have used a series of animal, human and computational studies to identify the optimal stimulation site and brain networks for achieving seizure control in ANT-DBS. It is worth mentioning that the evidence derived from these studies is different but complimentary, and overall limited by retrospective study designs, mostly small populations of patients studied and heterogenous datasets. Nonetheless, the basic premise of this thesis is to map a certain neuropsychiatric disease (e.g. epilepsy) or symptom (e.g. seizures) to a specific brain region and/or circuit. In the following paragraphs, we would like to take a "birds-eye view" and place the methods used in this thesis in a wider theoretical framework. Here, we describe the methods for studying relations between neuropsychiatric disease and brain regions/circuits, and how these methods have evolved to their use in this thesis.

Brain circuit mapping in animals and humans

In animal studies, we can precisely modulate different brain circuits in well- controlled conditions with methods such as DBS (as used in this thesis). This allows us to disrupt a specific brain region/circuit and measure its effects on a neuropsychiatric disease or symptom. In well-controlled experiments, we can hereby infer causal links between a specific brain region and symptom. While this approach has taught us a lot about the role of different brain regions/circuits in animals, it is well known that many findings in mice, rats or other animals do not translate well to humans. The equivalent experiment with comparable causal inference in humans would be a randomized-controlled trial (RCT). However, RCT's are lengthy, costly, and more importantly, require a significant body of evidence from case control or cohort studies showing potential clinical efficacy. An RCT is unlikely to be initiated or funded without this a priori evidence. Therefore, not every scientific question about the relationship between a specific neuropsychiatric disease or symptom and brain region/circuit can be answered by an RCT. Thus, in humans, we often turn to case control studies to investigate the role of specific brain regions/circuits associated with a neuropsychiatric disease, with are unfortunately inherently limited by their potential to infer causal links.

Neuroimaging

One approach to map neuropsychiatric symptoms to brain regions/circuits is to use neuroimaging techniques such as resting state functional MRI (fMRI). In these fMRI

studies, a group of patients with a specific neuropsychiatric disease or symptom receive a resting state fMRI scan and is compared to a group of controls without that disease or symptom. The findings from these studies are largely based on correlation, and by definition, cannot distinguish whether the difference in fMRI signal between the patient and control group is a cause or consequence of the disease. Case control studies of resting state fMRI have become an increasingly popular technique to map brain circuits over the past decades, but inherently suffer from a 'causality gap' when attempting to translate results into effective therapeutic targets. Bridging this causality gap is important to identify therapeutic targets. Furthermore, the field of brain-wide associated studies, including resting state fMRI, is currently experiencing a reproducibility crisis, since variations in preprocessing and sample size have shed light on important shortcomings in reproducibility across studies.[12] While many human studies mapping behavior or symptoms to brain regions are correlative in nature, there are incidental sources of causal information present in standard clinical care that offer a potential solution to bridging this causality gap.

Lesion mapping

Brain lesions are an incidental source of causal information and have been used for mapping symptoms and behaviors to brain regions for centuries. Well known cases include: 1) Patient Tan, where a stroke to an area the frontal lobe led to motor aphasia (i.e. Broca's area); 2) patient H.M, where bilateral resection of the hippocampus resulted in anterograde amnesia, and 3) patient Phineas Gage, where a lesion to the frontal lobe changed his personality. In the past decades, the study of mapping brain-behavior relationships using brain lesions has evolved to using more sophisticated computational methods. These methods include outlining the precise lesion locations that caused a specific symptom in a group of patients. These lesion locations are then normalized to a template atlas to compare the lesion locations across a group of patients with the same neuropsychiatric symptom. This technique is also called voxel-based lesion symptom mapping (VLSM).[13] VLSM can identify individual brain regions that are more lesioned in a group of patients with a specific symptom compared to a group of lesion patients without that symptom. A causal inference may therefore be drawn between a specific brain region and a specific symptom. The study of lesion mapping has instructed us about brain-behavior relationships and is a pivotal component of neurological localization in clinical practice. In fact, neurological localization is the first principle I was taught during my neurology rotation, similar to many students of neurology worldwide. However, everyday neurology practice quickly teaches us that lesions causing the same neuropsychiatric symptom do not always map to a single brain region. Lesions

to multiple different locations can cause the same neuropsychiatric symptom. It has thus become increasingly clear that brain-behavior relationships are likely the consequence of interactions of complex brain circuits. However, until recently, it has not been possible to map these brain circuits.

Lesion network mapping

Due to large-scale initiatives such as the Human Connectome Project and Genomes Superstruct Project, during which thousands of healthy participants received 3T high resolution MRI, we now have a detailed map of the functional and structural connections in the average human brain (also termed the human connectome).[11] The human connectome represents '*a wiring diagram*' of the average human brain, and is also called a normative connectome. With the use of normative connectomes, we can now use a template atlas of human brain circuits to identify the networks causally associated with a specific symptom in neuropsychiatric disease. This technique is similar to how an atlas of brain regions is used in VLSM to identify the brain regions causally associated with a specific symptom. However, instead of mapping brain regions causally related to a symptom, we can now map brain circuits causally related to a symptom. This method of combining brain lesions with the human connectome is also termed '*lesion network mappin.*'.[10] Lesion network mapping can thus overcome the limitations of lesion mapping, by defining the brain circuit that is causally associated with a specific behavior/symptom. Lesion network mapping may offer a potential solution to bridge the causality gap of brain wide association studies and move the field towards mapping brain circuits causally associated with a symptom to identify therapeutic targets.

ZAPPING BRAIN CIRCUITS: TOWARDS NETWORK-NEUROMODULATION FOR EPILEPSY

The findings from this thesis can be translated into a network-based neuromodulation treatment for patients with drug resistant epilepsy.

A 'sweet-spot' for seizure control in ANT-DBS for epilepsy

The first important finding in this thesis is that the optimal stimulation site in ANT-DBS for drug resistant focal epilepsy is at the ANT-MTT junction. This finding is consistent with an earlier study of 15 ANT-DBS patients that localized active contacts

associated with a successful treatment trial at a more anterior and superior location within the ANT.[14] Here, we externally validate these results in a cohort of 20 ANT-DBS patients and extend these findings by modelling the VAT to include information about the stimulation field. We show that 1) the ANT-MTT junction is a reliable neuroanatomical target that can be visualized across different MRI field strengths, 2) the shortest distance between the active contact and the ANT-MTT junction correlates to seizure control, and 3) the stimulation sites of responders (patients with >50% seizure reduction) overlap in a common 'hot-spot' at the ANT-MTT junction, while non-responders have no evident common neuroanatomical localization.

The ANT-MTT junction can be visualized using standard high resolution MR images that are available in most hospitals (preferably with a 3T or higher field strength scanner). Various MRI sequences can be used to visualize the ANT-MTT junction, including a T1 MPRAGE,[15] T2,[16] Short Tau Inversion Recovery (STIR),[17] or Fast Gray Matter Acquisition T1 Inversion Recovery (FGATIR).[18] Visualization of the ANT-MTT junction may even be improved by using a 7T MRI scanner and a white-matter nulled MPRAGE sequence[19] as illustrated in this thesis. Improvements in clinical accessibility of high-resolution 7T MRI scanners[20] are expected to improve targeting in ANT-DBS surgery for patients with epilepsy, and thus potentially clinical outcome.

The finding that the shortest distance between the active contact and ANT-MTT junction correlates with seizure control is interesting, as it may suggest a potential neuroanatomical 'sweet-spot' for ANT-DBS. For neurologists, it may thus be important to include both the ANT and the MTT in the stimulation field when programming patients after surgery. Overall, these results suggest a role for both the ANT and the MTT in seizure control, consistent with early animal studies[21] and some human studies.[22] Furthermore, this result is in line with recent findings in DBS for movement and psychiatric disorders, proposing that DBS may act through modulation of passing white matter tracts in addition to its local effects on gray matter.[23,24] We propose that the MTT has a role in the mechanism of action of ANT-DBS, as it is one of the fiber tracts that connects the hippocampal formation to the cortex along the Circuit of Papez.[6] However, it is important to highlight that the MTT may not be the only fiber tract that is modulated by ANT-DBS. While the MTT, connecting the ANT to the mammillary bodies, enters the ANT at the ANT-MTT junction, the connections that exit the ANT also originate here. These efferent connections of the ANT are called the anterior thalamic radiations.[25] The anterior thalamic radiations exit the ANT slightly anterior to the ANT-MTT junction and terminate in the anterior cingulate and frontal cortex. Unfortunately, it is not possible to visualize the anterior thalamic radiations using conventional anatomical MRI sequences. Our study is thus limited, by the fact that we did not perform diffusion tensor imaging of fiber tracts in our ANT-DBS cohort. We

could therefore not test whether overlap of the stimulation site and anterior thalamic radiations correlates with seizure control. A supplementary role for the anterior thalamic radiation in seizure control by ANT-DBS can thus not be excluded. Future studies directly testing this question are warranted. However, as the anterior thalamic radiations exit the ANT slightly anterior to the ANT-MTT junction, the optimal target identified here may serve as a good surrogate that can easily be visualized with conventional neuroanatomical MRI sequences. A surgical trajectory transecting an imaginary "triangle", spanning anterior to the ANT-MTT junction to the wall of the ventricles, may be an optimal neurosurgical target to stimulate both the ANT, MTT and anterior thalamic radiations. Recent evidence from a retrospective analysis of the DBS lead locations in the US-based SANTE trial supports this notion and externally validates our findings in a larger patient cohort (n=101).[26]

Overall, in this thesis, we contribute to the literature by showing that the stimulation site is crucial for clinical outcome in ANT-DBS for drug resistant epilepsy. Future studies combining DBS lead localization, VAT modelling, and tractography of white matter tracts may lead to substantial improvements in the consistency and magnitude of seizure reduction after ANT-DBS across patients.

Individual versus common brain networks in epilepsy

A second important finding in this thesis is that lesions related to epilepsy better map to a common brain network than an individual brain region. This finding is consistent with the network hypothesis of epilepsy[27] and lesion network mapping studies in other neuropsychiatric symptoms such as depression and Parkinson's disease (this thesis). We find that lesions causing epilepsy are more connected to the basal ganglia and cerebellum. We propose that intrinsic brain connectivity to the basal ganglia and cerebellum may thus play a causal role in why some lesions cause epilepsy and others do not. The concept that an intrinsic brain network, distant but connected to the lesion, may influence whether a lesion leads to epilepsy or not, is novel. At first sight, this concept stands in stark contrast to our current understanding of epilepsy and its diagnosis or treatment, which is focused on mapping a patient's individual epileptogenic zone or epilepsy network.

Since the discovery of the epileptogenic lesion by Penfield and Jasper, the diagnosis and treatment of drug resistant focal epilepsy has focused on identifying and removing the lesion location and spike zone hypothesized to cause seizures.[28] After important technological inventions such as the stereotactic frame and stereo encephalography in the 1950's, Bancaud and Talairach extended this lesion concept to a seizure network concept.[29,30] In this seizure network concept, the focus moved away from the

epileptogenic lesion, towards the spatiotemporal organization of the epileptic discharge across the brain. This seizure network concept increased our understanding of how spatio-temporal dynamic ictal patterns across the brain can lead to clinical symptoms (also termed anatomo-electro-clinical correlation). Epilepsy surgery following this seizure network concept focused on identifying where seizures start or spread, and removing those regions to treat seizures, with either surgical resections, disconnections and/or ablations. Building upon this seizure network framework, Spencer introduced the network hypothesis of epilepsy by compiling evidence from animal models, intracranial EEG, and modern neuroimaging and electrical stimulation studies. The epilepsy network hypothesis states that "*vulnerability to seizure activity in any one part of the network is influenced by activity everywhere else in the network, and that the network as a whole is responsible for the clinical and electrographic phenomena that we associate with human seizures*". In this context, a network is considered a functionally and structurally connected set of cortical and subcortical brain structures in which activity in any part affects activity in all others. Based on her extensive experience with a great number of human epilepsy patients, Spencer described three specific large human epilepsy networks that may be commonly affected across different patient groups. The medial temporal/limbic network (i.e. Circuit of Papez), the medial occipital/lateral temporal network, and the superior parietal/medial frontal network. Two additional networks, which are less commonly described, include the bifrontal/pontine/subthalamic network and the parietal/medial temporal network. This network framework has inspired and paralleled the application of advanced neuroimaging techniques in today's presurgical evaluation phase in epilepsy surgery. Increasingly, this framework is also guiding the treatment of DBS and RNS for epilepsy at the seizure-onset zone or to distant connected nodes. In summary, the prevailing dogma in our understanding of epilepsy and its treatment is focused on mapping the epileptogenic zone and epilepsy network that are unique to the individual patient.

In contrast, despite the many differences in lesion locations and types, we find that there is common, intrinsic brain network associated with lesion-related epilepsy across patients. Connectivity to this same network is associated with therapeutic response to ANT-DBS. It is important to highlight that our lesion network mapping findings do not necessarily contradict this prevailing dogma of individual epilepsy networks, but rather suggest the co-existence of an intrinsic brain network associated with epilepsy that is a common among patients, and may thus be independent of individual epilepsy networks. While we did not identify one of the hypothesized epilepsy networks as defined by Spencer, our lesion network mapping findings are consistent with the large-scale network hypothesis of epilepsy that "*one part of the network is influenced by activity everywhere else in the network*". In fact, digging into the historic literature of epilepsy

concepts, our results are more consistent with a relatively "forgotten" concept in epilepsy. This historic concept revolved around intrinsic anticonvulsant systems. As early as the 1950s, animal researchers resected parts of the cerebellum to study the effects on cerebral cortical excitability.[31] The neurosurgeon Cooper then introduced this concept as a potential treatment for patients with epilepsy and showed that cerebellar and thalamic stimulation could inhibit seizures[32]. Similar results were found with lesions and high frequency stimulation of the substantia nigra in various animal models of epilepsy.[33,34] According to these studies, the basal ganglia and cerebellum may be part of a "gating system" for seizures,[34] or play a role in seizure termination through acting like an "endogenous control center"[35] or "brake".[36] We propose that the activity of these "control centers" or "modulators" distant (but connected to) the lesion could influence whether a lesion leads to epilepsy or not, and whether a DBS site leads to seizure control or not.

While it is apparent that the number one goal of epilepsy surgery should still be to remove the epileptogenic zone, the proposed new concept that intrinsic brain connectivity distant from the epileptogenic zone can play a role in the efficacy of ANT-DBS raises important question how to optimize neuromodulation treatment with ANT-DBS. For instance, should we invest time and effort into meticulously mapping a patient's individual epilepsy network for the personalization of DBS; or just target the spot in the thalamus that is most connected to the basal ganglia and cerebellum? One possibility is that intrinsic brain connectivity dictates best ANT-DBS sites common to many patients, which would justify the latter. The other is that wide variability exists for individual efficacious stimulation sites within or outside the ANT, which would justify personalized mapping of epilepsy networks. I suspect that both will turn out to be partially true, but to what extent remains to be determined in further studies comparing different DBS targets and different stimulation sites within a DBS target.

Connectomic deep brain stimulation in epilepsy

The third important finding in this thesis is that the functional connections of the stimulation site may be crucial for seizure control in ANT-DBS. This finding is consistent with recent results from DBS in movement disorders and psychiatric disorders, that suggest that the clinical benefit from DBS may depend on connectivity between the stimulation and other brain regions.[23,24] For example, STN-DBS sites that successfully treat symptoms of Parkinson's disease are more connected to the motor cortex compared to STN-DBS sites that do not. Different DBS targets that successfully treat symptoms of OCD are all connected to a common brain network and a fiber tract that runs through the anterior limb of the internal capsule. In this thesis, we found that 1) ANT-DBS sites that successfully treat seizures are more connected to the basal

ganglia and cerebellum compared to ANT-DBS sites that do not, and 2) that lesions causing epilepsy and DBS sites treating epilepsy converge on a common brain network. We propose that an intrinsic brain network defined by functional connectivity to the basal ganglia and cerebellum may even be a potential novel therapeutic target. In the future, maps of connectivity to the basal ganglia and cerebellum could be used to guide DBS programming or eventually to refine neurosurgical implantation. These maps of connectivity, also termed 'connectomes', are typically derived from large cohorts of healthy human participants, but can be warped to patient space to target the neuroanatomical location in the anterior thalamus that should be most connected to the basal ganglia and cerebellum in the average human brain. One could use a variety of connectomes, such as functional and structural ones, or in the future maybe even neurophysiological connectomes based on (stereo) encephalography or MEG data. While there are already several connectomes available from healthy participants, DBS targeting may even be improved using a patient-derived connectome. Patient-derived connectomes are expected to better represent the functional and structural connections of that individual patient's brain. However, this would require high-resolution and advanced neuroimaging sequences, with a high signal-to-noise-ratio and clinically acceptable scanning durations. Until these technological barriers are overcome, normative connectomes based on healthy participants may function as a template atlas of intrinsic brain connectivity and provide a roadmap towards connectomic deep brain stimulation.[37]

Opportunities for non-invasive brain stimulation in epilepsy

In this thesis, we used causal information from incidental brain lesions and deep brain stimulation to identify an intrinsic brain network associated with epilepsy that may have therapeutic potential. Prospective studies that target this brain network are needed to answer the question whether this is an effective neuromodulation target. One way of testing this would be to use DBS to target this network. However, DBS is a relatively invasive procedure as it requires brain surgery and permanent implantation of a foreign device. DBS is therefore not a safe or practical method to test the therapeutic efficacy of a brain network that is hypothesized to improve clinical outcome. A less invasive way of testing the therapeutic potential of this brain network could be to use non-invasive brain stimulation methods. Non-invasive brain stimulation methods, such as transcranial magnetic stimulation (TMS) or transcranial electrical stimulation (TES), do not require brain surgery and can target superficial cortical nodes of distributed brain networks.[38] For example, TMS of the dorsolateral prefrontal cortex is an FDA approved therapy for drug resistant depression and has been shown to modulate a distributed brain network,

including the dorsolateral prefrontal cortex, subgenual cingulate, intraparietal sulcus, among others.[39] DBS targeted at a subcortical node within this same brain network (the subgenual cingulate) can also improve depressive symptoms.[40,41] In this thesis, we showed that TMS sites relieving depression and DBS sites modulating depressive symptoms map to a common brain network. Analogues to the use of DBS and TMS as a therapy that targets a common brain network in depression, we may hypothetically also use TMS or TES to target a common brain network in epilepsy.

Both TMS and TES have already been used to treat focal epilepsy with promising outcomes.[42] Yet, TMS and TES can only target the rare, superficially located cortical epileptic foci. This targeting strategy is similar to the target strategy in epilepsy surgery and RNS, as the target remains the epileptogenic lesion or seizure-onset zone itself. Unfortunately, as mentioned previously, in a large population of patients with drug resistant epilepsy we just cannot identify a clear seizure-onset zone. Our proposed common brain network for lesion-related epilepsy may thus be a potential novel target to treat a large population of patients with focal epilepsy using non-invasive brain stimulation. Using the human connectome, one could compute the cortical nodes that are most connected to the basal ganglia and cerebellum. We could then target these superficial cortical nodes and modulate a distributed brain network that may lead to seizure control. In line with the experimental stimulation studies by Cooper in the 1950's, non-invasive brain stimulation of a distributed brain network connected to the basal ganglia and cerebellum could lead to a novel brain network therapy for patients with drug resistant focal epilepsy.

KEY FINDINGS AND NEW INSIGHTS

The ***key findings*** of this thesis are as follow:

- ANT-DBS is a safe and efficacious therapy that can increase the seizure threshold, decrease seizure frequency and severity, and modulates a distributed brain network.
- Single-cell recordings can aid lead placement during ANT-DBS surgery, but do not relate to seizure control.
- The optimal simulation site for seizure control in ANT-DBS is at the junction of the mammillothalamic tract and ANT, also termed the ANT-MTT junction.
- Brain stimulation and brain lesions converge on common causal circuits in neuropsychiatric disease.
- Lesion-related epilepsy maps to a common brain network with prognostic and therapeutic potential.

– An intrinsic brain network defined by functional connectivity to the basal ganglia and cerebellum is a potential novel neuromodulation target for epilepsy.

In a wider theoretical framework, the most significant *new insight* gained from this thesis is as follows.

The current dogma in the treatment of drug resistant epilepsy is that we should develop tools to tailor epilepsy surgery or neuromodulation to target a patient's individual epilepsy network, not a common brain network across patients. First, this thesis raises the possibility that there is an intrinsic brain network universally related to epilepsy. Second, this common brain network across patients could potentially explain why some lesions cause epilepsy and others do not. Third, this thesis identifies this common brain network as a potential novel therapeutic target for the treatment of drug resistant epilepsy. If confirmed by future prospective studies, this new insight could represent a paradigm shift in how we view the role of brain networks in epileptogenesis, epilepsy, and its treatment.

However, before such provocative concept can be tested in RCTs, there remains a need for widespread testing of this hypothesis in multiple animal models of epilepsy and/or patients with drug resistant epilepsy that have no other therapeutic options left to attain seizure control.

STRENGTHS AND LIMITATIONS

This thesis has several *strengths*.

The strength of this thesis is that we narrowed our scope to investigate the optimal stimulation site in ANT-DBS for drug resistant epilepsy. We used both animal, human, and computational studies to investigate this aim and attempted to tackle the problem statement of this thesis from different angles. We subsequently stepped outside the traditional framework of mapping brain regions, towards a framework of mapping brain networks. To pursue this, we implemented a data-driven approach, using a combination of large lesion datasets, brain stimulation datasets, and brain connectivity datasets. We used incidental causal information that is present in daily clinical practice and replicated our results across multiple lesion types and datasets. Combined, this multimodal data-

driven brain network mapping strategy adds to the external validity and reproducibility of the findings in this thesis.

However, this thesis also has several *limitations*.

Selection bias

ANT-DBS may offer a chance for seizure control for a large population of patients with drug resistant epilepsy, but is (to this day) not a standard neuromodulation therapy in many epilepsy centers around the world. The patient numbers, in the range of 10-30 patients per center across Europe or US, remain low. However, it is worth mentioning that the cohort described in this thesis is one of the largest single-center ANT-DBS cohorts across Europe and the US. Nevertheless, this low patient number may still result in selection bias and may thus not reflect a representative sample of the target population of ANT-DBS patients. Selection bias may thus decrease the external validity of our findings to ANT-DBS patients from other centers with different inclusion criteria, neurosurgical practices, or DBS programming strategies. Future independent studies should replicate our results to increase external validity. In our lesion network mapping studies, we have increased our sample size to include data of over 1000 patients, derived from multiple datasets across different centers, countries and continents around the world. The external validity of the network findings in lesion-related epilepsy is thus expected to be relatively high.

Confounding bias

The studies performed in this thesis are retrospective in nature. Since we have FDA and CE approved data to support the use of the current DBS target, we could not perform a human randomized controlled trial of different stimulation sites within the ANT. Due to the retrospective design, the studies in this thesis may be subject to confounding bias. We have tried to minimize the risk of confounding by testing whether the effect of the variable of interest or outcome variable was different between different patient groups. However, the ANT-DBS patient population remains small and statistical adjustment for potential confounders in small cohorts is challenging. Therefore, we have performed several analyses in our larger lesion network mapping studies that control for potential confounders. Furthermore, we performed a subgroup analysis using propensity score matching, which is a validated method for accounting for covariates in retrospective studies. The results of these control- and subgroup-analyses showed that our results do not change when accounting for potential confounders. Nevertheless, in a retrospective

study design, the risk for unknown confounders remains present. Future larger and prospective studies are needed to externally validate our results.

Correlation versus causation

As the studies performed in this thesis are of a retrospective design, one could debate whether our findings are merely correlative or do provide some causal links. Identifying causal links in brain circuits is important, as these may lead to therapeutic targets for neuromodulation.

"*An event is causal if, with all else being equal, its presence or absence affects the probability of an outcome*". Causality is not binary (present or not present) but can be described along a spectrum (less causal or more causal) using criteria adapted from the Bradford Hill criteria for causation.[43] On this spectrum, a resting state fMRI case control study would be considered less causal and a randomized controlled clinical trial of DBS or TMS would be considered more causal. Brain mapping techniques can increase their causal inference by providing evidence for temporality, a clear counterfactual, specificity, dose-response, experimental manipulation, reversibility and coherence.[44] The lesion network mapping studies performed in this thesis have shown 1) temporality (patients did not have epilepsy or seizure control before the lesion or DBS), 2) a clear counterfactual (not having a lesion decreases the risk of epilepsy and not receiving DBS decreases the chance of seizure control), 3) specificity (other lesions and DBS sites that do not result in epilepsy or seizure control do not map to the identified common brain network), and 4) coherence (brain lesion data and brain stimulation data, that both modulate the risk of seizures, map to a common brain network). Only with prospective experimental manipulation could we test the other factors of the causality spectrum, such as reversibility and dose-response.

It is important to emphasize that we did not prospectively test whether DBS of the hypothesized optimal stimulation site and brain networks lead to increased seizure control in patients with ANT-DBS. We will need to design prospective studies that can test this hypothesis. One option would be to perform a DBS re-programming trial, where ANT-DBS non-responders will be called back for DBS re-programming to increase stimulation of the hypothesized optimal stimulation site and its connected brain networks. A different option could be to directly target the identified brain network with neuromodulation methods such as DBS, TMS or TES, and test clinical efficacy in a randomized sham-controlled trial.

Conclusion

In this thesis, we have performed a series of animal, human and computational studies to identify the optimal stimulation site and brain networks crucial for achieving seizure control in ANT-DBS for drug-resistant focal epilepsy. We find that the optimal stimulation site is at the junction of the ANT and mammillothalamic tract (ANT-MTT junction), a key hub in the anterior thalamus that connects the hippocampal formation with the cortex through the Circuit of Papez. DBS surgery and programming targeted at this brain region may result in improved seizure control after ANT-DBS. We learned that neurosurgical targeting and DBS programming to an exact spot is crucial for clinical outcome. Moving from a brain region- to a brain network-perspective, we combined data from '*a wiring diagram*' of the human brain with incidental causal information derived from patients with lesion-related epilepsy and ANT-DBS patients. We find that lesions causing epilepsy and ANT-DBS sites treating epilepsy share functional connectivity to a common brain network, defined by connectivity to the basal ganglia and cerebellum. This intrinsic brain network associated with epilepsy may be a novel therapeutic target, which warrants prospective testing in randomized controlled clinical trials.

In a wider theoretical framework, the most important new insight stemming from this thesis is that an intrinsic brain network distant from the lesion location or DBS site may influence whether a lesion leads to epilepsy or not, and whether a DBS site leads to seizure control or not. Yet, the prevailing dogma in our current understanding of epilepsy is that epilepsy diagnosis and treatment should be focused on a patient's individual epilepsy network, not a common brain network across patients. In conclusion, the new insight proposing intrinsic brain connectivity as a potential cause and modulator of focal epilepsy could represent a paradigm shift in how we '*map*' and '*zap*' brain networks to treat epilepsy.

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Summary

The aims of this thesis were to identify the optimal stimulation site and brain networks that are potentially crucial to engage for achieving seizure control with ANT-DBS in drug-resistant focal epilepsy. To address this aim, we have performed the following animal (*Part I*), human (*Part II*) and computational (*Part III*) studies:

The introduction **in Chapter 1** provides a brief background on the treatment of patients with drug resistant focal epilepsy and formulates the *problem statement* of this thesis. Patients with drug-resistant focal epilepsy are left with few therapy options if epilepsy surgery is not possible or fails to control seizures. Anterior thalamic DBS (ANT-DBS) is a potentially effective neuromodulation therapy for these patients, but knowledge gaps on the predictors of response, optimal stimulation site and brain networks that are potentially crucial for achieving seizure control with ANT-DBS, impede its widespread use as a standard neuromodulation therapy.

PART 1 - ON ANIMAL STUDIES

In Chapter 2, we review the literature on the animal evidence for ANT-DBS. We found that most rodent studies were performed in acute seizure models and focused on the modulation of the seizure threshold by ANT-DBS, not the modulation of spontaneous recurrent seizures. Only a few studies performed ANT-DBS in rodent models of chronic epilepsy, exhibiting spontaneous recurrent seizures, and results were mixed between different models of epilepsy. Some studies tested whether unilateral or bilateral thalamic stimulation is more effective and found that, in line with common clinical practice, bilateral stimulation was associated with more seizure reduction. No study tested different stimulation sites within the ANT and a comparison of different targets within the ANT could therefore not be made. Stimulation parameters differed between studies (high and low frequency) and, surprisingly, none of these studies performed the clinical stimulation paradigm that was used in the human trials: a cycled stimulation mode of 1 minute ON and 5 minutes OFF for 24 hours a day. In conclusion, rodent studies support the use of bilateral stimulation over unilateral stimulation, find that both low and high frequency stimulation can be effective in reducing seizures, but no study models the clinically used 24 hours a day cycled stimulation paradigm (1 min ON, 5 min OFF).

In Chapter 3, we report on a rat model of electrically induced epilepsy, exhibiting spontaneous recurrent seizures, that allows for the assessment of chronic ANT-DBS under controlled experimental conditions. To the best of our knowledge, this is the first

rodent study that applied cycled ANT-DBS for 7 consecutive days (24 hours a day), using a clinically relevant stimulation paradigm to test therapeutic efficacy and behavioural side effects. In the limited number of animals (n=5) that completed the study, we did not observe an effect of cycled ANT-DBS on seizure frequency. In this animal model of epilepsy, characterized by reduced spatial memory, cycled ANT-DBS did improve spatial memory, yet recognition memory, anxiety and locomotion remained unchanged. Due to the unexpectedly large loss to follow-up, the power of the current study, and the conclusions that may be made from it, are limited. We therefore turned to existing human data that was collected in patients receiving ANT-DBS to test whether neurophysiological or neuroanatomical characteristics of the ANT target area may identify an optimal stimulation site.

PART 2 - ON HUMAN STUDIES

In Chapter 4, we review the rationale, safety, clinical efficacy, and the proposed mechanisms of action of ANT-DBS in humans. We found that the clinical implementation of ANT-DBS in standard clinical practice still faces great challenges, as there is only a 50% chance of a 50% reduction in seizure frequency after 1 year of treatment. We focused on whether there are specific patient characteristics or other factors related to the treatment that could identify patients that respond or do not respond to ANT-DBS. We identified multiple opportunities for improving the localization of the DBS target area, including its neurophysiological characteristics and neuroanatomical surroundings, to eventually improve seizure control.

In Chapter 5, we explore the neurophysiological characteristics of the ANT in humans using microelectrode recordings obtained during DBS surgery (n = 10). The clinical utility of microelectrode recordings during ANT-DBS surgery remains unclear, as little is known about the neuronal firing properties of the ANT humans and even less about their relation to DBS lead placement or clinical outcome. Using data from 10 patients and a total of 19 trajectories, we found an incremental increase in firing rate when entering the ANT and a decrease in firing rate and burst rate when exiting the ANT. This pattern may hypothetically be associated with traversing the white matter of the medullary lamina of the thalamus. We then compared the trajectories of responders to non-responders and found no differences in the neuronal firing properties themselves nor their locations of peak firing/burst rate relative to the position of the active contact. We therefore conclude that single-cell firing rate detected by microelectrode recording

under general anesthesia can aid DBS lead placement within the ANT during surgery, but is not related to therapy response. In conclusion, single-cell recordings of the ANT target area did not identify neurophysiological characteristics of the ANT that could optimize seizure control by informing DBS surgery or programming.

In Chapter 6, we explore a potential role of white matter in seizure control by ANT-DBS and investigate whether stimulation of the mammillothalamic tract (MTT) is associated with optimal seizure control. Using data from patients that received ANT-DBS for drug resistant epilepsy (n = 20), we assessed the locations of both the ANT-MTT junction and the active contacts in stereotactic (or native) space. We found that the ANT-MTT junction can reliably be identified by two independent reviewers and can serve as a reproducible anatomical landmark for neurosurgical targeting. Active contacts more closely located to the ANT-MTT junction were associated with increased seizure control. The stimulation 'hot-spot' of responders was at the medio-ventral ANT in high vicinity to the ANT-MTT junction, in contrast to no evident hot-spot in non-responders. Accordingly, the ANT-MTT junction is not only a reliable neuroanatomical landmark for direct neurosurgical targeting, but also a potential optimal stimulation site for increased seizure control. In conclusion, we recommend planning a neurosurgical trajectory to target the ANT-MTT junction and programming the pulse generator to stimulate this region for optimal seizure control.

PART 3 - ON COMPUTATIONAL STUDIES

In Chapter 7, we introduce a novel technique, termed 'lesion network mapping' that combines a 'wiring diagram' of the human brain (the human connectome) with causal information from brain lesions and brain stimulation. Brain lesions cause damage to specific brain circuits which may lead to specific neuropsychiatric symptoms. Brain stimulation of these same circuits could potentially modulate these same neuropsychiatric symptoms. To determine whether brain lesion locations and brain stimulation sites modulating the same neuropsychiatric symptom converge on common brain circuits, we studied depression severity after brain lesions (n=461, five datasets), transcranial magnetic stimulation (TMS) (n=151, four datasets) and DBS (n=101, five datasets). We computed the functional connections between each lesion location and all other brain voxels using the resting state functional connectivity data from 1000 healthy participants (the human connectome). We found that lesions and brain stimulation sites most associated with depression severity were connected to a similar brain circuit.

Circuits independently derived from lesions, deep brain stimulation and transcranial magnetic stimulation were also similar, as were circuits derived from patients with major depression versus other diagnoses. Likewise, in an independent analysis in patients with Parkinson's disease, 29 lesions and 95 stimulation sites converged on a common brain circuit for motor symptoms of Parkinson's disease. We conclude that lesions, TMS and DBS map to common brain circuitry that may represent improved neurostimulation targets for depression or other neuropsychiatric diseases.

In Chapter 8, we apply lesion network mapping to epilepsy and test whether lesion-related epilepsy maps to a common brain network. We first studied lesion locations from patients with stroke-related epilepsy (n=76) and control lesions (n=625). Lesion locations were mapped to a common brain atlas and the brain network functionally connected to each lesion location was computed using the human connectome (n=1000). The functional connections associated with stroke-related epilepsy were identified. Generalizability to other lesion etiologies was assessed using four datasets with different lesion types (n=772). We then tested the therapeutic relevance of these connections using the outcome data from patients who received ANT-DBS for drug resistant focal epilepsy at our center (n=30). We found that lesion locations of stroke-related epilepsy map to a specific brain network defined by functional connectivity to nodes in the basal ganglia and cerebellum. Functional connectivity to these same nodes was associated with the risk of epilepsy across different lesion types and with therapeutic response to thalamic deep brain stimulation. We conclude that lesion-related epilepsy maps to a common brain network with therapeutic potential for neuromodulation.

The general discussion in **Chapter 9** provides an overview of the studies performed in this thesis, reflects on the methods used to map brain circuits, and provides a future perspective towards network-neuromodulation for epilepsy.

Impact paragraph

RELEVANCE

Epilepsy is one of the top 5 most common brain diseases and a major health problem, affecting more than 70 million people worldwide.[1] Persistent epileptic seizures carry serious neurological, cognitive, psychological and social consequences. On a societal level, epilepsy has significant economic implications due to chronic health care demands and lost productivity of work.[2] In addition to its high prevalence and burden, people living with epileptic seizures unfortunately still suffer from stigma, discrimination and even human rights violations which may lead to difficulties in education and employment (www.who.int). Consequently, epilepsy has a high medical, social and economic burden in our current society.

Most patients with epilepsy become seizure free with the first two antiepileptic drug regimens. However, for over 30% of patients, antiepileptic drugs do not result in seizure control and adding an additional drug only offers less than 5% additional probability of seizure control.[3] The burden of epilepsy is therefore highest among patients with uncontrolled seizures, also termed drug resistant epilepsy, accounting to 86% of all costs related to epilepsy in the United Stated (totalling $12.5 billion).[4] These numbers illustrate that we are still in need of an effective therapy control seizures in patients with drug resistant epilepsy.

Deep brain stimulation (DBS) of the anterior thalamus is a safe and potential treatment option for patients with drug resistant focal epilepsy, when epilepsy surgery or other less invasive neuromodulation strategies are not possible or failed to control seizures. DBS is thus a promising effective therapy for the most severely affected patients and may be a last resort for many patients. However, DBS response rates vary across patients and there is only a 50% probability that it will lead to 50% reduction in seizure frequency 1 year after surgery. Knowledge gaps on the predictors of response, optimal stimulation site and brain networks that are potentially crucial for achieving seizure control with DBS impede its widespread use as a standard neuromodulation therapy. With the aim of increasing the control of seizures by anterior thalamic DBS, we performed a series of animal, human and computational studies to identify the optimal stimulation site and brain networks that are crucial for achieving seizure control.

The main finding of this thesis is that there is an optimal stimulation site for seizure control in anterior thalamic DBS for drug resistant epilepsy and that these stimulation

sites are functionally connected to an intrinsic brain network that may play a causal role in lesion-related epilepsy.

TARGET AUDIENCE

The primary target audience will eventually be patients with severe epilepsy. The findings from this thesis could lead to better seizure control for patients with drug resistant epilepsy receiving anterior thalamic DBS and a better quality of life for patients and their family. Attaining seizure control is expected to result in patients being able to lead more normal lives, including a steady job, less dependency on relatives or healthcare workers, driving (when seizure free), and ultimately less risk of physical harm or sudden death in epilepsy. Effective treatment of patients with drug resistant epilepsy will also result in a marked decrease of the financial burden of epilepsy to our society, as productivity increases, and health care costs associated with uncontrolled seizures decrease.

However, the expected positive outcomes to patients and society may only be realized if our findings are *innovated* by the academic community, *adopted* by neuromodulation companies, and *implemented* by health care workers; constituting the other three target audiences of this thesis.

INNOVATION

In this thesis, we have contributed to the academic literature that studies effective stimulation sites in anterior thalamic DBS for drug resistant epilepsy. Our results were consistent with previously published smaller case series,[5,6] and innovative in three important ways: 1) we show that the junction between the mammillothalamic tract and anterior nucleus of the thalamus (ANT-MTT junction) is a reliable neuroanatomical target that can be visualized across different MRI field strengths, 2) we show that the stimulation sites (volume of activated tissue) of responders overlap at the ANT-MTT junction, and 3) we provide evidence and propose a role for modulation of fiber tracts in the efficacy of anterior thalamic DBS. In doing so, our study (among others) has inspired a retrospective analysis of the DBS lead locations implanted in the randomized controlled trial that led to FDA approval for anterior thalamic DBS (SANTE study),

which replicated our results.[7] In the short-term, we expect to see more replication studies of independent cohorts across the world and in the long-term, we hope that neurosurgeons and neurologists will adapt their surgical approach and DBS programming strategy to improve stimulation of this 'sweet-spot' for anterior thalamic DBS. Eventually we expect that identification of this optimal stimulation sites will lead to improved clinical outcome across patients and centers. One new insight gained from this research is that fiber tracts (or white matter) may have a role in the mechanism of action of anterior thalamic DBS, which warrants further studies using more sophisticated neuroimaging techniques that can visualize these fibers. In the future, this insight may even revive the use of several fiber tract targets historically used to treat epilepsy before the invention of modern neuromodulation devices. A second new insight is that intrinsic functional connectivity of the stimulation site is associated with clinical outcome after anterior thalamic DBS. This finding may seem surprising, but is in line with historical animal and human data showing that distant brain regions may have an effect on seizures, independent of where the seizures are starting in the brain (i.e. the seizure-onset zone).[8–12] We significantly extend this concept, by showing that lesions causing epilepsy and DBS sites treating epilepsy are functionally connected to a common intrinsic brain network, suggesting a role for a network distant from the seizure-onset zone in the cause and treatment of lesion-related epilepsy. We hope this finding leads to widespread testing by other academics into the role of this brain network in the cause and treatment of epilepsy.

Adoption

The findings of this thesis may also be relevant for neuromodulation companies such as Medtronic, NeuroPace, Boston Scientific and many others. Our results show that there is an optimal stimulation site and there are crucial network connections associated with increased seizure control after anterior thalamic DBS. By mapping increased seizure control to a brain region, the eventual template map of this region could be used to guide DBS surgery and programming. Neuromodulation companies are invested to deliver the best possible outcome for their patients, reduce side effects, shorten the time to therapeutic response, and streamline technology for clinicians to broaden adoption of the offered neuromodulation device. More specifically, Medtronic offers a software program, called Suretune (www.medtronic.com) that localizes the DBS lead in patient space and visualizes the lead location in respect to a thalamic atlas to assist postoperative programming by the neurologist. There are also open-access software tools available that

offer similar applications for research purposes, such as Lead-DBS (https://www.lead-dbs.org). The optimal stimulation site in this thesis may be converted into a region of interest (similar to an anatomical atlas) and transformed into patient space, to give the treating neurologist visual feedback of the optimal stimulation target to aim for during DBS programming. A similar approach can be taken using the optimal brain network connections identified in this thesis. A map of intrinsic connectivity to the basal ganglia and cerebellum could be derived from a normative connectome from healthy individuals, or even a patient specific connectome derived from functional MRI scans of that patient. This connectivity profile could be transformed into native space to assist the neurosurgeon and neurologist in modulating these connections with DBS. I hope and expect that widespread collaborations between engineers, clinicians, neuroimaging experts and medical device companies will adopt these findings and, in a wider framework, move the field toward network-neuromodulation for epilepsy.

Implementation

Knowledge obtained in this thesis has been and will be shared through paper contributions to the academic literature, presenting to scientific audiences in both Europe and the US, and inspiring new insights and studies in the field. We hope that the findings from this thesis will be picked up by both basic, translational and clinical researchers studying epilepsy and its treatment with neuromodulation. On the short term, the results of this thesis are relevant for neurosurgeons and neurologists treating drug resistant epilepsy with anterior thalamic DBS, who may use our stimulation site and intrinsic brain network to guide neuromodulation treatment in their daily clinical practice. These health care workers are also essential to independently validate and replicate our results across different centers and patient groups. I am currently collecting data around the world to test whether our identified intrinsic brain network associated with epilepsy may predict outcome in independent cohorts of anterior thalamic DBS. External replication is an important step towards testing whether this brain network may be used as an improved therapeutic target in randomized controlled clinical trials. Furthermore, the results of this thesis are relevant to brain stimulation neurologists, and to general and stroke neurologists treating patients with brain lesions. Brain lesions inherently increase the risk of epilepsy. Neurologists designing trials to test antiepileptogenic drugs may use our identified brain network to stratify patients into low and high risk for epilepsy. Using damage to our brain network as a selection criterion, one could enrich antiepileptogenic drug trials with patients at high risk of epilepsy.

Trials enriched with patients at high risk of epilepsy will drastically decrease the time to reach the specified inclusion numbers and eventually also the financial cost of such trials. In the long term, the findings of this thesis will need to be implemented in randomized clinical controlled trial designs that will be setup by health care workers specialized in stroke, epilepsy and neuromodulation.

While the findings of this thesis can already be used in the current daily clinical care of patients with anterior thalamic DBS, the new insight that 'intrinsic brain connectivity' may play a role in the cause and modulation of epilepsy may result in the largest scientific and social impact of this thesis. The current dogma in both the scientific and clinical arena of epilepsy and neuromodulation, is that epilepsy diagnosis and treatment should be focused on a patient's individual epilepsy network, not a common brain network across patients. The new insights from this thesis could therefore lead to a paradigm shift in how we understand the cause and perform the treatment of epilepsy. In the basic scientific community, studies that investigate whether intrinsic brain networks distant from the seizure focus may have a causal influence in epileptogenesis could open up new avenues. For example, one could design antiepileptic drug treatments or neuromodulation therapies that optimally modulate these distant brain networks. In the translational scientific community, studies that measure and perturb brain activity within these intrinsic brain networks may open up new treatment targets for neuromodulation. In the epilepsy centers, a focus away from mapping individual epilepsy network to common networks across patients may result in changes in how they work-up patients with drug resistant epilepsy to either treatment with epilepsy surgery or neuromodulation.

Overall, *innovation*, *adoption* and *implementation* of the findings described in this thesis may lead to a new way of '*mapping and zapping*' brain networks in epilepsy with substantial scientific, clinical and social impact.

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Biography

Frédéric L.W.V.J. Schaper was born in Heerlen on the 22nd of February 1990 and grew up in the town of Kerkrade in the far south-east of the Netherlands. He attended high school at College Rolduc, a medieval abbey located at the edge of Kerkrade. In 2008, he graduated from Rolduc and moved to Maastricht, the Netherlands to attend the bachelor program of Biomedicine (BSc). There, he became interested in Neuroscience, while listening to lectures on how brain stimulation could modulate specific human behaviours such as impulsivity, mania, laughter, or even movements; all with the switch of a button. As a research assistant and parallel to his bachelor's programme, Frédéric spent the next few years helping with brain stimulation experiments in the lab of Prof. Dr. Yasin Temel (neurosurgeon). For his bachelor internship, he spent 3 months as an Erasmus student in Samsun, a city in the east of Turkey, and studied cellular changes associated with Parkinson's disease. In 2011, he graduated from his Bachelor's and was accepted for the Physician-Scientist Research Master at Maastricht University, a dual degree programme with the goal of training students to become medical doctors and scientists (MD, MSc). For his senior clinical clerkship, Frédéric rotated in Neurology under the supervision of Dr. Rob P.W. Rouhl and Prof. Dr. Robert J. van Oostenbrugge (neurologists). He became fascinated by the multifaceted clinical presentation of seizures, a temporary excess of brain activity that (similar to brain stimulation) can evoke specific human behaviours. In 2015, he graduated *Cum Laude* (With High Honors) from his Master's and was awarded a Kootstra Talent Fellowship to start his PhD on '*deep brain stimulation for severe epilepsy*' in the lab of Prof. Dr. Yasin Temel. During his PhD, he presented at multiple (inter)national conferences and won several awards. In the last year of his PhD, he moved to Boston, U.S.A. for an 8-month PhD internship at the Berenson-Allen Center for Noninvasive Brain Stimulation. There, under the supervision of Dr. Michael D. Fox (brain stimulation neurologist), he learned to combine a '*wiring diagram of the human brain*' (the human connectome) with causal information from brain lesions and brain stimulation. For his postdoc, he re-joined the lab of Dr. Michael D. Fox in the newly founded Center for Brain Circuit Therapeutics at the Brigham and Women's Hospital of Harvard Medical School. Frédéric aims to become a brain stimulation neurologist and treat neurological diseases such as Parkinson's disease and epilepsy using (non)invasive brain stimulation.

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"Mischief Managed!"

Fred Weasley