1

A general introduction

1.1 Epilepsy – a disease once sacred

One of the major goals of fundamental brain research is to elucidate how groups of neurons communicate to generate behavior. To understand this we perform research in animals such as worms, flies, fish, mice and monkeys which in some cases is supported by computer simulations. We do so because our understanding of basic neurophysiology of a healthy brain is an important limiting factor when understanding mechanisms underlying neurological disorders and developing effective therapies for them. Epilepsy is one of these and is estimated to affect 1% of the population with 30% of patients having untreatable seizures despite optimal antiepileptic drugs [1]. It's a disease which is actually already known for at least 3000 years. One of the oldest accounts is a Babylonian tablet in the British Museum of London which describes one of the first reports with spectacular observations. This 'epilepsy tablet', which dates back to ~1000 B.C., describes features of epileptic attacks what we would recognize today as tonic-clonic, absence, Jacksonian, complex partial and even gelastic attacks of epilepsy [2]. The Babylonians considered epilepsy rightfully as abnormal, but described it as the result of demons in the body where each type of seizure was represented by a particular demon.

A different opinion about the cause of epilepsy other than a paranormal intrusion of the body was offered by the Greeks with the publication of 'on the Sacred Disease' (putatively written by one of the 'Fathers of Medicine'; Hippocrates), who regarded epilepsy as a physical disorder due to natural causes. It took however many centuries before the primitive concepts of making observations were replaced by rational and scientific notions, i.e., alike modern-day common practice in life science and medicine.

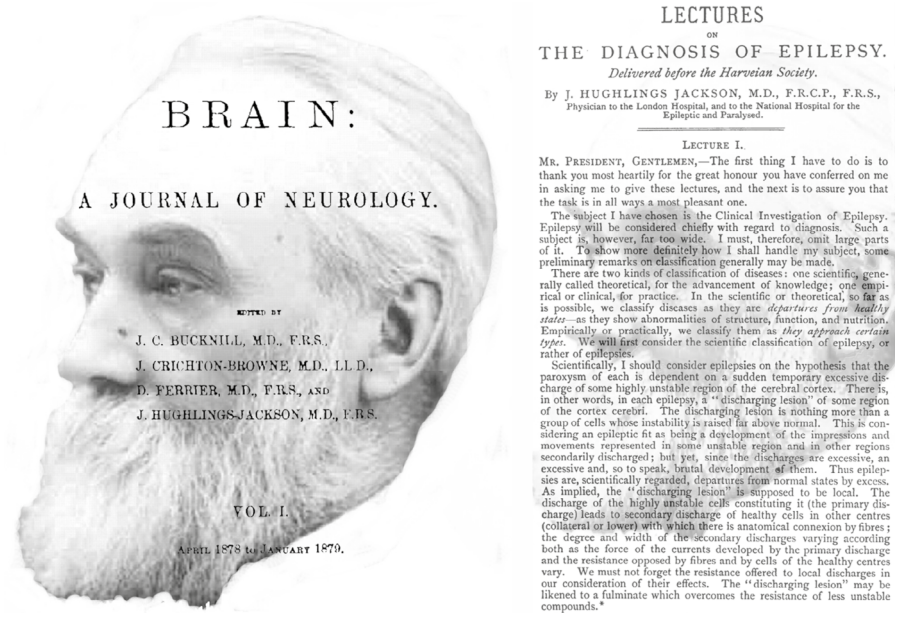
The start of this 'era' is probably best exemplified by the notifications and reports of John Hughlings Jackson (1835-1911). Widely recognized as one of the modern founding fathers regarding epilepsy research. One of the reasons for this is that he and his colleagues recognized the outcome of experiments being a matter of perspective and thus makes reporting and defining an essential task to do for any researcher. Furthermore is his 'founding father status' in part because of how remarkably accurate he was in his theories about epilepsy in a time where electroencephalographic recordings did not even exist.

1.2 Hughlings Jackson's; ahead of his time

Modern sophisticated neuro-diagnostic tools such as electroencephalic recordings and brain imaging were not available late 19th century. It was however the individual effort of many people who were responsible for the advance of knowledge in neurology. Under far from ideal circumstances people such as Nissl, Alzheimer, Golgi and Cajal performed

and achieved remarkable progress in the field of neurology and neuroanatomy. Regarding epilepsy there were many people who significantly contributed to the organized way of gaining knowledge about how to treat this disease.

Late summer 1909 there was a congress in Budapest attended by a few neurologists and physicians in the rising field of neurosurgery where the International League Against Epilepsy (ILAE) was launched. By that time Harvey Cushing was already pioneering in electrical stimulation during brain surgery since he just published a report in *Brain* (a journal founded by Hughlings Jackson starting with a lecture about the diagnosis of epilepsy, **Figure 1**) about electrical stimulation of the postcentral cortex evoking sensory responses and auras of focal attacks [3].



BRAIN:

A JOURNAL OF NEUROLOGY.

EDITED BY

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VOL. I.

APRIL 1878 to JANUARY 1879.

LECTURES
ON
THE DIAGNOSIS OF EPILEPSY.
Delivered before the Harveian Society.

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LECTURE I.

MR. PRESIDENT, GENTLEMEN,—The first thing I have to do is to thank you most heartily for the great honour you have conferred on me in asking me to give these lectures, and the next is to assure you that the task is in all ways a most pleasant one.

The subject I have chosen is the Clinical Investigation of Epilepsy. Epilepsy will be considered chiefly with regard to diagnosis. Such a subject is, however, far too wide. I must, therefore, omit large parts of it. To show more definitely how I shall handle my subject, some preliminary remarks on classification generally may be made.

There are two kinds of classification of diseases: one scientific, generally called theoretical, for the advancement of knowledge; one empirical or clinical, for practice. In the scientific or theoretical, so far as is possible, we classify diseases as they are *departures from healthy states*—as they show abnormalities of structure, function, and nutrition. Empirically or practically, we classify them as *they approach certain types*. We will first consider the scientific classification of epilepsy, or rather of epilepsies.

Scientifically, I should consider epilepsies on the hypothesis that the paroxysm of each is dependent on a sudden temporary excessive discharge of some highly unstable region of the cerebral cortex. There is, in other words, in each epilepsy, a "discharging lesion" of some region of the cortex cerebri. The discharging lesion is nothing more than a group of cells whose instability is raised far above normal. This is considering an epileptic fit as being a development of the impressions and movements represented in some unstable region and in other regions secondarily discharged; but yet, since the discharges are excessive, an excessive and, so to speak, brutal development of them. Thus epilepsies are, scientifically regarded, departures from normal states by excess. As implied, the "discharging lesion" is supposed to be local. The discharge of the highly unstable cells constituting it (the primary discharge) leads to secondary discharge of healthy cells in other centres (collateral or lower) with which there is anatomical connexion by fibres; the degree and width of the secondary discharges varying according both as the force of the currents developed by the primary discharge and the resistance opposed by fibres and by cells of the healthy centres vary. We must not forget the resistance offered to local discharges in our consideration of their effects. The "discharging lesion" may be likened to a fulminate which overcomes the resistance of less unstable compounds.*

Figure 1. First issue of *Brain* and its founding editor Hughlings Jackson (1835-1911).

Harvey Cushing and his colleagues (Sir Victor Horsley and later on Wilder Penfield) were among the first to use electrical stimulation to elicit electrical activity in the brain and monitor the behavioral readout.

Although Cushing is in many aspect a very important pioneering neurosurgeon in the 20th century it was Hughlings Jackson who laid the foundation for how we try to understand epilepsy nowadays.

In a series of landmark papers Hughlings Jackson published his observations on convulsions and seizures saying that there was an unstoppable force causing a sudden and temporary loss of nerve tissue function which was the underlying cause of epilepsy [4]. A short while longer he proceeded by saying that that 'this nerve-tissue should be considered as more unstable, over-ready and excitable; there is discharge too soon; its Time is shortened' [5] which were remarkable observations for that time. These clinical observations were enough for Hughlings Jackson to inform his colleague neurosurgeon dr. Horsley that it was probably a 'cortical irritation' causing focal attacks of epilepsy and he suggested to perform a craniotomy and remove it [6, 7]. Whether or not that was the last time for a neurologist to convince a neurosurgeon so easy to perform a procedure remains unclear. Nevertheless it was the beginning of a time where Horsley performed surgery on many patients with an encouraging result; his patients survived [8]. It is remarkable how the combination of local anesthesia and intraoperative cortical stimulation aiming for reproduction of a seizure was the essential of epilepsy surgery performed in a time when neuromodulation by electrical stimulation was at its infancy. It forms however the fundamentals by how we perform awake cranial surgery nowadays.

Not only was Hughlings Jackson a remarkable clinician, he also proved himself capable of fundamental research. He performed several experiments together with Charles E. Beevor observing thumb and finger responses in monkeys after electrical cerebral cortex stimulations. Parallel to this, Hughlings Jackson's colleague and former student, David Ferrier, started to experimentally proof the observations made by Hughlings Jackson. It was this combination which supported Hughlings Jackson in his observations and recommendations to perform surgeries on patients. The close translational collaboration between experimenters/students (Ferrier and Beevor), neurosurgeon (Horsley) and neurologist (Hughlings Jackson) still serves today as a crucial bench to bedside model for modern day epilepsy research and translational neuroscience in general.

1.3 Electrical stimulation – why and how?

Horsley and colleagues were using electrical stimulation knowing that the brain functions by electrical signals and that stimulation can evoke normal responses (like the thumb movements mentioned earlier) and abnormal responses (like the auras of focal attacks reported by Cushing). Probably they didn't foresee that precise and standardized electrical stimulation came on the rise a few decades later as a treatment for abnormal responses. The use of precise localization is nowadays called functional stereotactic surgery and many patients cannot benefit neuromodulation treatment without the landmark paper

introducing this. That moment is marked a few years after the first publications from Horsley and Cushing when neurologist dr. Spiegel and neurosurgeon dr. Wycis reported in the cross-discipline journal Science the creation of a stereotactic apparatus and its use in humans to perform ablative procedures [9].

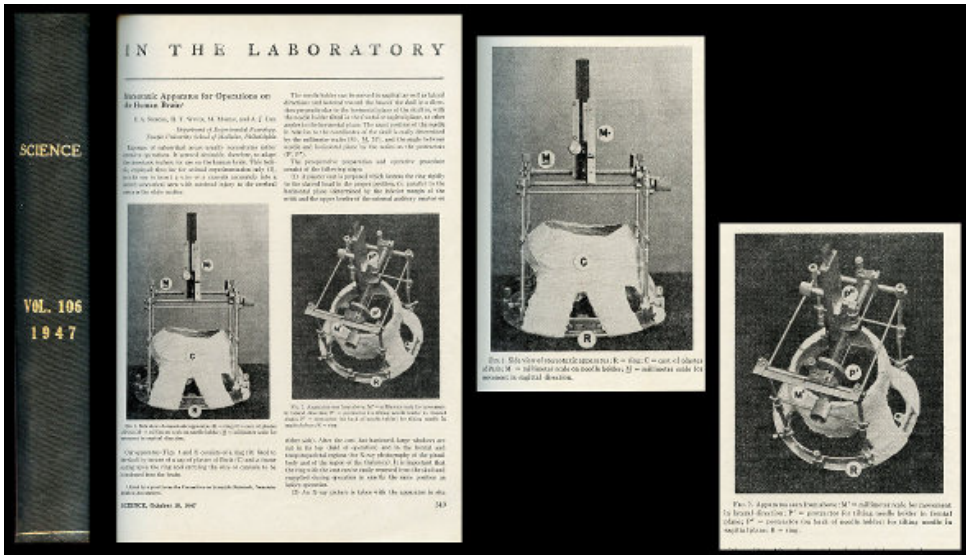

Figure 2. Image of the first publication of a stereotaxic apparatus for implantation of electrodes in the human brain in Science.

Many people fail to recognize this original publication as aiming to refine the rough methods used for performing e.g. lobotomies in psychiatric patients by replacing it for stereotactic procedures. Instead many think it is designed for treating movement disorders such as M. Huntington and Parkinson.

The fact that neuromodulation was indicated for treating psychiatric disorders and the pioneering work of Horsley, Cushing, Penfield and others in the identification of epileptic foci using electrical stimulation made it easy to hypothesize that these foci could be treated with the technique of electrode implantation and intermittent stimulation [10]. Subsequently therapeutic chronic stimulation in thalamus and cerebellum was introduced for patients having refractory epilepsy [11-13]. Over the years many trials have been performed with inconsistent results in the beginning. This was arguably due to unprecise localization methods and limited knowledge about physiology, mechanism of action and anatomy connections (**Chapter 3** of this thesis focuses further on these issues). Recently more and stronger evidence in larger studies came available indicating that neuromodulation

of thalamus or cortex can be an additional treatment for epilepsy patients since seizure reduction up to 68% has been reported after 5 years of follow-up [14, 15]. Fact remains that nowadays epilepsy is still an invalidating disease that leaves many patients without any adequate treatment options. This and proceeding knowledge are one of the reasons that my thesis (re)focuses on the question if cerebellar output can function as a remote site to control epilepsy. To understand this it is important to know the differences between normal and abnormal brain functioning.

1.4 Do oscillations occur in normal brain activity?

A lot of essential brain functions such as memory consolidation, navigation and sleep are based upon synchronized, rhythmic firing among smaller or larger neuronal cell populations; i.e. oscillations [16]. Neural oscillations can be seen as the rhythmic glowing of an army of fireflies where every firefly represents a brain cell. Different groups of fireflies and their frequency of lighting represent different oscillatory frequencies. Another example would be that neural oscillations can be seen as the many different ripples in a pool of water after a stone bouncing in.

Pioneering work on oscillations was published by Vladimir Práwdicz-Neminski from the Kiev University. His 1912 publication of an 'elektrocerebrogramm' in dogs pioneered recording electrical brain activity. He also identified two different rhythms what he referred to as 'waves of the first and second order', nowadays better known as alpha and beta waves. Práwdicz-Neminski was very likely not the first with his recordings (Richard Caton recorded already in 1875 electrical potentials of rabbit and monkeys) and a little later Hans Berger followed publishing an 'elektrenkephalogramm' recording in humans which subsequently changed into the English version 'Electroencephalogram' or EEG [17]. The first discovered and best-known frequency band is alpha activity (8 – 13 Hz); prominent during relaxed wakefulness or the awake resting-state in the absence of sensory inputs. Other frequency bands are delta (1 – 4 Hz), theta (4 – 8 Hz), beta (13 – 30 Hz) and gamma (30 – 70 Hz) (as reviewed by [18] (**Figure 3**).

All frequency bands have their specific properties and relations to brain functioning. Delta band frequencies are classically known to occur during deep sleep and appears to be controlled by cerebral cortex in the absence of sensory input. Theta frequency is important for working memory and emotional arousal and arises from cortical GABAergic interneurons impacting corticothalamic connectivity. Beta frequency is prominent during vigilance and attention and gamma frequency highlights feature integration involved in perception.

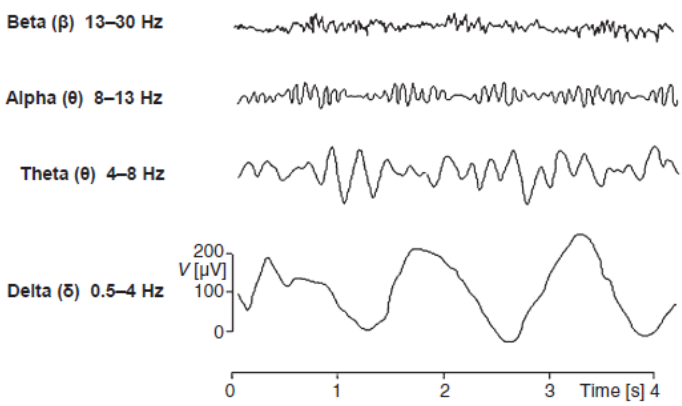

Figure 3. Main oscillation frequencies in EEG. (Constant 2012 et al.)

All of these frequency bands can be considered as global network processes and thus hard to attribute to one specific brain region. Cerebral cortices affect their downstream connective hubs such as thalamus, basal ganglia and others with these rhythms. These structures give feedback in return which can modify the main cerebral oscillation frequencies. To exemplify how natural oscillations enable different brain activity patterns I will explain this by using two recognizable brain activities; i.e. sleep and memory processes. Although the exact mechanism is far from elucidated in general it is likely that different stages of sleep coordinate the (re-)activation and distribution of other brain-region dependent memories [19]. Slow-wave sleep (using oscillatory frequencies < 1 Hz) are thought to re-activate memory traces of events whereas REM sleep (characterized by theta (4 – 8 Hz) oscillations) is thought to contribute to consolidation of these memory traces. This indicates that different brain oscillations have their own function in activating and synergizing different brain regions to accomplish their specific goals. Taken together, specific brain functions need different oscillatory patterns to establish a temporal and spatial relation with a well-defined event. More specific this means that establishing memory will likely fail or be significantly hampered if sleep patterns are lacking.

1.5 Sleep and epilepsy are(n't) the same

Not all oscillations are necessarily good oscillations. Numerous pathological oscillations are known to form the basis of neurological disorders. One of them is Alzheimer's disease (AD) which is associated with different than normal slow-wave activity in neocortex, thalamus and hippocampus [20]. Other examples are traumatic brain injury and epilepsy.

A common misconception about epilepsy is that seizures occur due to short-circuited brain activity. A more accurate description is that seizures are typically due to run-away excitation of brain network activity. I will now introduce how these conditions come about and will utilize the commonalities between oscillations in the healthy brain during sleep stages and in the epileptic brain during seizures. Using this frame-of-reference I hope to enhance the general understanding of the impact of cerebellar stimulations on generalized seizures.

Both sleep and epilepsy arise predominantly in networks where the thalamus forms a critical structure for generating normal and abnormal oscillations. *Thalamos* is the Greek word for innermost room or sleeping chamber which was used in the Archaic Greek house as a room connecting (in)directly the many other chambers and passages. Prior to modern neuroscience it was thought that thalamus acted as a reservoir where vital spirits were stored. Nowadays thalamus is generally recognized as the coordinating center for the flow of information between the senses and cerebral cortices, although recent findings expand this functionality extensively [21-23]. The reciprocal connectivity between the thalamus and cerebral cortex is crucial to generate flow of oscillations that encode, for instance, sensorimotor integration patterns that are essential for daily functioning. Feedforward and feedback connections within and between the thalamic and cortical areas ultimately lead to a dynamic regulation of oscillatory patterns that may synchronize distant thalamocortical networks. The amount of synchrony in groups of cells in the thalamic network seems to be crucial in determining whether normal or pathological oscillations occur. Normal synchronous oscillations occur, as explained earlier, during various behavioral states, e.g. sleep, but hypersynchronous discharges are associated with generalized epilepsy. Both normal and abnormal oscillations can be detected using EEG recordings (see **Figure 4**). An example rhythm that reflects normal activity of ensembles of thalamocortical cells are so-called sleep spindles whereas the pathological variant are Spike-Wave Discharges (SWDs). Although significantly different a close correlation can be found between sleep-spindles and SWDs (Fig. 4). One of the arguments for this is that we can assume that the circuit used for generating spindles is comparable with the circuit that generates absence seizures. Prominent in this is the finding that thalamic activity was in phase with the timing of spike-wave discharges in patients with absence epilepsy [24]. This was confirmed when thalamus and cortex were found firing together during spindles [25].

Moreover the generation of a pattern of sleep spindles or SWDs seems to critically depend on the amount of cells recruited to participate in the spindle sequences or SWDs [26-29].

Furthermore similar features such as being intermittent, lasting a few seconds and present throughout the cortex are soft arguments that absence seizures and spindles use the same circuit. Finally and most important is the proof that there is no need for rewiring

the thalamic circuit to initiate spike-wave discharges. This was shown many times when infusing GABAa receptor antagonists in thalamic slices changed spindle-oscillations in epileptiform oscillations [30-32].

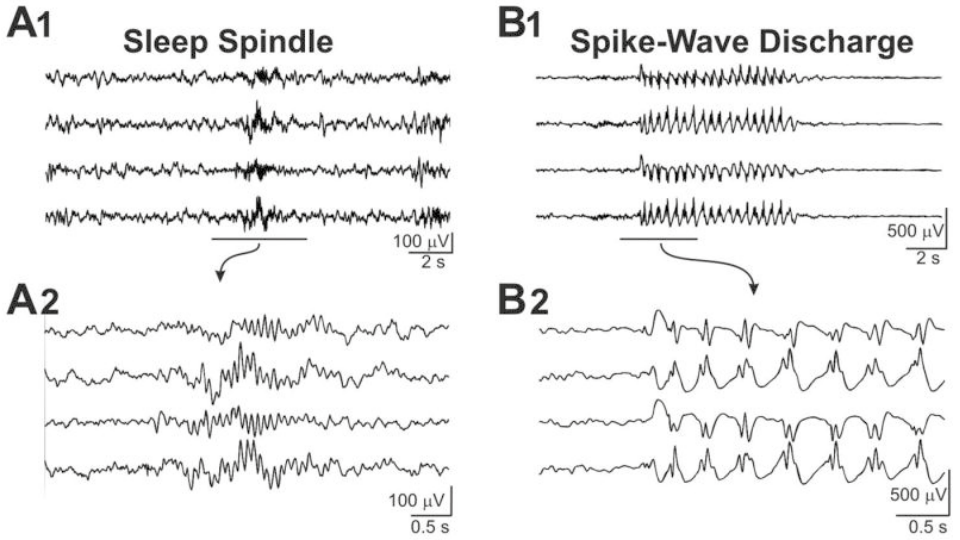

Figure 4. Cortical LFP recordings showing sleep and epileptic signals. (Beenhakker 2009)

Although the circuit might be the same it is far from clear what initiates such oscillations. Several groups suggested that cortical activity is responsible based in part on the observation that cortical cells start oscillating before cerebral EEG recordings follow [33-35]. And indeed terminating cortical 'foci' activity using lidocaine reduced the occurrence of SWDs. However, unpublished observations from our groups' cerebellar nuclei recordings also did show oscillatory firing prior to subsequent spike-wave discharges and upgrading its activity using gabazine completely abolished spike-wave discharge occurrence (**this thesis**).
Furthermore it was shown that the start of absence epilepsy in a genetic mouse mutant, i.e. *tottering,* coincided with impaired feedforward thalamocortical inhibition onto layer IV neurons [36]. While this can still be seen as the problem residing in the cortex another group revealed for several absence epilepsy animal models that compromised GABA uptake directly in the thalamus because of aberrant GABA transporter GAT-1 functioning resulted in both behavior and ECoG correlates of absence seizures [37]. And who else, if not Hughlings Jackson, reported about a patient exhibiting epilepsy when having cerebellar pathology [38]. This shows that the question if cerebral cortex is the main initiator of epileptic oscillations remains a very open one and is not for sure to be answered with a yes.

And if or not the cortex is the focus another interesting questions in the past and future of treating epilepsy is how to intervene in epileptic networks to regain control.

1.4 The thalamus as a hub between cerebellum and cerebrum

To know how to intervene in epileptic networks it is essential to know how thalamic neurons generate (spindle) oscillations (**Figure 5**). In the thalamus this occurs because a thalamic subdivision, i.e. reticular thalamus (RT), directs inhibitory feedback to another subdivision, i.e. dorsal thalamus relay nuclei (TRN).

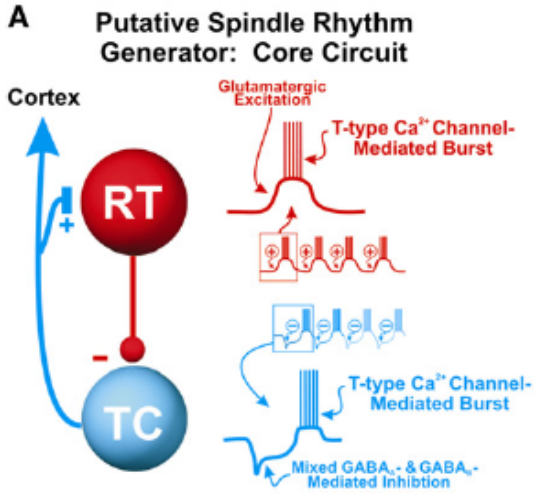

Figure 5. Schematic representation of thalamocortical interaction (Beenhakker 2009).

These relay nuclei receive excitatory and inhibitory input from other subcortical nuclei and send (at least in rodents) excitatory feedforward and feedback information to reticular thalamus and cerebral cortex. One of these subcortical nuclei is the cerebellum which influences TRN neurons with excitatory input. This mechanism is further introduced and explained in Box 3 in **Chapter 2.**

In principle, this back and forth excitation and inhibition between RT and TRN is the fundamental circuitry of a very important oscillation generator in the brain and provides support for the 'waxing and waning experience' most often seen in oscillations.

Over the years several brain targets for intervention in pathological oscillations underlying refractory epilepsy have been proposed [39]. Question remains if the cerebellum is well-positioned for intervention using its excitatory output predominantly on TRN neurons.

Therefore **Chapter 2** introduces the cerebellum as a potential candidate and serves as a kick-off for the rest of this thesis focusing on the question whether cerebellum can function as a remote control site to control thalamocortical (pathological) oscillations.

1.6 Aims of this dissertation

The aim of this thesis is to further elucidate neural mechanisms underlying the role of the cerebellum in generalized epilepsy and more specifically if the cerebellum can be used as a remote control site to influence thalamocortical networks in health and during epilepsy.
Following an introduction on electrical brain stimulation in general and fundamental brain processes at first I aimed to provide an overview in **Chapter 2** of commonly used stimulation targets. In this context we implemented cerebellar studies and propose several new stimulation strategies to maximize impact on thalamocortical networks.
This chapter raised the question which (optogenetic) research tools were best to study effects of directly impacting cerebellar neurons and its long ranging projections to downstream targets (**Chapter 3**).
After these introductory chapters we first aimed to question in **Chapter 4** to what extent modulation of simple and specifically complex spike activity (crucial identifiers of Purkinje cell activity; the sole output of cerebellar cortex) is related to Generalized Spike-and-Wave Discharges occurring in awake *tottering* mice and what this tells us about the involvement of the inferior olive. After identifying the upstream firing activity related to the occurrence of GSWDs I continue by investigating the role of Cerebellar Nuclei in two different generalized absence epilepsy models and if (optogenetic) short- and (pharmacologic) long-term modulation of firing activity can impact the occurrence of epileptic attacks (**Chapter 5**).
How and to what extent cerebellum can impact thalamic nuclei remains largely unknown. Therefore we aimed to elucidate this with an *in vitro* study (**Chapter 6**) in wildtype animals by demonstrating the effect of cerebellothalamic synapses.
Next we investigated what effect synchronizing cerebellar output has on thalamic nuclei in an in vivo mouse model of absence epilepsy. Using the results of this chapter we come to a candidate mechanism underlying cerebellum reestablishing control in thalamocortical networks (**Chapter 7**).
Finally I analyze if cerebellum is capable of stopping generalized epilepsy in another much more severe generalized epilepsy mouse model, i.e. Dravet mouse (**Chapter 8**).