Seizures, spreading depolarizations and sudden death

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Chapter 1

General introduction

GENERAL INTRODUCTION

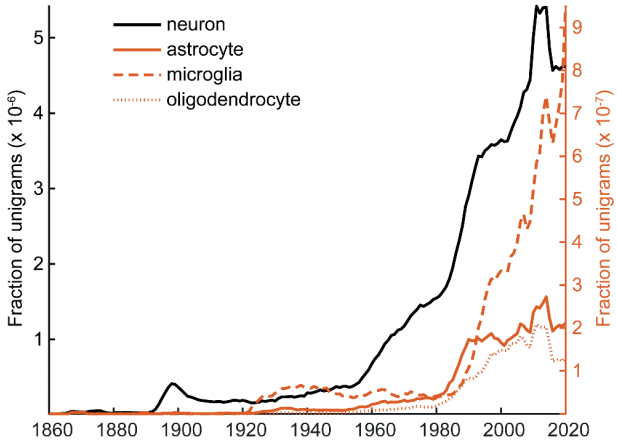
The brain and its episodic disorders

The structure of the brain and the dynamics it produces to drive thought and behavior are complex. Naturally, brain dynamics – often studied by the electrical fields they produce – require time to be expressed, and over time these dynamics show recurring patterns, or *periodicity*. This periodicity has been hypothesized to allow the brain to guide behaviors and anticipate perturbations, in effect functioning as a "foretelling device".[1] To achieve this, recurring patterns produced by oscillators far removed from one another in the brain need to synchronize. The architecture required to produce such synchronization however may result in an inherent susceptibility to episodic neurological disorders. These disorders are characterized by recurring changes in brain dynamics that cause episodes of neurological dysfunction. Two episodic neurological disorders that are associated with particularly dramatic changes in electrical fields are epilepsy and migraine with aura, which will be the focus of this thesis. Paradoxically, the same architecture that allows hard-to-predict epileptic seizures and migraine episodes to occur, is believed to allow the brain to function as a "foretelling device".

The structure of the brain and spinal cord (together referred to as the central nervous system) was at first considered a continuum – that is, it was believed to lack membrane boundaries. Since the late 19th and early 20th century, the "neuron doctrine" became the dominant theory, dictating that the central nervous system is composed of individual cells.[2] Although neurons have received most attention in the literature, glial cells – which support neuronal functioning and include astrocytes, oligodendrocytes and microglia – have become of growing interest later over the course of the 20th and 21st century (Figure 1).

A neuron may be conceptualized as a battery: its plasma membrane is polarized, which is brought about by an unequal distribution of cations and anions on either side. Similar to a battery, this chemical energy can be "utilized" by the neuron to generate electric potentials or action potentials, which are considered the dominant mode of neuronal communication. The neuronal plasma membrane is more permeable to K^+ than to other cations, resulting in a net outflow of K^+ and hence a negative membrane potential. During an action potential, the membrane is depolarized by small ion fluxes through opening of specific (mainly Na^+) ion channels. Repolarization is mainly achieved by opening of K^+ channels, allowing even more outflow of K^+. Although action potentials are an efficient means of communications by having relatively little effect on ion concentrations in the bulk intra- and extracellular space,[3] this efficiency relies on fast recovery of these ion distributions. This is mainly achieved by the Na^+/K^+ ATPase pump, which transports Na^+ out and K^+ into neurons, with the chief aim to maintain a polarized membrane (i.e. excitability). In addition, to control the (gradual) buildup of extracellular K^+ due to neuronal activity, Na^+/K^+ ATPase pumps in glial cells are required. By opposing the concentration gradients of these cations, this process costs approximately 50% of the total energy consumed by the brain.[4]

FIGURE 1. Trends in usage of the term *neuron* and three subtypes of glial cells from English sources contained in the Google Books corpus.



Note that the term *neuron* increased in frequency from the late 19th century onward, while the other terms followed later. Source: Google Books Ngram Viewer.

Excessive neuronal activity, seizures and spreading depolarizations

These electrochemical characteristics underlying membrane potential changes allow neurons to "fire" action potentials at high metabolic efficiency. A potential downside of this high efficiency is that neurons can become excessively activated and, by recruiting sufficient neurons through synchronization, produce *seizures*.[5] Seizures can be induced in a large variety of experimental (animal) models.[6] Seizures that occur in humans and in these models show similar features and are therefore considered naturally inherent to brain tissue.[7]

Almost 80 years ago, the pioneering Brazilian neuroscientist Aristides Leão attempted to characterize the electrical activity associated with cortical seizures in rabbits. When creating conditions of high excitability in the cortex, he surprisingly found a lasting reduction in spontaneous neuronal activity.[8] This decrease in activity was associated with a slow negative voltage shift in the recordings, which he could replicate by occlusion of the major brain arteries.[9] Later studies showed that this phenomenon was caused by sustained depolarization of neurons,[10] resulting from a profound decrease in ion gradients across their membrane.[11] This sustained depolarization propagated slowly through brain tissue, at a rate of a few millimeters per minute, and is therefore now referred to as a *spreading depolarization* (SD). The lasting reduction in spontaneous activity that was already observed by Leão was termed *spreading depression*,[8] that is initiated by the sustained depolarization exceeding the inactivation threshold of action potential-generating membrane channels.[12, 13] These characteristics of SD are fundamentally different from those of a seizure: in

general, seizures propagate at much faster rates throughout the brain, while neurons affected by it maintain their ability to fire action potentials. In keeping with the neuron-battery analogy: while a seizure-state is equivalent to a battery discharging in tandem, an SD-state corresponds to it being short-circuited.[14]

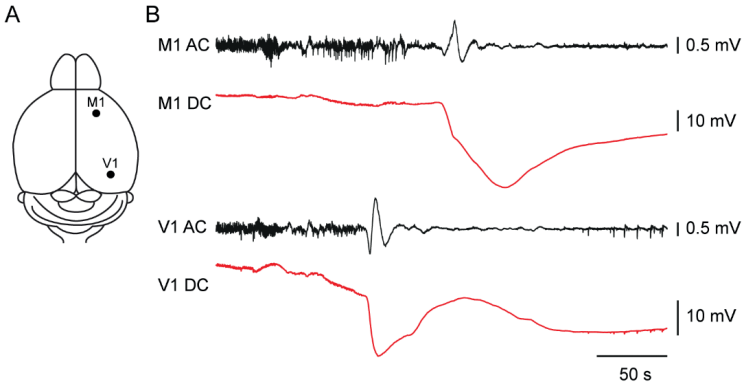
Despite these fundamental differences, both seizures and SDs result from conditions of high neuronal excitability. Similar factors promote seizures and SD, including high concentrations of K^+ and glutamate receptor agonists, low concentrations of Mg^{2+}, and electrical stimulation.[6, 15, 16] In addition, seizures and SDs have been observed in close association under experimental conditions,[17-20] as well as in the human brain for instance following trauma.[21] The deleterious effects of seizures and SDs on functioning of neuronal populations underscore the need to understand the mechanisms of these phenomena in episodic neurological disorders. In epilepsy and migraine with aura, the role of seizures and SD, respectively, are already well-established. Therefore, the remaining paragraphs of this introduction outline the involvement of seizures and SD in the pathophysiology of these two common episodic neurological disorders.

Spreading depolarizations in migraine with aura and epilepsy

Hyperexcitability within neuronal populations may result in SD that, in contrast to seizures, can only be appreciated using direct current (DC) coupled recordings,[22] which reliably capture the "infraslow" (<0.1 Hz) nature of the SD (Figure 2).

Spreading depolarizations are considered a very relevant aspect in the pathophysiology of migraine, as it is believed to be the underlying substrate of the aura phase.[23] Migraine is characterized by recurrent episodes of severe unilateral headache accompanied by nausea, photophobia and phonophobia.[24] Two main types of migraine are distinguished: migraine with aura, and migraine without aura.[24] The term "aura" refers to focal symptoms that are characteristically of visual nature, but may involve other sensory, phatic, motor or brainstem functions. These symptoms often show a characteristic spreading pattern, such as a scintillating scotoma that slowly expands within a visual field. SD was hypothesized to be the underlying cause of the aura symptoms already soon after its discovery,[25] and this hypothesis became (albeit many years later) widely accepted.[26, 27] Experimental evidence indicates that SD can activate trigeminal nociceptive pathways, potentially producing migraine headache.[28] As such, understanding the origin of SD in migraine is believed to allow improvement of migraine treatment.

FIGURE 2. DC recordings are required to reliably detect SD.



Example of a spontaneous SD recorded in a mouse model of familial hemiplegic type 2, detected by microelectrodes implanted in the primary motor (M1) and visual (V1) cortex **(A)**. Simultaneous alternating current (AC, bandpass filtered at 0.05 – 500 Hz) and DC (lowpass filtered at 500 Hz) recordings at these locations show slow potential changes during SD **(B)**. DC recordings show the magnitude of the depolarization that is lost in the AC recordings, despite the relatively wide bandpass filter settings. Note the suppression of AC signal following SD, representing the spreading depression. Source: Jansen NA et al., Spontaneous spreading depolarizations originate subcortically in a novel mouse model of familial hemiplegic migraine type 2, Neurobiology of Disease 2024;202:106714. Image reproduced with permission of the rights holder, Elsevier.

Experimental models of spreading depolarizations

An SD is an all-or-none depolarizing wave that propagates through gray matter.[15] The fact that it is an all-or-none phenomenon can be explained by the net cation flux across the neuronal membrane that turns persistently inward.[13] This inward current is generated by voltage-gated channels and/or increased extracellular K^+. SD can be initiated by depolarization of neurons via two major mechanisms: activation of neuronal voltage-gated Na^+ and/or Ca^{2+} channels, or inactivation of the Na^+/K^+ ATPase pump that is present on both neurons and astrocytes.[15, 29] Experimental stimuli that induce SD via (in)direct involvement of voltage-gated channels include chemical/pharmacological (with agents such as KCl, glutamate or N-methyl-D-aspartate (NMDA) receptor agonists), electrical or mechanical stimuli.[15, 30] On the other hand, experimental ischemia and/or hypoxia may induce SD via impairment of the Na^+/K^+ pump, which provides a mechanism for the observation of SD and the associated neuronal suppression in stroke.[14, 31] Although these experimental studies have informed us on diverse potential mechanisms of SD, they fail to faithfully model migraine-related SD, since this would require a model in which SDs occur spontaneously.

Genetic models of migraine and neuronal hyperexcitability

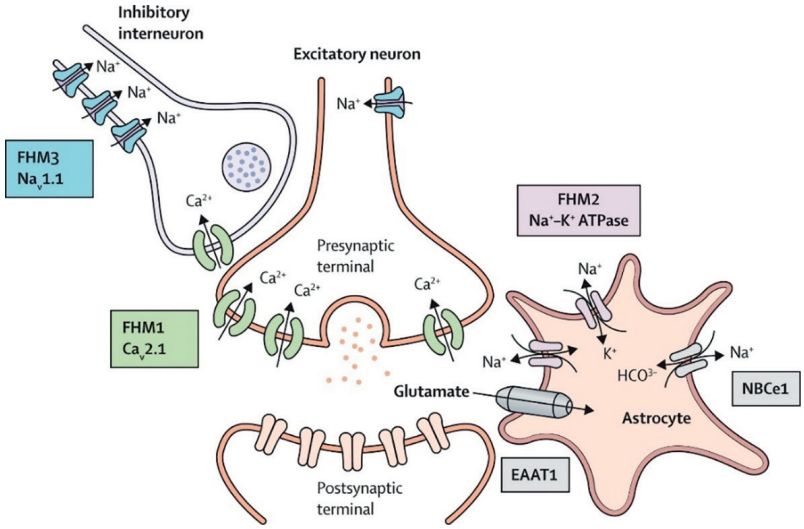
Relatives of migraine patients have an increased risk for the same disorder.[32] Whereas polymorphisms in many different genes in concert with environmental factors contribute to common forms of migraine, mutations in single genes underlie forms of familial hemiplegic migraine (FHM), a

subtype of migraine characterized by particularly severe aura, which may include hemiparesis, decreased consciousness, and coma.[33, 34] In the majority of hemiplegic migraine cases, a mutation in one of three genes is found: *CACNA1A* (FHM1), which encodes the pore-forming α1 subunit of $Ca_V2.1$ voltage-gated calcium channels expressed in neurons, *ATP1A2* (FHM2), which encodes the α2 subunit of the Na^+/K^+ ATPase pump, which is almost exclusively expressed in astrocytes in the adult brain, and *SCN1A* (FHM3), which encodes the α1 subunit of voltage-gated $Na_V1.1$ sodium channels expressed in neurons. The discovery of these pathogenic mutations has allowed the translational study of mechanisms underlying migraine-associated symptoms in model systems.[35] For all three FHM subtypes, specific mutations have been found in patients presenting both with migraine and epilepsy,[34, 36] providing a genetic target for research on these often comorbid disorders. In FHM1, the *CACNA1A* S218L missense mutation has been reported to cause both hemiplegic migraine and (lethal) seizures.[37] In FHM2, certain missense mutations in the *ATP1A2* gene can result in hemiplegic migraine and childhood epilepsy.[38] For FHM3, several missense mutations in *SCN1A* have been reported to cause childhood epilepsy,[39] in addition to hemiplegic migraine. Most importantly, increased susceptibility to experimentally induced cortical SD has been demonstrated in mouse models of FHM1[40, 41] and FHM2 (e.g.[42]). Such findings are attributed to enhanced neuronal excitability, albeit via different mechanisms (Figure 3). Similarly, common migraine is generally considered a condition characterized by hyperexcitability, which it shares with epilepsy. [43]

Why model spontaneous spreading depolarizations?

Similar to seizures in epilepsy, SD may be considered a paroxysmal manifestation of neuronal hyperexcitability underlying the aura phase in migraine. Yet, whereas spontaneous seizures have been widely reported in animal models of epilepsy, spontaneous SDs have not been reported in animal models of migraine. That this is a relevant shortcoming may be appreciated by reviewing recent progress in the field of epilepsy research. For example, inhibitory activity was found to be paradoxically increased prior to seizures in various epilepsy models,[44-46] which was also found in a model of Dravet syndrome (DS)[47], a severe epilepsy syndrome that in a majority of cases is caused by a mutation in the *SCN1A* gene. This illustrates that a theoretical framework that may provide a convincing explanation for hyperexcitability (in the case of DS, a decrease in GABAergic inhibition by *SCN1A*-encoded $Na_V1.1$ loss-of-function) may not be sufficient to explain the initiation of a seizure. Similarly, the initiation of an SD may not be accurately explained by enhanced release or impaired uptake of glutamate and/or K^+. Studying the conditions prior to spontaneous SD events could first of all allow a better understanding of how attacks of migraine (in particular those preceded by an aura) initiate. Second, it could help us understand why hyperexcitability sometimes results in seizures, but not in SD, and *vice versa*. And finally, the apparently intricate relation between seizures and SD could be better understood.

FIGURE 3. Affected proteins underlying neuronal hyperexcitability at the glutamatergic and GABAergic synapse in three FHM subtypes.



Source: Russell MB et al., Sporadic and familial hemiplegic migraine: pathophysiological mechanisms, clinical characteristics, diagnosis, and management, The Lancet Neurology 2011;10:457-470. Image reproduced with permission of the rights holder, The Lancet Neurology.

Spreading depolarizations in epilepsy

In its first description, SD-related spreading depression was already associated with seizure activity.[8] Since then, various studies have reported SD following induction of seizure activity in animal models,[18, 48-52] as well as following seizures in patients with cortical damage.[21] In addition, SD may occur following spontaneous seizures, as was shown for a DS mouse model.[53] It is important to note that although results from intracranial alternating current (AC) recordings are usually described in epilepsy animal models, direct current (DC) recordings much more rarely are. In addition, technical difficulties in acquiring a stable DC signal from scalp-EEG may explain the current lack of clinical evidence for seizure-related SD in epilepsy patients. It is thus very well possible that SD is a ubiquitous phenomenon in the seizing brain. The fact that SD is followed by neuronal inactivity is of potential benefit in this regard, since it may promote termination of seizure activity, although this has only been tested for chemically-induced prolonged seizure activity.[54] However, SD could be associated with adverse outcome if vital functions, that are otherwise preserved in the seizing brain, may become affected by it. A lethal consequence of such adverse outcome is *Sudden Unexpected Death in Epilepsy* (SUDEP).

Sudden Unexpected Death in Epilepsy

SUDEP is a post-mortem diagnosis defined as an unexpected non-traumatic and non-drowning death of a patient with epilepsy, in the absence of a structural or toxicological cause of death.[55] SUDEP can be diagnosed regardless of the presence of a seizure immediately preceding death, but prolonged seizures lasting ≥30 minutes are regarded as *status epilepticus*, an exclusion criterion for SUDEP.[56] The incidence rate of SUDEP has been established at 1.16 per 1,000 epilepsy patients,[57] but this is likely an underestimation: SUDEP reports mostly rely on death certificates, and incidence rates were found to be higher when medical charts and autopsy reports were available and taken into consideration.[58] Occurrence of generalized tonic-clonic seizures is the most important SUDEP risk factor, and thus development of treatment strategies to limit such seizures is considered to contribute to mitigating SUDEP risk.[59]

Mechanisms of Sudden Unexpected Death in Epilepsy

In the field of forensic medicine, the *cause* of death is distinguished from the *mechanism* of death. Whereas the first entails the disease, trauma or substance causing death, the latter entails the physiological sequence that results in death. In that sense, the knowledge gap between the cause and mechanism of death is substantial for SUDEP. Filling this gap is complicated by the fact that (1) SUDEP is rarely witnessed or recorded, and (2) measurement modalities are limited.

To address the former problem, studies have relied on data from non-lethal seizures to infer hypotheses on SUDEP mechanisms. Since cardiac arrythmias and changes in blood pressure are relatively common during seizures, failure of cardiac function was initially considered as the primary mechanism for SUDEP.[60] Later, a study that included a (relatively) large collection of SUDEP recordings – resulting from a collaborative effort involving epilepsy monitoring units around the world – collated electroencephalogram (EEG), respiratory and electrocardiogram (ECG) activity from 16 definite SUDEP cases.[61] Two important findings emerged from this study: (1) most SUDEP cases had proven seizure activity immediately prior to death, and (2) terminal apnea preceded cardiac arrest in all patients with sufficient quality cardiorespiratory recordings. A later study suggested postictal apnea as a promising biomarker for SUDEP risk.[62] Although seizure activity may not always be evident prior to SUDEP,[63] these recordings favor respiratory failure over cardiac arrythmia/arrest as a primary SUDEP mechanism.

Nevertheless, clinical SUDEP studies are severely limited by the measurement modalities employed, which is inherent to their retrospective nature. Development of preclinical models of SUDEP may help address this problem, since it allows measurements that are ethically or practically difficult to obtain clinically. Although in various models induced seizures can be lethal, animal models should ideally present spontaneous seizures to reproduce clinical SUDEP.[64]

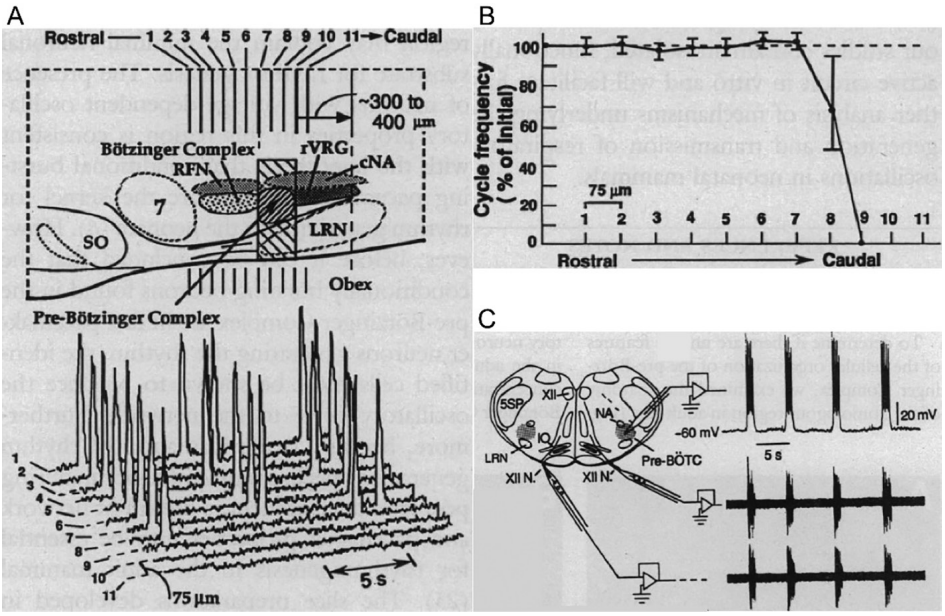
Sudden Death in Epilepsy: a role for spreading depolarizations?

Various animal models have been developed that display fatal and non-fatal seizures. The DS mouse model is interesting in this regard since clinical DS is associated with an unusually high incidence

of seizure-related mortality and SUDEP.[65, 66] DS mice display a SUDEP phenotype that was initially explained by seizure-related severe bradycardia.[67] However, later this was refuted when parallel recordings of respiratory activity showed that apnea preceded bradycardia in DS mice.[68]

Although the ultimate "effector" of SUDEP may have shifted from cardiac to respiratory arrest, the effectual trigger is unknown. A focus on cardiac mechanisms and findings of mutated genes that are expressed in both brain and heart, explains why terms such as "neuro-cardiac channelopathies"[69] and "arrhythmogenic epilepsy"[70] have emerged that propose a mechanism for SUDEP by suggesting a predilection to cardiac arrythmias exacerbated by seizures. However, DS mice in which disruption the *Scn1a* gene was limited to brain tissue could still display fatal seizures.[67] Similarly, disruption of *Kcna1* – which was proposed to increase SUDEP risk by yielding a greater risk for cardiac arrythmias[71] – could still result in fatal seizures when expressed only in brain tissue.[72] Thus, seizure-related failure of neuronal control over vital functions is of increasing interest for SUDEP research.

FIGURE 4. The pre-Bötzinger complex is critical for respiratory rhythmogenesis.



(A) Sagittal view of the rat medulla showing the level of serial microsections performed in a rostral to caudal direction (numbered) and their corresponding effect on breathing rhythm as recorded from phrenic motoneurons from C4 spinal ventral roots. Note the disruption (at location 8) and cessation (from location 9) of breathing rhythm, also shown in **(B)**. **(C)** Recordings in a thick medullary slice that include the pre-Bötzinger complex show respiratory rhythmogenesis, measured by motor output from the nucleus hypoglossus (XII N.). Source: Smith JC et al. Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals, Science 1991;254:726-729. Image reproduced with permission of the rights holder, The American Association for the Advancement of Science.

Whereas the (extracranial) sinoatrial node is the pacemaker of the cardiac rhythm, an intracranial structure is required for respiratory rhythmogenesis: the brainstem ventral respiratory column.[73, 74] At the core of this "central pattern generator" lies the pre-Bötzinger complex, which is both necessary and sufficient to produce the inspiratory phase of the breathing cycle (**Figure 4**).[74, 75] Disturbance of the rhythmic bursting of this small population of neurons may have disastrous consequences. Indeed, suppression of its glutamatergic neurons can induce fatal apnea in anaesthetized rats.[76] Intriguingly, also *awake* rats did not resume breathing up to the point of severe hypoxia that required mechanical ventilation, indicating that also voluntary breathing control was completely blocked.[76]

The brainstem is crucially involved in "low-level" control of cardiorespiratory function. Seizures may affect brainstem function by recruiting local neurons, and/or by the release of neuromodulators. Although obtaining local electrophysiology from brainstem structures is practically constrained in humans, blood flow in brainstem regions is increased during tonic-clonic seizures.[77] Interestingly, clonic seizure components require forebrain circuitry, but brainstem circuitry is sufficient to produce the tonic component.[78, 79] The tonic seizure component is strongly associated with post-ictal EEG suppression,[80] a proposed SUDEP risk factor,[81] and monitored SUDEP cases all showed a tonic-clonic seizure prior to death in another study.[61] As such, spread of seizures – and seizure-associated SD – to vital brainstem areas may contribute to SUDEP.

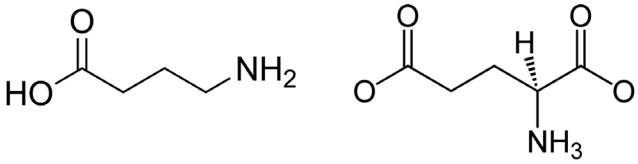
Epileptogenesis and the origin of seizures in epilepsy

Epilepsy (from ἐπιλαμβανω, "to seize") is characterized by the propensity of brain areas to spontaneously generate seizures.[82] Seizures that are restricted to a limited area of the brain are referred to as *focal* seizures, whereas seizures that involve large, bilateral, areas of the brain are referred to as *bilateral tonic-clonic* (in case of focal onset) or *generalized* (in the absence of focal onset) seizures.[83] The process by which the healthy brain develops into one that generates spontaneous seizures is termed *epileptogenesis.*[84]

The study of epileptogenesis is a relatively recent affair. The importance of a proper understanding of why and how epilepsy develops is reflected in the limited efficacy of treatments in a large proportion of patients up until recently: approximately one-third of epilepsy patients have seizures that are poorly controlled by medication,[85] a proportion that has barely changed in almost 50 years.[86, 87] Classical anticonvulsive drugs are suitable for the termination and prevention of seizures, but are ineffective at preventing epileptogenesis,[88] which may explain the lack of improvement in treatment efficacy. A more thorough understanding of epileptogenesis is thus critical to advance epilepsy treatment in coming years.

Traditionally, epilepsy is regarded as a brain disorder resulting from an imbalance between excitatory and inhibitory neurotransmission.[89] The structurally very similar molecules glutamate and γ-aminobutyric acid (GABA; Figure 5), which are respectively excitatory and inhibitory neurotransmitters, mediate the majority of neuronal synaptic communication.[90] Furthermore, removal of these neurotransmitters from the extracellular space by astrocytes is essential for effective synaptic transmission.[91]

FIGURE 5. The chemical structure of GABA (left) and (L-)glutamate (right).



Inhibitory GABAergic neurons are vastly outnumbered by excitatory glutamatergic neurons as it is estimated that only ~15-20% of cortical neurons are GABAergic.[92, 93] Rather than simply opposing the effects of excitatory neurotransmission, inhibitory neurotransmission controls the transfer of information.[90] This is classically illustrated by the research of Charles Sherrington, who observed that a stimulus that induces muscle contraction in parallel inhibits its antagonist muscle.[94] Since cortical excitatory and inhibitory neurons are reciprocally connected, increases in excitatory and inhibitory neurotransmission occur in concert, with changes in inhibitory neuronal function causing pronounced effects on a large number of connected excitatory neurons.[95] Selectively decreasing inhibitory function by specific manipulations therefore results in pathological activity such as seizures.[96] Inhibitory dysfunction is considered importantly implicated in the mechanisms underlying epileptogenesis and seizure generation.[97]

Inhibitory dysfunction in epileptogenesis and epilepsy

Epilepsy can be *acquired*, i.e. caused by an injury in a healthy brain, or *genetic*, i.e. resulting from a genetic mutation. The most common acquired epilepsy in adults is temporal lobe epilepsy (TLE), in which seizures originate from the temporal lobe. It is believed that a precipitating factor – such as a prolonged initial seizure, infection, ischemia, head trauma or a tumor – is the underlying trigger for the development of this epilepsy syndrome. Mesial temporal sclerosis often develops, in which part of the temporal lobe, in particular the hippocampus, is affected by gliosis and atrophy. Both the electroclinical phenotype of TLE and the associated mesial temporal sclerosis have been replicated in various animal models.[88] In such animal models, loss of hippocampal somatostatin-positive GABAergic interneurons is a consistent finding.[98, 99] Notably, this finding has been confirmed in the hippocampus of TLE patients,[100] suggesting impaired inhibition as a mechanism for epileptogenesis in TLE.

Modeling genetic epilepsy syndromes provides another means to study epileptogenesis. Genetic manipulations allow us to examine whether inhibitory dysfunction is necessary and/or sufficient (that is, without the presence of another precipitating factor) to cause epilepsy. For example, genetic modification of a gene involved in regulating the development of cortical and hippocampal interneurons resulted in impaired inhibition and epilepsy in mice.[101] Genes that are predominantly

or specifically expressed in inhibitory interneurons and affect their excitability, which include various genes encoding voltage-gated channels, have been implicated in epilepsy syndromes. Disruption of *KCNA1*, a gene encoding a voltage-gated potassium channel that importantly modulates the excitability of parvalbumin-positive (PV) GABAergic interneurons,[102] results in epilepsy in mice[103] and patients.[104] On the other hand, recruiting PV interneurons using optogenetic activation effectively terminated seizures in a TLE mouse model.[105] Studying how modulation of GABAergic interneuron excitability affects network activity and seizure susceptibility may thus allow a better understanding of epileptogenesis.

Inhibitory dysfunction in Dravet syndrome

Dravet syndrome (DS) is a severe epilepsy syndrome in which inhibitory dysfunction has been particularly well described. Seizures usually initiate within the first year of life,[106] and are followed by cognitive and behavioral deficits later during development.[107] In the majority of cases, a loss-of-function mutation is found in the *SCN1A* gene.[108, 109] This gene encodes the pore-forming α1 subunit of voltage-gated sodium channel type 1 ($Na_V1.1$). In the hippocampus and cortex, $Na_V1.1$ expression appears restricted to PV GABAergic interneurons.[110] Since Na_V channels are critical for the initiation and propagation of action potentials, $Na_V1.1$ loss of function would be expected to specifically result in hypoexcitability of GABAergic interneurons, which indeed was demonstrated for hippocampal interneurons.[111] Decreased versus intact excitability in inhibitory compared to excitatory neuronal populations, respectively, have since been demonstrated in the hippocampus and cortex in DS mouse models.[112-114] These data suggest that network hyperexcitability and seizure activity in DS result from impaired inhibitory functioning. This was further supported by evidence showing that an interneuron-specific knockout of *Scn1a* resulted in spontaneous seizures.[115] In fact, such interneuron-specific knockout may result in a more severe phenotype than observed in mice with global knockout of *Scn1a*, since additional deletion of $Na_V1.1$ in excitatory neurons ameliorated the DS-related phenotype.[116]

Yet, loss-of-function mutations in *SCN1A* do not always result in the severe phenotype associated with DS. As an example, the same *SCN1A* mutation was associated with a mild epilepsy phenotype (without developmental delay) in one family[117] but with DS in another.[118] In addition, carriers without any symptoms were found in both families. Hence, the genotype does not necessarily predict the phenotype. In order to improve disease prediction and allow early treatment, a better understanding of how inhibitory dysfunction may affect network dynamics and promote epileptogenesis in DS is required.

Signatures of inhibitory functioning in local field potential

Neuronal excitation and inhibition are "as thick as thieves", as in, both are recruited during evoked and spontaneous activity, resulting in patterns of feed-forward and feed-back inhibition. In contrast to the many cortical excitatory neurons that have axons projecting to distant brain areas, the majority of inhibitory neurons form local synapses.[95] As a consequence of reciprocal connections

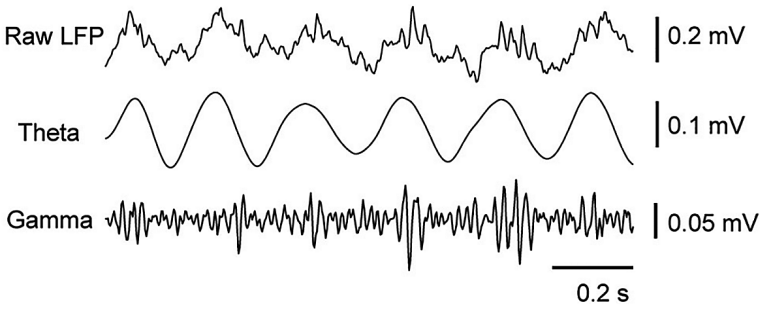
within anatomically well-organized networks, neuronal networks composed of excitatory and inhibitory neurons produce oscillatory activity that can be recorded using extracellular recording electrodes that measure *local field potential* (LFP). Different frequencies of these oscillations have been defined, that span from low frequencies in the delta range (0.5-4 Hz) to high frequencies in the (high) gamma range (30 Hz and above). When a sufficient proportion of neurons fire action potentials in a synchronized and regular manner this will produce membrane potential fluctuations in postsynaptic target neurons,[119] resulting in an oscillation of a certain dominant frequency that can be detected in the LFP signal. At the cellular level, these membrane potential oscillations may remain below the threshold to produce an action potential, but at the network level they do result in temporal windows of increased/decreased excitability.

Excitatory and GABAergic inhibitory synapses differently contribute to oscillatory activity. In particular, GABA is critical for the production of local field gamma oscillations: blockade of $GABA_A$ receptors (i.e. GABA receptors that are fast-responding) abolishes gamma oscillations in the hippocampus.[120, 121] In contrast, antagonists of the AMPA receptor, a glutamate receptor, are ineffective[120, 121] or only partially inhibit gamma oscillations.[122, 123] Thus, gamma oscillations are of particular interest to probe inhibitory functioning. More specifically, PV fast-spiking interneurons are critical for gamma oscillations[119]: they fire phase-locked and with high fidelity to gamma oscillations.[17] As their name suggests, PV fast-spiking interneurons can fire action potentials at high rates, in the range of several hundred Hz.[124] Also, since PV interneurons are widely interconnected, their ability to synchronize their own activity makes them well-positioned to generate (high) gamma oscillations.[119] In addition to gamma, PV interneurons fire phase-locked to theta oscillations[17] and importantly contribute to theta rhythm generation.[125] Therefore, these neurons are considered crucial for another phenomenon that may assist in neuronal communication across brain regions: cross-frequency coupling.

Theta-gamma coupling, inhibition and epileptogenesis

Gamma oscillations are typically very localized. Their amplitude may be modulated by the phase of lower frequencies, which was suggested to underlie the coordination of activity in neuronal populations that are distant from one another.[126] This mode of cross-frequency coupling is termed *phase-amplitude coupling*, which is considered an efficient way of neuronal communication across multiple spatial scales.[127] A ubiquitous form of phase-amplitude coupling in the brain is the coupling of gamma amplitude to theta phase (Figure 6). For example, hippocampal theta modulates both local gamma oscillations,[17, 128] as well as gamma oscillations in distant cortical areas.[129]

FIGURE 6. Cross-frequency coupling of gamma amplitude to theta phase.



LFP recorded from mouse visual cortex (upper) and the results of bandpass-filtering this signal in the theta (5-10 Hz, middle) and gamma (40-160 Hz, lower) ranges. Note that gamma frequency amplitude tends to increase in the midcycle (or ~180°) of the theta frequency wave. Source: Jansen NA et al., Impaired θ-γ coupling indicates inhibitory dysfunction and seizure risk in a Dravet syndrome mouse model, Journal of Neuroscience 2021;41(3);524-537. Image reproduced with permission of the rights holder, Society for Neuroscience.

Such theta-gamma coupling is considered to underlie cognitive processes including memory function.[126, 130] Since dysfunction of PV interneurons may disrupt theta-gamma phase amplitude coupling,[131] and dysfunctional PV interneurons have been implicated in memory dysfunction in Alzheimer's disease,[132] theta-gamma phase-amplitude coupling may be a sensitive readout for PV interneuron functioning. Impaired functioning and/or loss of PV interneurons have been demonstrated for patients with TLE[133] and animal models of TLE[134, 135] and DS.[112-114] In addition, selective impairment of PV interneurons is sufficient to induce spontaneous seizures in mice.[136] As such, theta-gamma phase-amplitude coupling may hold promise as a measure to monitor epileptogenesis.

Outline of the thesis

The general aim of this thesis is to characterize brain network dynamics underlying spontaneous spreading depolarizations (I), as well as sudden death (II) and epileptogenesis (III) in genetic animal models of migraine and epilepsy.

To study the effects of cortical spreading depolarizations, these events are generally locally induced by suprathreshold stimuli, which poorly model the spontaneous events that are thought to underlie the migraine aura. This approach inherently complicates the study of spreading depolarization initiation mechanisms. We introduced the FHM type 3-associated *Scn1a*^{L263V} mutation in a mouse model, predicted to result in aberrant neuronal $Na_V1.1$ sodium channel functioning, to examine whether spreading depolarization could occur spontaneously in these animals (**Chapter 2**). To generalize the results to FHM, we established a mouse model of FHM2

with a genetic mutation affecting the Na^+/K^+ adenosine triphosphatase (ATPase) pump, which is chiefly expressed in astrocytes in the adult brain. This approach allowed us to evaluate whether pharmacologic modulations could differently affect spreading depolarization initiation and propagation (**Chapter 3**).

The mechanisms of *sudden unexpected death in epilepsy* (SUDEP) and *sudden infant syndrome* (SIDS) remain elusive and may be better understood by the development of animal models of spontaneous (seizure-related) death. In **Chapters 4 and 5**, we present a mouse model of spontaneous seizure-related sudden death. We used ambulatory recordings to relate brain activity to seizure-induced death (**Chapter 4**). We performed further recordings of cardiorespiratory and brainstem activity in these mice to evaluate whether vital functions may be impaired by aberrant brainstem functioning and whether pharmacological modulation thereof may prevent death (**Chapter 5**). In a translational study, we combined data from a clinical case and a mouse model to study the mechanisms of life-threatening apnea in the absence of seizure activity associated with the *SCN1A*^{L263V} mutation (**Chapter 6**).

Epileptogenesis is mostly studied in experimental models following an induced status epilepticus or brain-damaging insult, as opposed to genetic models that would better model genetic epilepsy syndromes. On the other hand, the same genetic mutation may or may not result in an epilepsy syndrome. We developed a mouse model of Dravet syndrome to study the epileptogenic potential of global *versus* local cortical or hippocampal *Scn1a* knockout (**Chapter 7**). We further used this model to identify potential electrophysiological metrics of seizure risk in Dravet syndrome (**Chapter 8**).

A general discussion of these chapters will follow in **Chapter 9.**

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