Seizures, spreading depolarizations and sudden death

Jansen, N.A.

Chapter 9

General discussion

GENERAL DISCUSSION

The main aim of this work was to establish mouse models of seizures and spreading depolarizations (SDs), in an effort to study their mechanisms and potentially debilitating consequences. To this end various transgenic animal models were generated and characterized in which aberrant neuronal excitability was a common denominator, but each model had otherwise unique features. An important contribution to the field is that all models showed seizures and/or spreading depolarizations in an apparent unprovoked fashion, increasing their clinical significance and allowing the study of initiation mechanisms of these events. These models may thereby prove a valuable tool to understand how excessive neuronal excitability results in episodic disorders as distinctive as epilepsy and migraine, as well as their complications, aiding the development of (preventative) treatment strategies.

In this Chapter, I will summarize, integrate and discuss the main findings from preceding chapters, and finally provide a perspective for future research.

Part I: Spontaneous spreading depolarizations

Cortical SD as first described by Leão[1] is considered the underlying substrate for the migraine aura.[2] This theory has gradually displaced the "vascular theory" of the migraine aura, which posits that the focal (visual) symptoms of the migraine aura are the result of vasoconstriction.[3] The latter theory has failed to explain the highly stereotypical propagating nature of the migraine aura.[4] This is often illustrated by the scintillating scotoma in visual auras, which starts in the center of the visual field and propagates to the periphery over the course of several minutes.[5] Early imaging studies in migraine patients demonstrated a "spreading oligemia" that propagated in a posterior-to-anterior pattern over the cerebral cortex.[6-9] However, it was already early recognized that the timing of this oligemia poorly correlated with the focal symptoms of the migraine aura.[9] Later measurements of cortical magnetic resonance imaging (MRI) blood oxygenation level dependent (BOLD) signal during migraine auras found similar patterns within the visual cortex, which commenced with initial hyperemia of a duration and propagation characteristic of cortical SD, correlating with aura symptoms, and which were later followed by hypoperfusion.[10] Thereby, an SD propagating through the visual cortex is considered the homotopic equivalent of the experience of a scintillating scotoma in the visual field.

Shifting focus to spreading depolarization initiation

Despite progress in our understanding of factors that may evoke SD and facilitate or inhibit their propagation, little is known about the physiology underlying the spontaneous initiation of SDs. Experimental studies have resorted to actively inducing SD, which is convenient, reliable, and replicable. Models allowing the study of unprovoked SD initiation were hitherto lacking, limiting our ability to identify clinically relevant mechanisms of SD initiation. In **Chapters 2 and 3**, we present two novel models of familial hemiplegic migraine (FHM) in which SDs occur

spontaneously. The mouse models harbor fundamentally different pathogenic mutations: the FHM3-related *Scn1a*^{L263V} mutation, expected to result in $Na_V1.1$ gain of function, presumably resulting in interneuron hyperexcitability, and the FHM2-related *Atp1a2*^{T345A} mutation, expected to result in decreased Na^+/K^+-ATPase pump function in glia. Thus, although different proteins expressed in different cell types are involved, these models share a hyperexcitability phenotype that results in spontaneous SDs. In both models, SDs in the cortex propagated in a caudal-to-rostral (i.e. posterior-to-anterior) direction, similar to studies of cerebral blood flow and MRI BOLD signal in patients during migraine attacks described above. Interestingly, we found that the large majority of spontaneous SDs in homozygous *Atp1a2*^{T345A} mice occurred during awakening. Although we cannot at this point rule out that animals are in fact awakening *from* the SD, clinical studies indicate that migraine attacks (with and without aura) have a predilection to occur in the early morning[11, 12] and upon awakening.[13] The question that follows is: what could initiate these SDs?

Previous literature convincingly showed that inhibition of N-methyl-D-aspartate receptors (NMDARs) blocks experimentally induced SD, although uncertainty persists whether this is caused by impairment of SD initiation or propagation, or both.[14] Studies in another FHM2 model harboring the *Atp1a2* W887R missense mutation have suggested impaired glutamate clearance as a primary trigger of SD.[15, 16] Treatment of these FHM2 mice with a compound that was expected to increase glutamate uptake – by inducing upregulation of a glutamate transporter – resulted in an increased threshold for induced SD.[15] Since glutamate would facilitate SD by activation of NMDARs, allowing regenerative influx of K^+ and other cations through their pores,[17] these data support the view that NMDAR activation is crucial for SD initiation. Indeed, MK801, a potent NMDAR antagonist, blocked the spreading depolarization near the site of initial depolarization evoked by high levels of KCl.[18] In contrast, our results in *Atp1a2*^{T345A} mice indicate that MK801 is ineffective at blocking spontaneous hippocampal SD in homozygous *Atp1a2*^{T345A} mice, while SD propagation to the cortex was blocked. Mei et al.[19] similarly found that blockade of NMDAR, by intracellular administration of compounds, did not affect initiation of SD in the hippocampus of wildtype mice. To explain these apparent discrepancies, we have to consider the area studied: we found a hippocampal origin of SD in *Atp1a2*^{T345A} mice, and Mei et al.[19] studied hippocampal slices. Indeed, NMDAR blockade was previously shown not to prevent SD in hippocampal slices, although its timing and waveform were affected.[20] In contrast, Vitale et al.[18] studied cortical slices. SD mechanisms may vary depending on the area studied, as was earlier proposed given the higher dosage of NMDAR antagonists required to block SD in hippocampus *versus* cortex.[14] Regardless, former studies have relied on some form of experimental induction of SD, be it current injection, pinprick or application of KCl to the dura or brain slice. Given the "all-or-none" character of SD – which entails that once started, its amplitude is independent from the trigger[21] – studying the eventual threshold is key to understanding SD initiation. This was indeed also recognized by Vitale et al.,[18] who found a specific level of NMDAR activation to be required for SD initiation. And yet, this is especially discrepant with our data, as we found that spontaneous SDs in homozygous *Atp1a2*^{T345A} mice were in fact more likely to occur *earlier* after MK801 administration (**Chapter**

3). Although we did not investigate why this occurred, NMDAR antagonists may paradoxically increase excitability by preferential inhibition of inhibitory neurons,[22] possibly by higher ambient glutamate in excitatory to inhibitory synapses.[23] In any case, our data argue against a crucial role for glutamate and NMDARs in SD *initiation*, at least in the context of the T345A FHM2 mutation, in contrast to the widely appreciated crucial role for glutamate and NMDARs in SD *duration* and *propagation*.[17] This should compel us to look for potential alternative mechanisms of SD initiation.

Van Harreveld *versus* Grafstein: potassium persists

A crucial role for increases in extracellular K^+ in the initiation and propagation of SD was suggested in an early study by Grafstein.[24] Based on previous research, increases in extracellular K^+ under conditions of increased excitability could be a common trigger for SD in our FHM2 and FHM3 models. In FHM2, the *Atp1a2*^{T345A} mutation results in impaired clearance of extracellular K^+ in transfected cell cultures.[25] In FHM3, since *Scn1a*-encoded $Na_V1.1$ channels are being predominantly expressed in inhibitory interneurons, the gain-of-function effects of the *Scn1a*^{L263V} mutation[26] could result in accumulation of extracellular K^+ due to increased intensity of interneuron firing.[27] Indeed, experimentally induced activation of $Na_V1.1$ channels or hyperactivity of inhibitory interneurons is sufficient to induce cortical SD, even when neurotransmitter release is blocked.[28] Increases in extracellular K^+ can generate a self-sustaining inward current if exceeding K^+ clearance, resulting in SD.[17]

In mice, cortical extracellular K^+ is relatively low during sleep[29] and increases throughout the cortex immediately prior to brain state transitions.[30] Such state-dependent changes in K^+ occurred much faster – within seconds – than changes in other cations such as Ca^{2+}.[29] Following these changes, a relatively stable K^+ concentration was maintained during each brain state.[29] Interestingly, since we found in **Chapter 3** that the majority of spontaneous SDs in homozygous *Atp1a2*^{T345A} mice occurred during awakening, this raises the possibility that "physiological" changes in extracellular K^+ could contribute to SD initiation, especially when increased release (such as in FHM3) or decreased clearance (such as in FHM2) cause extracellular K^+ rises to overshoot its clearance. Hence the question arises: how would such overshoots cause SD?

For SDs to initiate, inward currents are required that inactivate only slowly.[31] Persistent sodium currents have been identified in neurons and are thought to act as amplifiers of synaptic potentials,[32] regulating the excitability of neurons. Although the magnitude of these sodium currents is relatively small during resting conditions, it is greatly enhanced when extracellular K^+ is increased,[31] as well as during hypoxic conditions.[33] This points to the relevance of overshoots in extracellular K^+ for SD initiation. If large enough, such overshoots may render NMDAR antagonists ineffective against SD.[34] Similarly, we found that a potent NMDAR antagonist did not block SD in our FHM2 mouse model (**Chapter 3**), or massive depolarization in our FHM3 mouse model (**Chapter 6**). Instead, the persistent sodium current blocker GS967[35] effectively reduced SD frequency in homozygous FHM2 mice and prevented lethal massive brainstem depolarization in FHM3 mice, indicating

that persistent sodium currents are a promising target for SD prevention when extracellular K^+ overshoots are suspected.

Future research on initiation mechanisms of spreading depolarizations

Recently, increased concentrations of extracellular K^+ were demonstrated during SD in a different transgenic FHM3 mouse model that expresses the L1649Q missense mutation,[36] previously found in a Dutch family with hemiplegic migraine.[37] Similar to our data (**Chapter 2**), these mice showed a decreased threshold for induction of cortical SD (i.e. with electrical stimulation), with no differences in SD propagation velocity when compared to wildtype animals. This suggests a selective effect on SD initiation by increased extracellular concentrations of K^+. However, spontaneous SDs were not demonstrated in this FHM3 model. Indeed, there is currently no direct evidence of a critical role for K^+ overshoots – or any other mechanism – in SD initiation, since there is a paucity of models available to study spontaneous SDs reliably. One study demonstrated spontaneous cortical SDs in a mouse model with a knock-out of *Atp1a2* specifically in astrocytes.[38] It may be argued that such a model is not clinically representative of FHM2 because of the genetic approach used (homozygous knockout of *Atp1a2* limited to astrocytes), or because of the observed premature mortality such as in our models, as has been argued by some.[36] Indeed, in the models presented here, spontaneous (cortical) SDs always occurred in genotypes that were also prone to early mortality (see next paragraph). And yet, these models provide a unique possibility to study SD initiation mechanisms. Most notably, the complete penetrance of the SD phenotype shown for *Atp1a2*^{T345A} mice (**Chapter 3**) should facilitate the study of spontaneous SD. Longitudinal recordings and modulation of local extracellular K^+ [29, 30, 39] in such models would be a promising research strategy to identify alterations in network dynamics that culminate in SD initiation.

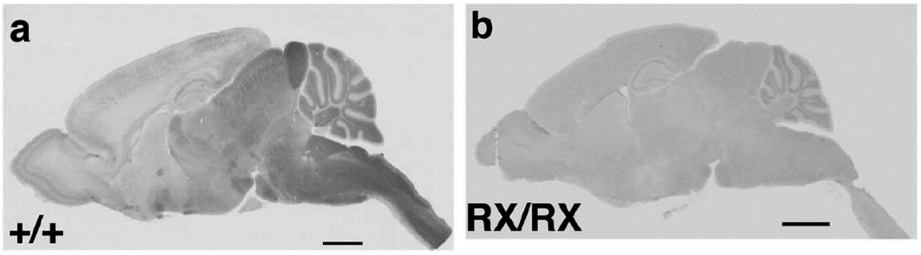
Part II: Mechanisms of sudden death

Although we have managed to breed mice with a monogenic mutation causing FHM1, -2 and -3, early mortality occurred in all models. Early mortality is not a typical feature of the clinical FHM phenotype, which may tempt us to perceive this as an oddity resulting from genetic dosage effects in these model organisms. However, in addition to homozygous animals, early mortality was especially striking in heterozygous *Scn1a*^{L263V} FHM3 mice (**Chapters 2 and 6**), which showed ~90% mortality at P60 (compared to ~30% in homozygous *Cacna1a*^{S218L} FHM1 mice[40] and ~40% in homozygous *Atp1a2*^{T345A} FHM2 mice (**Chapter 2**)). In addition, sporadic deaths were noted in heterozygous FHM2 mice to occur spontaneously and over the course of hippocampal kindling (**Chapter 3**). It should be noted that in all models deaths occurred suddenly, i.e. not secondary to impaired mobility or prolonged seizure activity. In fact, behavioral seizure activity was only a consistent phenotypical feature in *Cacna1a*^{S218L} mice. As described in **Chapters 4-6**, sudden deaths in homozygous *Cacna1a*^{S218L} and *Scn1a*^{L263V} mice were mediated by brainstem (spreading) depolarizations.

Brainstem SD in FHM models

Requirements for SDs to occur in brain tissue have been proposed to include tissue homogeneity, high neuronal density and absence of myelinated borders.[41] As such, the brainstem – which constitutes a region with nuclei of relatively low neuronal density and bordered by myelin-rich structures – appears cytoarchitecturally impervious to SD. *In vitro* and *ex vivo* experiments in the rat and mouse brainstem have indeed shown that brainstem regions are highly resistant to K^+-induced SD.[42, 43] However, SD could be induced by K^+ in the rat brainstem, be it after extensive preconditioning in order to create hyperexcitable conditions.[44] It is likely that in our studies, such hyperexcitable conditions are intrinsically met by the pathogenic mutation, rendering the brainstem much more sensitive to SD. For example, the $Na_V1.1$ sodium channel, encoded by the *Scn1a* gene, is abundantly expressed in the pons and medulla (**Figure 1**).[45] *Scn1a*^{L263V} gain-of-function effects may thus disproportionally affect brainstem excitability, culminating in the SD-induced apnea observed in *Scn1a*^{L263V} mice (**Chapter 6**).

FIGURE 1. Expression of NaV1.1 protein as detected by immunohistochemistry in mice pups with WT *Scn1a* (a) and (b) a homozygous R1407X nonsense mutation (RX/RX), resulting in truncated NaV1.1.

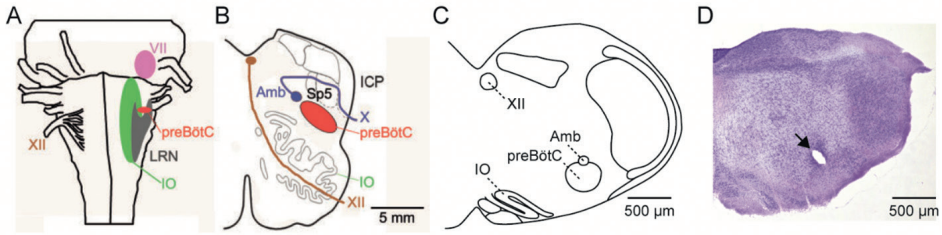


Note the marked differences in expression in the mid- and hindbrain. Scale bars indicating 1 mm. Source: Ogiwara et al. Nav1.1 Localizes to Axons of Parvalbumin-Positive Inhibitory Interneurons: A Circuit Basis for Epileptic Seizures in Mice Carrying an Scn1a Gene Mutation, Journal of Neuroscience 2007;27(22):5903-5914. Image reproduced with permission of the rights holder, Society of Neuroscience.

Despite a common sudden death phenotype, our studies reveal notable differences between the FHM1, -2 and -3 models. First of all, in *Cacna1a*^{S218L} mice all deaths were preceded by seizure behavior (**Chapters 4 and 5**). Second, brainstem SD evidently spread slowly – over the course of tens of seconds – throughout the brainstem. And finally, brainstem SD was not necessarily lethal in these animals, although it always suppressed breathing activity when involving the ventrolateral medulla. In contrast, *Scn1a*^{L263V} mice did not show behavioral or electrophysiological evidence of seizures, and brainstem "SD" in fact showed sub-second delay between brainstem regions, and was lethal in all cases (**Chapter 6**). As such, the expected propagation rate is an order of a magnitude greater in *Scn1a*^{L263V} mice when compared to *Cacna1a*^{S218L} mice, suggesting different mechanisms

of the observed depolarizations. This is further reflected by a different treatment response: whereas the sudden death phenotype in *Cacna1a*^{S218L} mice is completely rescued by NMDAR antagonist MK801 (**Chapter 5**), this drug was ineffective in *Scn1a*^{L263V} mice (**Chapter 6**). In accordance with the reasoning in the first paragraph, these results may be due to a selective effect of MK801 on inhibition of *SD propagation*, as occurring in *Cacna1a*^{S218L} mice, whereas modulation of brainstem depolarization in *Scn1a*^{L263V} mice may in fact require inhibition of *SD initiation*.

FIGURE 2. Location of the pre-Bötzinger complex in humans (A and B) and mice (C and D).



(A) Schematic ventral view of the human brainstem. (B and C) Schematic transversal view of the human (B) and mouse (C) brainstem, at the level of the pre-Bötzinger complex (preBötC). In both species, the pre-Bötzinger complex is located (latero)dorsal from the inferior olive (IO), interpositioned between the facial nucleus (VII) and lateral reticular nucleus (LRN) at the transversal level of the nucleus ambiguus (Amb) and hypoglossus (XII). (D) Lesion (arrow) produced at the electrode tip that recorded SDs in a *Cacna1a*^{S218L} mouse, located in the expected location of the pre-Bötzinger complex (**Chapter 6**). Source: panels A and B are adapted from Schwarzacher et al.,[47] panels C and D are adapted from Jansen et al.[51]

The vital node

Early descriptions by Pierre Flourens, a French physiologist and physician, identified a *noeud vital* – or "vital node" – in the medulla oblongata: a region critical for respiratory pacemaking. Later efforts, most notably a lesioning study,[46] have demonstrated that the *noeud vital* was in fact the pre-Bötzinger complex, a paired nucleus consisting of a few hundred neurons located in the ventrolateral medulla oblongata, which was later also identified in humans.[47] Since animal studies have shown that bilateral,[48, 49] but not unilateral,[50] disruption of the pre-Bötzinger complex results in lethal apnea, the slow propagation of SD would theoretically pose a less severe threat to humans when compared to smaller mammals such as mice: the distance between the bilateral nuclei is roughly a factor 5 – 10 times bigger in humans than in mice (**Figure 2**), with similar differences in nucleus size. Similar cortical SD propagation velocities in humans and smaller mammals[1, 7, 10] would thus predict a sub-minute delay between SDs in the pre-Bötzinger complex of mice, *versus* a delay of several minutes in humans, presuming an SD propagation rate of 2 – 4 mm/min in the brainstem.[44] Such timing differences could be crucial, since duration of non-fatal neuronal suppression by SD was around 1 – 1.5 min in our study in FHM1 mice (**Chapter 5**). These interspecies differences, in addition to cytoarchitectural differences such as increased thickness of myelin-rich borders,

could contribute to a lower risk of SD-induced fatal apnea in humans with similar genetic dosage compared to our model organisms.

Brainstem spreading depolarization: are humans at risk?

And yet, certain conditions may enhance the risk of SD-induced fatal apnea in both mice and humans. Up until now, we have presumed that SD originates from a single source, consecutively spreading through a heterogenous brainstem and impeded by white matter structures. However, SDs can occur in the setting of seizure activity.[52-54] Seizures can spread throughout brain tissue via contiguous grey matter, similar to SDs, clinically referred to as "Jacksonian march".[55] Alternatively, seizures can spread via white matter structures to homotopic regions.[56] As such, brainstem areas may become involved in the seizure network expanding from local[57] or more distant[58] epileptogenic foci, perhaps initiating SDs in multiple brainstem areas. This hypothesis would need verification in different SUDEP models, including larger model organisms.

In our FHM2 and FHM3 mouse models (**Chapters 2, 3 and 6**), sudden death occurred always (in FHM3) or mostly (FHM2) in the absence of electrographic or behavioral seizure activity. In *Scn1a*^{L263V} mice, fatal apnea coincided with profound depolarizations in two (relatively) distant brainstem electrodes, with often sub-second delay between the electrodes (**Chapter 6**). Such propagation velocities are incompatible with unifocal SD, and rather support a preceding synchronizing event. Such "prodromal" events have been demonstrated in the setting of hippocampal SD,[20] presumed to be mediated by gap junctions. Whether such a mechanism could premediate the massive brainstem depolarization observed in *Scn1a*^{L263V} mice remains a topic for future investigation. In any case, *SCN1A* mutations are clinically strongly associated with SUDEP, the leading cause of death in Dravet syndrome,[59] as well as sudden death in infancy (SIDS) and childhood (sudden unexpected death in pediatrics (SUDP)).[60-62] Although these mutations are typically considered to result in loss of function, missense variants may be rescued *in vivo*.[63] In addition, despite the tendency of sodium channel blockers to increase seizure frequency and severity in Dravet syndrome,[64] they effectively *reduced* lethality in *Scn1a* knockout mice.[65] This effective dissociation of seizure incidence and mortality risk was related to a decreased occurrence of tonic seizure behavior (tonic hindlimb extension),[65] which was associated with lethal apnea in another SUDEP mouse model.[66] Thus, our findings in *Scn1a*^{L263V} mice may extend to other *SCN1A* variants – in addition to the clinical homozygous *SCN1A*^{L263V} carrier we presented in **Chapter 6** – supporting a crucial role for brainstem (spreading) depolarization in sudden death.

Future research on mechanisms of sudden death

How then does the "brainstem SD" hypothesis relate to other hypotheses regarding sudden death pathophysiology? Both SUDEP and SIDS are considered to result from "cardiorespiratory dysfunction" and decreased arousal.[67] Slowly, this "cardiorespiratory dysfunction" has been specified as a sequence of respiratory arrest followed by cardiac arrest, most notably motivated by the MORTEMUS study.[68] Of course, cardiac-driven SUDEP/SIDS may occur, but the latter data

suggest that respiratory arrest underlies a majority of cases. This shift in thinking is aptly illustrated by data from animal studies, which demonstrated lethal bradycardia in a Dravet syndrome mouse model,[69] which was later shown to result from peri-ictal apnea.[70] Several mechanisms underlying sudden (peri-ictal) apnea have been proposed, including impaired CO_2-mediated arousal (whether or not related to brainstem serotonergic dysfunction),[67, 71] increased adenosine release,[72] and laryngospasm – either due to increased reflexes[73] or seizure activity in brainstem motor nuclei.[74, 75] In fact, several of these mechanisms may co-occur in SUDEP/SIDS cases. What these hypotheses have in common, however, is that integrity of neuronal activity is the *sine qua non*. The occurrence of brainstem SD under normoxic conditions – as demonstrated in our studies – would thus effectively decrease the relevance of these other hypothetical mechanisms to modulating factors (i.e. affecting SD risk and/or recovery of physiological activity following SD). As such, efforts should be made to investigate the plausibility of brainstem SD in patients at risk of sudden death. To do so, a first step would be to replicate our results in other SUDEP and SIDS animal models. Second, experiments in larger model organisms should be considered to approach the dimensions of the human brainstem. Similar to the postictal changes we observed in heart rate parameters in *Cacna1a*^{S218L} mice (**Chapter 5**), such studies could allow identification of SD-related changes in neurophysiology, behavior and vagal output to establish more readily available SD biomarkers that could facilitate its detection in patients.

Part III: Epileptogenesis in Dravet syndrome

A variant in the *SCN1A* gene is the most frequently encountered cause of monogenic epilepsy.[76] The associated phenotype, however, can range from no/mild epilepsy burden to the epileptic encephalopathy that constitutes Dravet syndrome, characterized by early-onset seizures and severe cognitive and behavioral deficits.[77, 78] In this respect, age of seizure onset was shown to be a better predictor than mutation type,[78] although a recent study showed increased prediction performance when a more elaborate *SCN1A* genetic score was added.[79] These improvements in outcome prediction will improve patient and caregiver counselling, yet improvement in treatment outcomes, including seizure control, will ultimately rely on more than baseline patient characteristics.

Probing for inhibitory dysfunction

Patients with Dravet syndrome will likely benefit from recent clinical treatment advances,[80, 81] as well as promising targeted interventions aimed at enhancing $Na_V1.1$ activity developed in pre-clinical research.[82-85] However, biomarkers for treatment monitoring are currently lacking, although the pathophysiological basis for Dravet syndrome is relatively well-understood. Haploinsufficiency of the *SCN1A* gene, encoding the α1 subunit of $Na_V1.1$ channels, is expected to result in decreased voltage-dependent sodium currents specifically in GABAergic interneurons,[86, 87] evidenced by selective expression of $Na_V1.1$ in inhibitory neurons[45, 88] and reproduction of the epileptic phenotype when *Scn1a* knockout is limited to inhibitory neurons.[89] These findings have helped explain clinical observations of adverse outcome in Dravet syndrome following treatment with lamotrigine, a

sodium channel blocker.[64] Studies have suggested, however, that interneuron impairment is not permanent in Dravet syndrome, as cortical GABAergic neurons show only transient impairment of excitability in *Scn1a*$^{-/+}$ mice,[90] while in the hippocampus only fast-spiking GABAergic neurons remain impaired.[91] Although *homozygous* knockout of *Scn1a* resulted in spontaneous seizures when limited to hippocampus or visual cortex (**Chapter 7**), local *heterozygous* knockout resulted in seizures only for hippocampus and not visual cortex (**Chapter 8**), indicating regional differences in epileptogenesis susceptibility. Since recently developed treatment strategies aim to increase transcriptional[84] or post-transcriptional[82] expression of the healthy *SCN1A* allele, it appears ever more crucial to know when and where to introduce such therapies for the individual patient. A good disease biomarker should ideally reflect both underlying neuronal dynamics, thereby for example preventing efforts to increase *SCN1A* expression in individuals that have $Na_V1.1$ functional dominant negative effects due to a gain of function,[92] as well as the clinical phenotype including seizure burden and cognitive deficits. In **Chapter 8**, we propose local field potential (LFP) theta-gamma phase-amplitude coupling as such a biomarker, by showing that it correlated with the expected transience in cortical inhibitory dysfunction, as well as with expression of seizure activity in mice with local or global *Scn1a* knockout. Further, these changes were shown to be amenable to acute cannabidiol treatment, which improved seizure burden in Dravet syndrome [80] and increased inhibitory functioning in *Scn1a*$^{-/+}$ mice.[93] The pattern of theta-gamma coupling, i.e. coupling of theta to high *versus* low gamma, correlated with the locality of $Na_V1.1$ dysfunction (i.e. neocortex *versus* hippocampus). As such, theta-gamma coupling may aid in determining the strategy, timing and location of treatment in Dravet syndrome.

Future research on theta-gamma coupling

However, challenges await before such a biomarker can be clinically implemented. First, detection of oscillations noninvasively, such as in scalp EEG, is generally limited to frequencies <100 Hz. Second, localization of subcortical activity from scalp EEG is often not considered feasible. Lastly, although neocortical theta-gamma coupling has been demonstrated in human subjects during cognitive processing,[94] it is likely much more abundant in the neocortex of mice due to volume conductance of theta oscillations from hippocampal sources in the latter.[95, 96] Yet, relatively recently have investigators managed to record (biologically relevant) higher frequencies such as high-frequency oscillations,[97, 98] as well as subcortical activity[99-101] from scalp EEG and/or MEG. Since fast-spiking GABAergic interneurons importantly contribute to (high) gamma oscillations[102, 103] and remain impaired in the hippocampus of *Scn1a*$^{-/+}$ mice,[91] such advances may be critical for informative recordings in Dravet syndrome.

The quest for a biomarker of inhibitory dysfunction will not only benefit patients with Dravet syndrome. Impaired hippocampal theta-gamma coupling has been associated with dysfunctional fast-spiking GABAergic interneurons in animal models of temporal lobe epilepsy[104] and Alzheimer's disease.[105-107] Clinically, these disease processes show overlap, as epilepsy and epilepsy-related cognitive decline are associated with amyloid β aggregation[108] – a characteristic

finding in Alzheimer's disease – whereas increased seizure risk[109] as well as subclinical epileptiform activity,[110] which may remain undetected on EEG,[111] are associated with Alzheimer's disease. Similarly, identical treatment strategies aimed at improving inhibitory dysfunction could benefit multiple patient groups, as has been demonstrated for models of Alzheimer's disease and Dravet syndrome.[112] As such, a primary aim should be to establish a clinically reproducible measure of theta-gamma coupling or other markers of dysfunctional network inhibition in order to guide treatment and provide insight in pathophysiological similarities and differences between these neurological disorders.

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