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Dysregulation of Sodium Channels in a Rat Model of Absence Epilepsy

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**DYSREGULATION OF SODIUM CHANNELS IN A RAT MODEL
OF ABSENCE EPILEPSY**

YALE SCHOOL OF MEDICINE SENIOR THESIS

By

DAVENDER KHERA

2006

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DYSREGULATION OF SODIUM CHANNELS IN A RAT MODEL OF ABSENCE EPILEPSY. Davender S. Khera, Josh P. Klein, Hrachya Nersesyan, Eyal Kimchi, Stephen Waxman, Hal Blumenfeld. Department of Neurology and Department of Neurobiology, Yale University School of Medicine, New Haven, CT.

Abstract

Absence epilepsy is a generalized form of epilepsy where spike-wave discharges (SWDs) involve both hemispheres of the brain and thereby alter consciousness. Recent evidence by Meeren et al (2002) in the WAG/Rij rat model of absence epilepsy points to a cortical focus of SWDs before rapid generalization of the SWDs. This focus belongs in the peri-oral area of the somatosensory cortex, and it was found to consistently lead SWDs in other cortical and subcortical areas. With this recent finding, it seems plausible that a defect lies in this focal region of the cortex, leading to SWD in the WAG/Rij model. It is likely that an alteration of one or more ion channels leads to seizure generation in this rat model, as ion channels are what produce the hyperexcitability of seizures.

In this study, our laboratory performed three consecutive days of scalp EEG recordings on WAG/Rij animals at different ages and compared this to control rat EEGs. As has been found before, we saw an increase in time spent in SWDs as the WAG/Rij animals aged. After completing EEG recordings, the animals were sacrificed and quantitative PCR and immunocytochemistry was performed on six regions of the cortex. In comparison to control animals, WAG/Rij rats had an increase in sodium channel subunits $Na_v1.1$ and $Na_v1.6$ in the region corresponding to the seizure focus identified by Meeren et al. In addition, as WAG/Rij rats aged, the amount of $Na_v1.1$ and $Na_v1.6$ also steadily increased in the peri-oral region of the somatosensory cortex. These findings suggest that specific sodium channelopathies may initiate SWD generation in this rodent model.

The results of our study have many implications. Perhaps many, if not all, forms of human absence epilepsy are rooted in ion channelopathies which could be limited to specific regions of the brain. If this is so, and if the specific channelopathies are identified, it is also possible that very targeted therapies could be devised – either medically or surgically – to treat both “benign” and refractory absence epilepsies.

Future studies are needed to determine whether the sodium channel dysregulation found in this rodent model is the cause or effect of SWDs and whether other channelopathies or dysregulation of channels exists. Our lab is currently looking at what effects ethosuximide, an anti-absence drug, has on sodium channel composition in the cortex of the WAG/Rij rat.

I. INTRODUCTION	1
HISTORY OF EPILEPSY	1
SEIZURES AND EPILEPSY	1
SEIZURE CLASSIFICATION	2
ABSENCE EPILEPSY	3
ION CHANNELS AND EPILEPSY	7
ABSENCE EPILEPSY GENETICS AND ION CHANNELOPATHY.....	11
ABSENCE EPILEPSY IS CONFINED TO CERTAIN NETWORKS	13
ANIMAL MODELS OF ABSENCE EPILEPSY.....	16
ETHOSUXIMIDE AND ABSENCE EPILEPSY	19
II. HYPOTHESIS	20
III. METHODS	21
ANIMALS	21
ELECTRODE PLACEMENT.....	22
EEG RECORDING	24
RNA EXTRACTION AND CDNA SYNTHESIS	24
QUANTITATIVE REAL-TIME PCR	25
IMMUNOCYTOCHEMISTRY	27
DATA ANALYSIS.....	27
IV. RESULTS	28
WAG/RIJ VS. CONTROL EEG DATA.....	28
AGE OF RAT AND SEVERITY OF EPILEPSY	29
SODIUM CHANNEL mRNA IN WAG/RIJ RATS AND CONTROLS	30
SODIUM CHANNEL EXPRESSION IN WAG/RIJ AND CONTROLS.....	32
V. DISCUSSION.....	34
VI. REFERENCES.....	37

I. **Introduction**

History of Epilepsy

Throughout history and in different cultures, epilepsy has undergone many different interpretations and understandings. Epilepsy can cause marked behavioral changes in an individual that can rouse panic and fear in those who witness a seizure. In the book *The Spirit Catches You and You Fall Down*, the Hmong parents interpret Lia's epileptic seizures as a loss of the spirit causing young Lia to "fall down" (Fadiman, 1997). This supernatural interpretation contrasts with the neurologic one proposed by modern medicine and by the American doctors of young Lia.

Descriptions of epilepsy are found as early as 2500 years ago, where references to the affliction are found in Babylonian texts (Wilson, et al 1990). Supernatural and magical interpretations of epilepsy persisted in Roman times, and later in the Middle Ages where these cultures branded epilepsy as a magical, mystical experience (Wyllie, 1993). Hughlings Jackson began the modern thinking on epilepsy with his 1861 description of the generation and spread of seizures from a grey matter focus in the brain.

Seizures and Epilepsy

A seizure causes a sudden change in behavior as a result of hyper-synchronization of neuronal networks in the central nervous system (Chang and Lowenstein, 2003). A seizure and epilepsy are not synonymous. A seizure is an isolated event. Epilepsy is the condition when an individual develops recurrent seizures.

Patients develop seizures for multiple reasons. Metabolic abnormalities (e.g. hypocalcemia) and drugs (e.g. alcohol) can trigger seizures. In addition, systemic

diseases (e.g. hypertensive or hepatic encephalopathy) and CNS insults (e.g. trauma or stroke) may also cause seizures (Aminoff, 2001; Kotagal and Luders, 1999).

Whether the above causes of seizures lead to epilepsy depends on whether the insult causes a permanent change in the brain, creating a preponderance for future seizures. In addition to the insults listed above, congenital causes (e.g. ion channelopathies or structural brain defects) are possible causes of epilepsy.

Seizure Classification

A wide variety of seizures can occur in an epileptic patient. There are two main categories of seizure: partial and generalized.

Partial seizures are limited to specific networks or regions of the brain, most often localized to one side of the brain. Partial seizures are subdivided into three different classes: simple partial seizures, complex partial seizures, and partial seizures evolving to secondarily generalized seizures. Simple partial seizures do not involve impairment of consciousness, whereas complex partial seizures do. Impairment of consciousness here is defined as an impairment of memory and/or the development of disorientation during a seizure. Some partial seizures secondarily generalize, i.e. they begin in one region of the brain but subsequently spread to the entire brain.

Generalized seizures involve both hemispheres of the brain. However it is becoming clear that in many generalized seizures, only certain neuronal networks are involved (Blumenfeld, 2003). There are many different types of generalized seizures, including absence (petit mal), tonic-clonic (grand mal), tonic, myoclonic, atonic seizures, and others (Rosenberg, 2003).

Absence Epilepsy

The International League Against Epilepsy (ILAE) has classified the different human generalized epilepsies as shown in ***Error! Reference source not found.***

(Commission on Classification and Terminology of the International League Against Epilepsy, 1981 and 1989).

- Tonic-clonic seizures (includes variations beginning with a clonic or myoclonic phase)
- Clonic seizures
 - Without tonic features
 - With tonic features
- **Typical absence seizures**
 - Childhood absence epilepsy
 - Juvenile absence epilepsy
 - Juvenile Myoclonic Epilepsy
 - Myoclonic Absence Epilepsy
- Atypical absence seizures
- Myoclonic absence seizures
- Tonic seizures
- Spasms
- Myoclonic seizures
- Massive bilateral myoclonus
- Eyelid myoclonia
 - Without absences
 - With absences
- Myoclonic atonic seizures
- Negative myoclonus
- Atonic seizures
- Reflex seizures in generalized epilepsy syndromes
- Seizures of the posterior neocortex
- Neocortical temporal lobe seizures

Table 1: Generalized Epilepsies (*From: Panayiotopoulos, 2004*)

In this study, we will focus on a rat model of typical absence seizures in humans, as the rat model that we have chosen most closely resembles typical absences. By definition, typical absence seizures are generalized epileptic seizures with a sudden onset and sudden termination (Panayiotopoulos, 2004). In humans, the EEG shows 2.5-4 Hz spike-wave discharges and patients experience impairment of consciousness.

The impairment of consciousness in typical absence seizures ranges from inconspicuous to severe (Commission on Classification and Terminology of the International League Against Epilepsy, 1981 and 1989). Impairment of consciousness usually manifests itself with a blank stare and cessation of activity, but without loss of muscle tone. Classically, an individual will stop speaking in mid-sentence or stop a certain activity, only to resume the activity once the seizure is over. External stimulation can sometimes abort the seizure. While impairment of consciousness may be the only clinical manifestation of typical absence seizures, impairment of consciousness is often accompanied by other clinical features. Automatisms of the eyes or mouth frequently occur. Some patients also experience either localized or widespread myoclonic jerks. In addition, some patients have atonic or tonic components, and some have autonomic manifestations (e.g. pallor or dilated pupils). Absences may be the only seizure type in an epileptic patient (e.g. in many cases of childhood absence epilepsy), or they may simply be one of a host of seizure types that the patient experiences (e.g. in juvenile absence epilepsy: absence seizures, myoclonic jerks, and generalized tonic-clonic seizures often all coexist).

The ILAE currently identifies four types of absence epilepsy syndromes in which typical absence seizures occur (Engel, 2001): childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and myoclonic absence epilepsy (MAE).

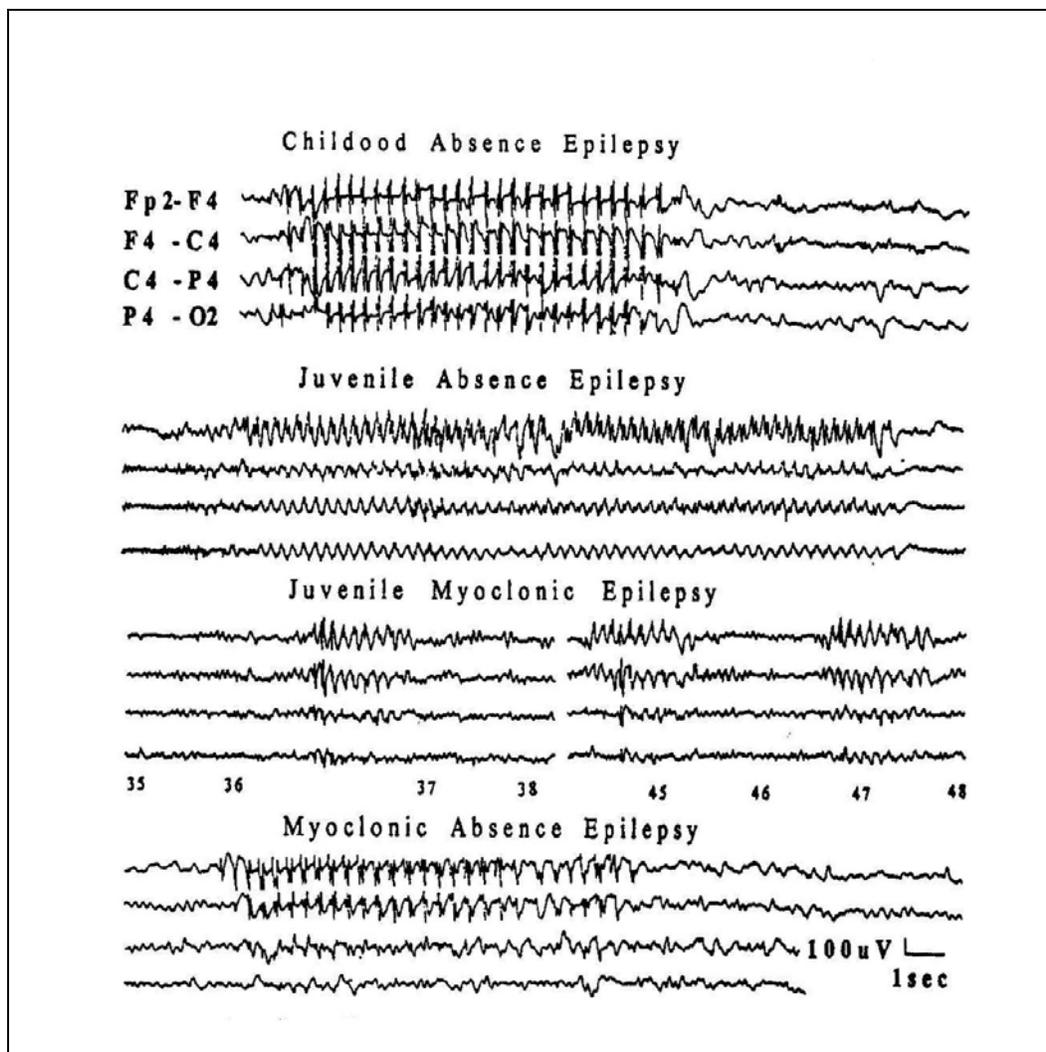


Figure 1: The four epileptic syndromes with typical absences – (1) childhood absence epilepsy, (2) juvenile absence epilepsy, (3) juvenile myoclonic epilepsy, and (4) myoclonic absence epilepsy. (From Engel and Pedley, 1997)

Each of the four typical absence epilepsy syndromes have different clinico-EEG characteristics. The EEG patterns are shown in *Figure 1*. For example, in childhood absence epilepsy, seizures usually begin between the ages of three and eight years of age, patients experience multiple seizures (up to 200) per day, and seizures are not triggered by sensory or visual stimuli (Crunelli and Leresche, 2002). *Table 2* lists the typical features of CAE. The majority of patients with CAE (70%) have remission of their seizures around the time of puberty. Many of those patients with CAE who do not undergo remission of their epilepsy have generalized tonic-clonic seizures. The other typical absence syndromes differ from CAE in their clinical presentation, EEG characteristics, and prognosis.

<p>Clinical ictal symptoms</p> <ul style="list-style-type: none"> • Severe (more rarely moderate or mild) impairment of consciousness; for example, no response to commands or recollection of ictal events • Eyes are open and may stare • Automatisms (often not stereotyped) are visible in many patients with severe impairment of consciousness <p>Ictal electroencephalogram</p> <ul style="list-style-type: none"> • Spike (usually 1, maximum 3) and slow-wave discharge is generalized, bilateral and synchronous • Frequency: 3 Hz (range 2.5–4 Hz) with a gradual decline in frequency during the seizure • Duration: ~10 s (range 4–20 s) <p>Interictal electroencephalogram</p> <ul style="list-style-type: none"> • Normal (rhythmic posterior delta activity in two-thirds of patients)
<p>Table 2: <i>Clinical and EEG Characteristics of Childhood Absence Epilepsy (Table from: Crunelli and Leresche, 2003)</i></p>

The prognosis and complications of the four typical absence epilepsy syndromes differ (Panayiotopoulos, 2004). Childhood absence epilepsy usually remits within 2 to 5 years from onset. The other three syndromes usually have a life-long risk of absences, myoclonic jerks, and generalized tonic-clonic seizures, and therefore these patients

usually require life-long medical treatment. Though the response to treatment in this latter group is often quite good, 10-20% do not achieve control of their seizures.

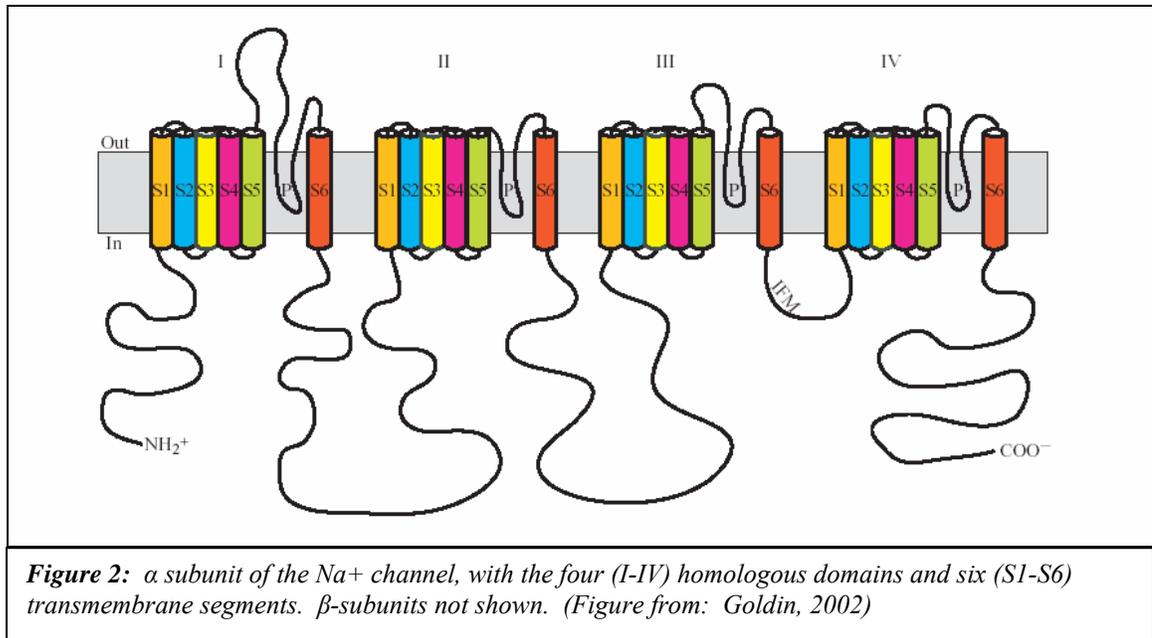
Typical absences are quite heterogeneous and are by no means uniform in terms of clinico-EEG characteristics (Futatsugi, 1998). Typical absences are only labeled “typical” to contrast them from atypical absences. Atypical absences occur mainly in the context of severe symptomatic epilepsies of children with learning difficulties who also suffer from frequent seizures of other types (e.g. atonic or tonic seizures). Atypical absences also do not have the abrupt onset and extinction of seizures that typical absences do. Changes in tone are more pronounced in atypical absences. Finally, the ictal EEG of atypical absence seizures is generally slower than typical absence seizures, with a frequency less than 2.5 Hz SWDs. These slower discharges are often heterogeneous, asymmetrical, and may include irregular complexes.

Ion Channels and Epilepsy

Genetic studies have shown that many different channels, from GABA to Ca^{2+} to Na^+ channels, are possibly defective or are dysregulated in epileptic brains (Crunelli and Leresche, 2003; Futatsugi and Riviello, 1998). As noted above, there are many different types of human absence epilepsy, each with different clinical presentations. This may indicate that defects in several ion channels could produce absence in different forms, and/or each ion channel can have multiple defects that create different clinical presentations.

Our study will look at several subtypes of the Na^+ channel in a rodent model of absence epilepsy. The sodium channel is made up of two subunits, α and β . The α

subunit is approximately 260 kDa and contains four homologous domains named I-IV as shown in *Figure 2*. Within each of these domains I-IV, there are 6 transmembrane segments, S1-S6. The α subunit is the pore-forming portion of the sodium channel, through which the sodium ion traverses the cell. The α subunit is associated with the β subunits by either non-covalent bonds or covalent, disulfide linkages (Goldin, 2001). Although the α subunit can function without the β subunits, it is the β subunits that regulate the kinetics and voltage dependence of the channel.



A sodium-channel nomenclature has been established. The prefix Na_v indicates the principle ion that crosses the channel, in this case, Na^+ . The subscript 'v' indicates that the principle regulator of channel function is voltage. Another type of channel, Na_x , exists. This channel is not thought to be voltage gated, but since it is not functionally expressed, it is not completely known. A number following the subscript indicates the

gene subfamily, and the number following the decimal point indicates the specific channel isoform, e.g. $Na_v1.3$ (Goldin, 2000).

Table 3 lists each Na_v isoform's tissue distribution, e.g. $Na_v1.1$ is found only in the CNS. Moreover, each isoform probably has its own unique distribution within the tissue(s) it is expressed.

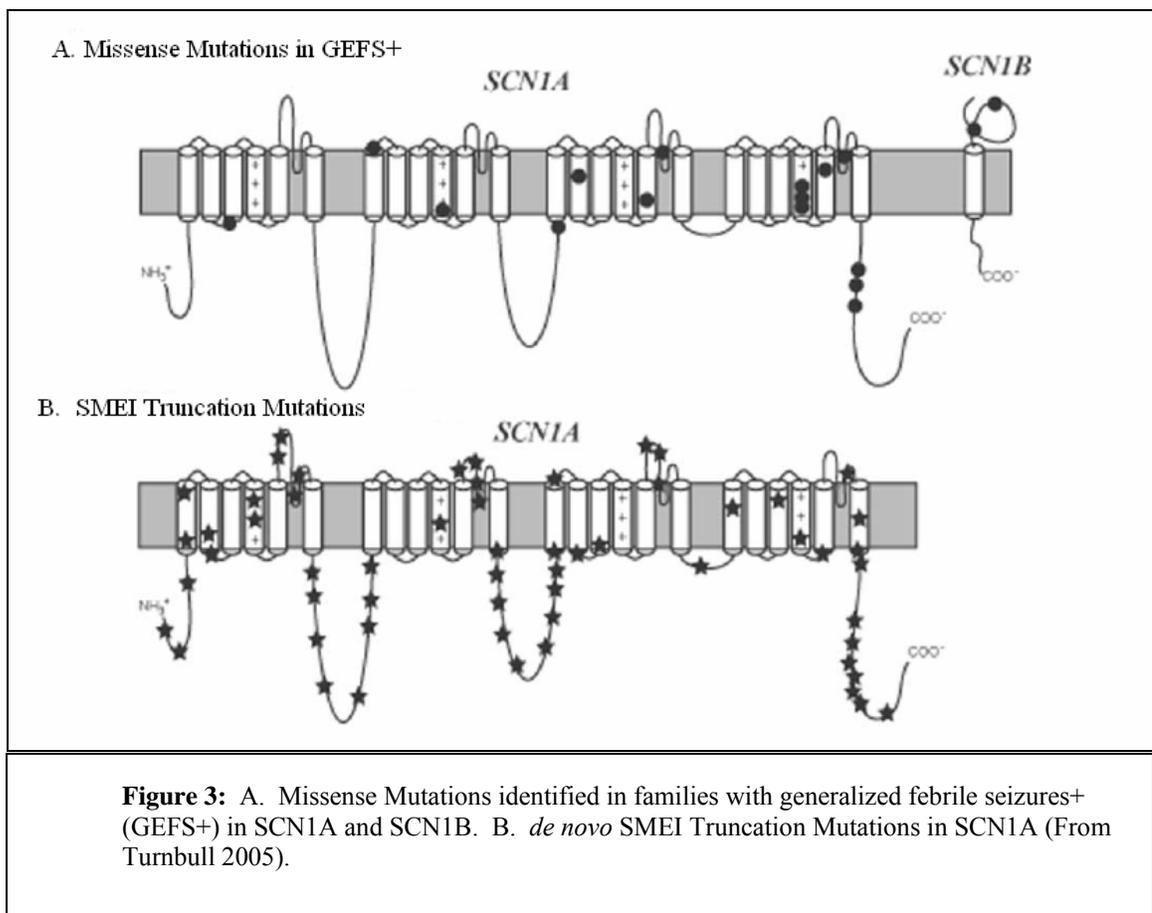
<i>Table 3: Human Sodium Channel Subunits (Goldin, 2000)</i>	
<u>Channel Name</u>	<u>Tissue found in</u>
$Na_v1.1$	CNS
$Na_v1.2$	CNS
$Na_v1.3$	CNS
$Na_v1.4$	Skeletal muscle
$Na_v1.5$	Skeletal muscle, heart
$Na_v1.6$	CNS, PNS
$Na_v1.7$	PNS, Schwann cells
$Na_v1.8$	DRG
$Na_v1.9$	PNS
Na_x	Heart, uterus, skel. Muscle, astrocytes, DRG
CNS, central nervous system; PNS, peripheral nervous system; DRG, dorsal root ganglion.	

Each sodium channel isoform has distinctive properties. For example, $Nav1.1$ mediates mainly a transient current, while $Nav 1.6$ mediates a persistent current (Vega-Saenz de Miera, 1997). The combination of sodium channels with different distributions in each tissue gives each tissue its electrogenic properties.

In animal models, it has been shown that the number of specific types of sodium channel mRNAs increase following induced seizures. Kainate-induced seizures in rats produced an increase in two types of sodium channel mRNA and an increase in the $\beta 2$ subunit mRNA, suggesting that kainate induces seizures by somehow altering sodium

channel compositions (Bartolomei et al, 1997; Gastaldi, 1998). In an electrically-induced status epilepticus animal model, it was shown that certain sodium channel subtypes increased compared to controls (Aronica et al, 2001).

In humans, several sodium channel gene mutations have been identified that lead to different forms of epilepsy (Turnbull et al., 2005). Turnbull et al. notes three sodium channel mutations that have been identified in human epilepsy. The first is a mutation in the sodium channel gene *SCN2A*. This mutation is linked with benign familial neonatal and infantile seizures (BFNIS) and generalized epilepsy with febrile seizures plus (GEFS+).



Mutations in SCN1B and SCN1A have been linked with GEFS+, and mutations in SCN1A are also linked with severe myoclonic epilepsy of infancy (SMEI). SMEI is an early-onset syndrome which is also marked by intellectual deterioration. SMEI demonstrates how many mutations of a channel can cause a spectrum of disease, as seems to be the case in absence epilepsy. For SMEI, 200 independent mutations in individuals have been identified in SCN1A (see *Figure 3*), with more than 90% of these cases being sporadic mutations. Since there are so many different mutations causing SMEI, it is no surprise that there is a spectrum of disease, from moderate to severe symptoms. As for SCN1B, two mutations in the β -1 subunit (as mentioned above, the β -subunit regulates the kinetics of the Na⁺ channel) have been identified, which is thought to delay sodium channel inactivation. With time, more mutations in sodium channels as well as in other ion channels and other non-ion channel genes will be found. It is certainly becoming clear that in most instances of epilepsy, the genetics is complex, often involving many different ion channelopathies.

The importance of sodium channels in epilepsy is further highlighted by the use of anti-epileptic drugs like ethosuximide that are thought to act, to some extent, on the sodium currents in the brain. This is discussed below in the section “Ethosuximide and Absence Epilepsy”.

Absence epilepsy genetics and ion channelopathy

Absence epilepsy seems to be a genetic condition where no apparent insult is necessary for seizures to begin and where the disease tends to exist within certain families. In most cases, absence epilepsy has a complex inheritance pattern. One study

looked at 55 families with idiopathic generalized epilepsy (IGE), where IGE includes four different types of epilepsy: childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and idiopathic generalized epilepsy with tonic-clonic seizures (IGE-TCS) (Marini et al, 2004). The study found that of the 55 families with IGE probands, 34% of the probands had concordance with first-degree relatives and 14% had concordance with second-degree relatives.

Crunelli and Leresche (2002) have summarized many of the genetic studies of IGE that have been performed both in humans and in rodents. An excerpt of their review is shown in *Table 4*. As of that publication, only two single point-mutations – in GABA_A and a voltage-gated Ca²⁺ channel subunit (CACNA1A) – had been associated with CAE (Wallace RH et al, 2001; Jouveneau A et al., 2001). Perhaps this is not a surprise, as

<i>Table 4 : Various Linkage and Genome Scan Studies of Idiopathic Generalized Epilepsies (from Crunelli and Leresche, 2002)</i>				
Linked gene or association	Protein	Subjects	Phenotype	Reference
GABRB3	GABA A receptor B3 subunit	50 probands	40 CAE only, 10 with infrequent GTCS	Feucht M et al. (1999)
OPRM1	μ-Opioid receptor type 1	72 probands	26 CAE, 46 JAE	Sander T et al. (2000)
CHRNA4	nAChR α4 subunit	108 probands	IGE	Steinlein et al. (1997)
KCNQ2	Voltage-gated K ⁺ channel Q2	115 probands	44 CAE or JAE; 71 JME	Steinlein et al. (1999)
CACNA1A	Voltage-gated Ca ²⁺ channel α1A-subunit	55 families	42 CAE or JAE; 26 JME	Sander T et al. (1998)
SCN1A	Voltage-gated Na ⁺ channel α1 subunit	165 families	83 CAE or JAE; 72 JME; 4 GTCS	Escayg et al. (2001)
CAE = Childhood absence epilepsy; JAE = Juvenile absence epilepsy; JME = Juvenile myoclonic epilepsy; GTCS = Generalized tonic-clonic seizures; IGE = Idiopathic Generalized Epilepsy				

most of the patients with IGE probably have multiple genetic “hits” in one or many types of channels rather than a single-point mutation.

Absence epilepsy is confined to certain networks

Absence seizures have characteristic, bilateral spike-and-wave discharges. As a result, absence seizures are considered “generalized”. However, evidence demonstrates that although much of the brain is involved, only certain networks of the brain are affected.

Human EEG recordings of absence seizures reveal a frontal predominance to the seizures (Rodin and Ancheta, 1987). In the WAG/Rij rat model, anesthetized rats were found to have focal increases in BOLD fMRI signal in anterior brain regions during spike-wave discharges that correspond to a frontal EEG predominance (Nersesyan et al, 2002).

Further, it seems that absence seizures occur along the same thalamocortical network as do normal sleep spindle oscillations. Depth recordings in humans revealed highly synchronized oscillations within the thalamocortical network (Williams, 1953). *Figure 4* shows the classical view of how absence seizures are generated, where the discharges originate from thalamocortical neurons (TC) and quickly generalize to the cortex. As is discussed below, it is now believed that (at least in some types of absence epilepsy) the seizures originate in the cortex and spread to the thalamus from there.

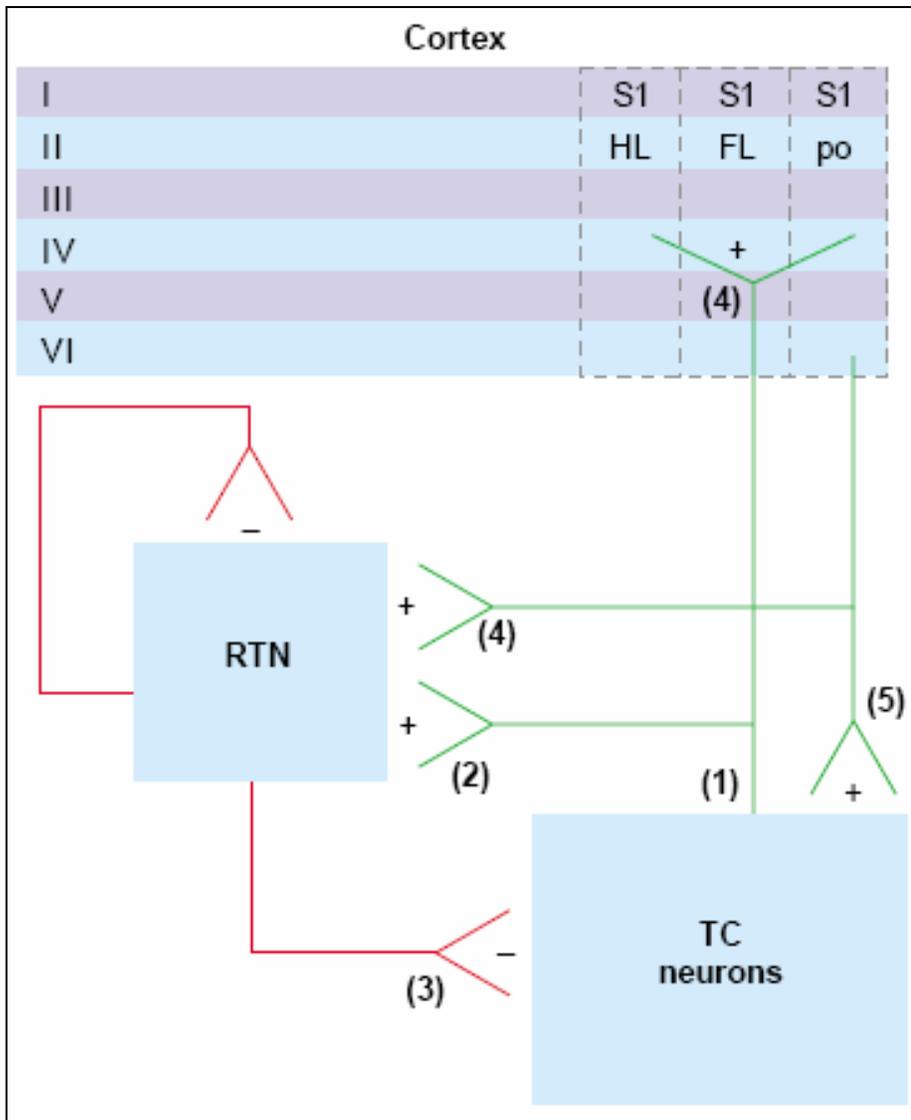
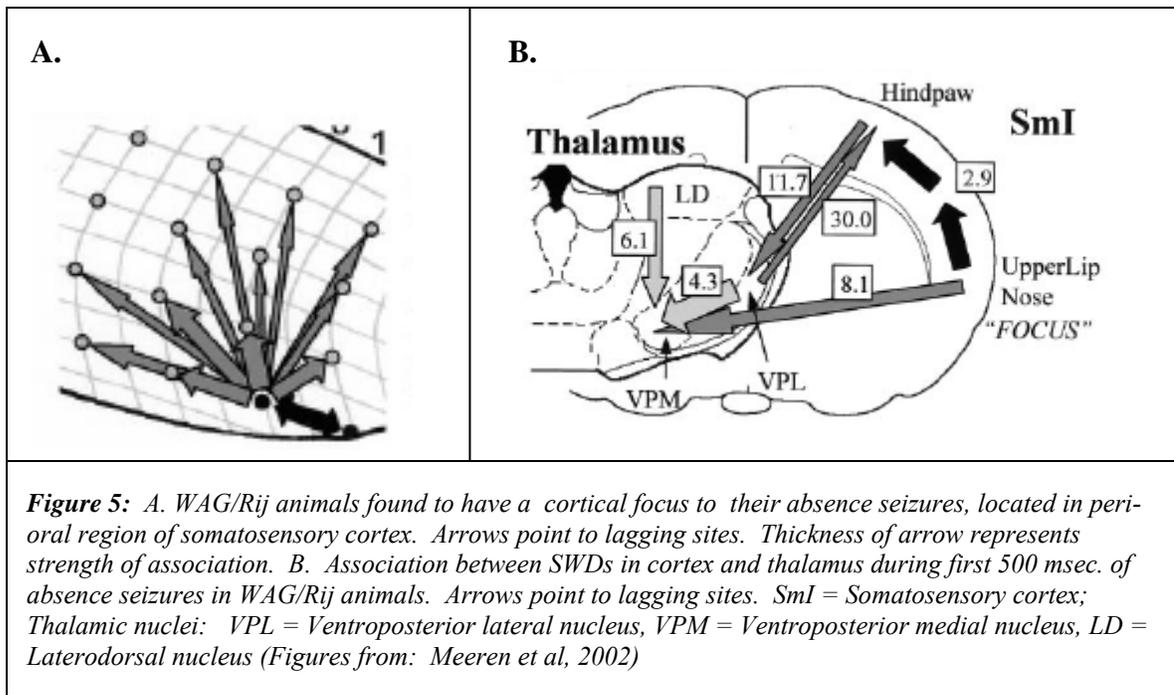


Figure 4: The classic view of absence seizure pathophysiology from Manning et al (2003). Activation of low-voltage Ca^{2+} (1) current in the thalamocortical (TC) neurons potentiates a burst of action potentials (2) in the reticular thalamic nuclei (RTN), leading to a high-frequency spontaneous burst of action potentials. Consequently, GABA is released (3) on TC neurons, (4) leading to further excitation via glutamate of RTN as well as the peri-oral region of the somatosensory cortex (S1po), as well as the forelimb (S1fl) and hindlimb (S1hl) regions of the somatosensory cortex. New evidence by Meeran et. al (2002) suggests that the initial discharge potentiating the SWD occurs in the cortex at the somatosensory cortex, leading to a spread to the thalamus and other regions of the cortex.

In vitro work on ferret lateral geniculate nuclei slices were performed to show how spindle waves and spike-wave discharges are generated (Blumenfeld and McCormick, 2000). If a single shock was given to simulate normal cortical firing, the result was 6-10 Hz spindle wave oscillations seen during normal sleep. However if a burst of shocks were applied to simulate increased cortical firing, the result was slower 3-4 Hz absence-like oscillations in this thalamocortical network. Therefore a laboratory induced over-excitation of this network produced the electrophysiologic equivalent of an absence seizure. It seems then that an in vivo overexcitation of the same network could produce spontaneous absence seizures. As discussed in the previous section, a dysregulation or dysfunction of ion channels can predispose an individual to increased burst firing in the cortex or thalamus, leading to 3-4 Hz spike-wave discharges.

In addition to the finding that absence seizures spare certain areas of the brain, recent evidence demonstrates that absence seizures may in addition have a focus that secondarily generalizes (Meeren et al, 2002). In the WAG/Rij rat, one rodent model of absence epilepsy, a consistent cortical focus was found within the peri-oral region of the somatosensory cortex. This cortical focus consistently preceded the thalamus and other cortical areas during the first 500 milliseconds of each spike-wave discharge. As illustrated in *Figure 5*, this initial SWD was then found to rapidly generalize to both hemispheres.



Animal Models of Absence Epilepsy

Table 5 lists many of the rodent models of absence epilepsy. Other models of absence epilepsy exist involving other species, as well as models in which absence epilepsy is induced by pharmacologic or other means (Danober, 1998). All of these animal models have both similarities and differences with human absence epilepsy. Our study will look at the mechanisms underlying the generation of absence seizures in the WAG/Rij rat model. WAG/Rij animals were derived by breeding a subset of normal Wistar rats that had been incidentally found to have SWDs on EEG.

WAG/Rij animals and humans with absence epilepsy share many things in common. WAG/Rij rats have “staring spells” and minor oro-facial movements during spontaneous SWDs on EEG. During these subtle motor events, the animals are otherwise immobile. Similar to humans, approximately 80% of the WAG/Rij SWDs occur during

quiet wakefulness or during slow-wave sleep (Drinkenberg et al, 1991). During the other 20% of seizures, the rats are usually actively awake or in deep slow-wave sleep.

WAG/Rij animals have no other obvious clinical abnormalities in contrast to some of the other rodent models (e.g. ataxia in some of the mouse models) listed in *Table 5*. The SWDs in WAG/Rij animals are effectively treated with ethosuximide and other absence drugs commonly used in humans (Coenen and van Luijtelaar, 2003). As with humans, WAG/Rij animals have a circadian pattern to their absence seizures and the SWDs are also aggravated by sleep deprivation (Burr et al, 1991; Drinkenburg et al, 1995).

There are also differences between the WAG/Rij model and human absence epilepsy. In human absence, SWDs are 3-4 Hz, while in WAG/Rij animals the SWDs are faster at 7-11 Hz. In humans, absence epilepsy most often begins and remits before puberty. This compares to the WAG/Rij model, where SWDs most commonly begin after puberty and increase in both duration and frequency until the rat dies (Coenen and van Luijtelaar, 2003). The absence epilepsies that do persist into adulthood – JAE, JME, and MAE – often have other seizures along with SWDs, which is not completely consistent with the WAG/Rij model either. Perhaps there is no fully analogous animal model to typical absence epilepsy, the most common being CAE.

More is known about animal SWDs versus human SWDs, as depth EEG recordings cannot be warranted in young children with benign absence seizures. In the WAG/Rij rat model, SWDs are bilaterally symmetric and generalize over the entire cortex, albeit with a frontal predominance (Coenen and van Luijtelaar, 2003). WAG/Rij seizures have recently been found to originate from a focus in the peri-oral region of the somatosensory cortex as depicted in *Figure 5* (Meeren et al, 2002).

Although some of the mice models listed in *Table 5* have SWDs more similar to the 3-4 Hz of humans, most of these mice have other behavioral abnormalities not seen in the human form. Therefore the WAG/Rij and GAERS rat models are perhaps the best clinico-EEG correlates to human absence epilepsy.

Table 5: Rat and mouse models of absence epilepsy (Table from: Crunelli and Leresche, 2002)

<u>Model</u>	<u>SWD frequency and duration</u>	<u>Age at onset of seizures</u>	<u>Neuropathological and behavioral abnormalities</u>	<u>Chromosome and gene/protein</u>
GAERS rat	7-11 Hz; 0.5-75 sec	>30 days (100% at 4 months)	Astrocytic alterations	
WAG/Rij rat	7-11 Hz; 1-45 sec	>75 days		
HVS rat Fisher 344 Brown Norway	7 Hz 8 Hz (not females)	Myoclonic movements		
Tottering mouse	6-7 Hz; 0.3-10 sec	>30 days	Increased noradrenergic innervation; cerebellar degeneration; moderate/severe ataxia; dystonia; rare tonic-clonic movements	Chromosome 9; P/Q Ca ²⁺ channel α 1 subunit
Lethargic mouse	5-6 Hz; 0.6-5 sec	>3 weeks	Ataxia and loss of motor coordination; focal myoclonus	Chromosome 2; Ca ²⁺ channel β 4 subunit
Stargazer mouse	6 Hz; longer than in tottering and lethargic	>2 weeks	Ataxia; impaired vestibular function	Chromosome 15; Ca ²⁺ channel g2 subunit (stargazin)
Mocha2j mouse	6 Hz		Hyperactivity	Chromosome 10; adaptor-like protein complex (AP-3)
Slow-wave-epilepsy mouse	3-4.5 Hz	Depends on genetic background	Ataxia; tonic-clonic seizures; neurodegeneration	Chromosome 4; Na ⁺ /H ⁺ exchanger (Nhe1)
Ducky mouse	6 Hz		Ataxia; paroxysmal dyskinesia	Chromosome 9

Ethosuximide and Absence Epilepsy

Both ethosuximide and valproate stop SWDs in human absence epilepsy as well as in the WAG/Rij and GAERS rat models of absence epilepsy (Manning et al, 2003). The mechanisms of action of anti-absence drugs ethosuximide and valproate have been elucidated for the most part. An understanding of these drugs' mechanisms helps us better understand the pathogenesis of absence epilepsy.

The anti-absence drug valproate has been shown to increase levels of GABA in specific regions of the brain. However it is unlikely that this is the mechanism by which valproate suppresses absence seizures, as GABA agonists more often exacerbate absence epilepsy. Instead, valproate's main effect is probably in its ability to inhibit voltage-gated sodium channels (Manning et. al 2003). In contrast to ethosuximide, valproate has been shown to have no effect on calcium currents in thalamic neurons (Coulter et al, 1989).

It has been postulated that ethosuximide controls absence seizures by reducing calcium currents via T-type calcium channels in the thalamus (Coulter DA et al., 1989). Ethosuximide has also been shown to suppress the persistent sodium current (I_{NaP}) and sustained Ca^{2+} -activated potassium currents ($I_{K(Ca)}$) which may also contribute to ethosuximide's anti-absence properties (Crunelli and Leresche, 2002; Leresche et al., 1998).

Although it was once thought that ethosuximide's main action was suppression of the aforementioned T-type calcium channels in the thalamus, recent evidence suggests that ethosuximide's main action is in the cortex. As mentioned earlier, Meeren et al. (2002) identified a seizure focus in the WAG/Rij animal corresponding to this

somatosensory cortex. Further, ethosuximide infused into thalamic nuclei of WAG/Rij rats did not fully suppress absence seizures (Richards et al, 2003), whereas bilateral infusion of ethosuximide in the somatosensory cortices fully suppressed seizure generation in GAERS animals (Manning et al., 2003).

Some have suggested that ethosuximide and valproate suppress absence seizures by blunting the persistent Na^+ current (I_{NaP}). I_{NaP} is a slowly inactivating and depolarizing current. Since I_{NaP} activates near firing threshold, increased activation of this channel can lead to neuronal hyper-excitability and thus seizure activity. However in vitro experiments of whole-cell patch-clamp recordings on thin rat hippocampal slices (in CA1 pyramidal neurons) have shown that ethosuximide only mildly reduces I_{NaP} while valproate has no effect at all (Niespodziany et al., 2004). This study only looked at I_{NaP} in hippocampal neurons, while it seems the region of interest is in the cortex, and further investigations at the persistent current in this region are warranted (Sue, 2001).

Now that we know that the WAG/Rij animals' seizures originate from the peri-orral region of the somatosensory cortex, the next question is: Why does ethosuximide infused into the cortex prevent SWDs? Is there a specific channelopathy in the WAG/Rij rat that is to blame for this animal's pathology?

II. Hypothesis

In the human brain, ion channels are responsible for both normal and abnormal electrical activity. Several single-channel defects have been identified as the likely cause of absence epilepsy, and many other ion channel defects have been linked to either individuals or families with absence seizures (Crunelli and Leresche, 2002). It seems

plausible that multiple defects of multiple ion channels can cause the many manifestations of absence epilepsy, and although some individuals may have a single channelopathy leading to epilepsy, it is more likely that individuals will have multiple “hits” that result in their epilepsy. Perhaps someday, individuals with absence epilepsy will be identified by their specific ion channelopathies and/or channel dysregulation and will be treated with targeted agents.

Recent evidence in the WAG/Rij rat – one rodent model of absence epilepsy – reveals that spike-wave discharges (SWDs) consistently begin in the peri-oral region of the somatosensory cortex and rapidly generalize to involve both hemispheres as well as subcortical structures. Our hypothesis is that either defective or abnormally regulated ion channels in the somatosensory cortex are the cause of SWDs in the WAG/Rij rat. Because one of the major central nervous system ion channels is the sodium channel, we will look at 3 subunits of sodium channels that are plentiful in the CNS of both humans and rats – $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ – to see if their expression is altered in the cortices of WAG/Rij versus control animals.

III. **Methods**

Animals

The epileptic WAG/Rij rats were compared to the age-matched Wistar control animals. The details of the WAG/Rij animals are included in the Introduction. The WAG/Rij animals were originally bred at the Radiobiological Institute, TNO in Rijswijk (Reinhold, 1966) and a colony of WAG/Rij rats has been maintained at Yale University for the past 40 years. The Wistar rats were obtained from the Charles River Laboratories

in Wilmington, MA. The animals were housed in an animal facility, kept on a 12 hour light/dark cycle with unlimited access to food and water in accordance with the NIH guidelines for the care and use of laboratory animals. Animal protocols were approved by the Yale University Institutional Animal Care and Use Committee.

In all, 25 animals were used in this study. As shown in *Table 6*, 18 of the animals were used for quantitative PCR of sodium channel subunits and 7 were used for immunocytochemistry. In addition to these 25 animals, two 18-month old WAG/Rij animals had EEG data recorded, but because no age-matched controls were available, no PCR or immunocytochemistry was performed on these animals.

Table 6: Animals used in this study

		# of animals	# of animals
Animal	Age	for PCR	for immunocytochemistry
<i>WAG/Rij</i>	2 month	2	
<i>(epileptic)</i>	4 month	3	4
	6 month	4	
<i>Wistar</i>	2 month	2	
<i>(control)</i>	4 month	3	3
	6 month	4	
	Total	18	7

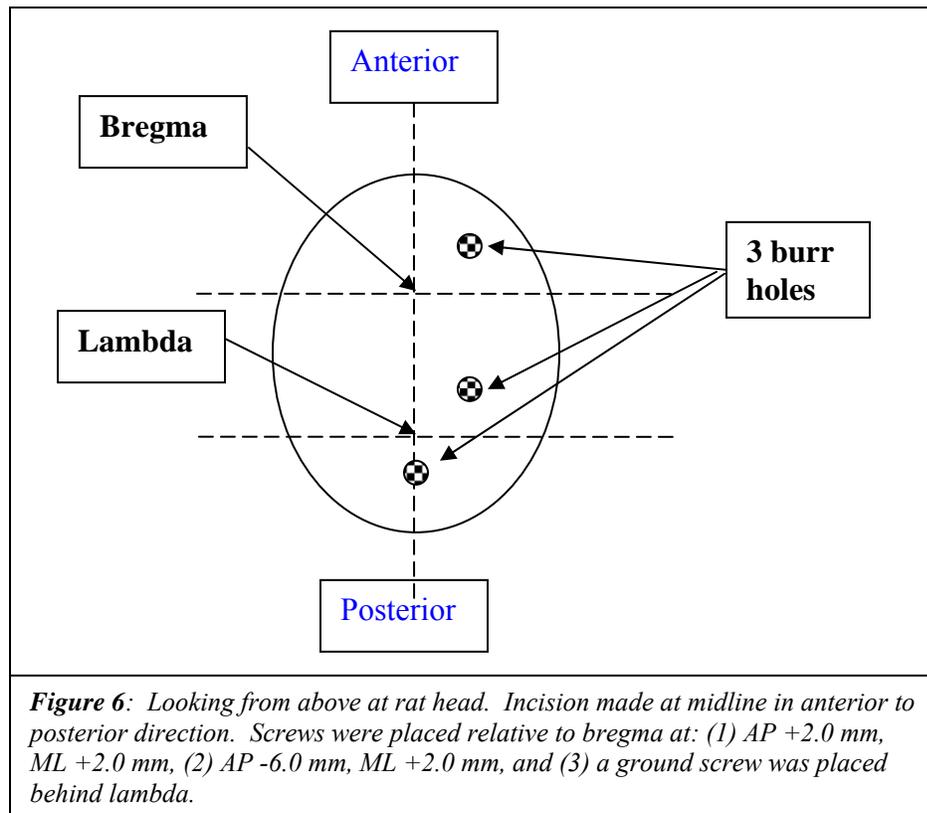
Electrode Placement

I performed all of the following electrode placements and subsequent EEG recordings, as well as the data analysis.

Fully awake animals were weighed and then anaesthetized with 4 MAC halothane for ~4 minutes until the animal became unresponsive to footpinch. The animal was then deeply anaesthetized with an intramuscular cocktail of ketamine (100 mg/kg), xylazine (5.2 mg/kg), and acepromazine (1.0 mg/kg) and placed in a stereotactic frame (David

Kopf Instruments, Tujunga, CA). During the procedure, the level of anesthesia was monitored by respirations, heart rate, skin perfusion, and response to foot pinch. If the rat began responding to foot-pinch, 0.02 cc's of IM anesthetic cocktail was administered.

Once placed in the stereotactic frame, the scalp was shaved and a mid-line incision was made over the skull. Bleeding was controlled with electrocautery. Burr holes were made in 3 locations as shown in *Figure 6*, with care taken not to disturb the dura. 1.60-mm stainless steel screws (Plastics One) were partly screwed into these burr holes, a tripolar electrode was attached to the three screws, and then the screws were completely fastened to the skull. Dental acrylic (Lang Dental Mfg, Wheeling, IL) was used to secure the electrode apparatus.



EEG Recording

After a one-week recovery period, EEG recordings were performed on awake-behaving rats, 2 hours/day over a 3-day period. Recordings took place between 10am-4pm each day. EEG signals were amplified with a Grass CP 511 unit (Grass-Telefactor, Astro Med) with band pass filter settings of 1-100 Hz. A CED Power 1401 digitized the EEG signals, and EEG was stored and analyzed using Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

SWDs were defined as large-amplitude ($>400\mu\text{V}$ peak-to-peak) rhythmic 7-11 Hz discharges with spike-wave morphology lasting >0.5 seconds. After recording, each WAG/Rij and Wistar animal's 3 recordings were analyzed for # of SWDs and % time in SWD.

RNA extraction and cDNA synthesis

The following methods for RNA extraction and cDNA synthesis, quantitative real-time PCR, immunocytochemistry, and data analysis are from Klein et al (2004).

Rats were deeply anaesthetized with CO_2 , decapitated, and brains were quickly removed. Twelve plugs of tissue, each measuring approximately 1 mm^3 were dissected from the left and right cortex of each rat using iridectomy scissors. For each hemisphere, three plugs at AP +3, 0, -6 and ML +6 mm, and three plugs at AP +3, 0, -6 and ML +2 mm were taken (see *Figure 9* inset). Tissue plugs included all six layers of cortex but did not extend into subcortical tissue. Tissue was immediately frozen in dry ice and stored at $-80\text{ }^\circ\text{C}$ until use.

Total RNA from brain tissue was extracted using RNeasy mini-columns (Qiagen). The purified RNA was treated with RNase-free DNase-I (Roche) and re-purified using an RNeasy mini-column (Qiagen). RNA was then eluted in 50 μ l of water. First-strand cDNA was reverse transcribed in a final volume of 50 μ l using 5 μ l purified total RNA, 1 mM random hexamer primer (Roche), 40 U SuperScript II reverse transcriptase (Life Technologies), and 40 U of RNase inhibitor (Roche). The buffer consisted of (in mM): 50 Tris-HCl (pH 8.3), 75 KCl, 3 MgCl₂, 10 DTT, and 5 dNTP. The reaction proceeded at 37 °C for 90 min and 42 °C for 30 min, and was then terminated by heating to 95 °C for 5 min. A parallel reaction was performed as a negative control to demonstrate the absence of contaminating genomic DNA (data not shown) by using all identical reagents except for the reverse transcriptase enzyme.

Quantitative real-time PCR

The relative standard curve method was used to quantify and compare RNA extracted from different regions of cortex in epileptic (WAG/Rij) and control (Wistar) rats. An 18S rRNA primer-probe set (Applied Biosystems) was used as an endogenous control to normalize the expression level of the sodium channels. Standard curves for 18S rRNA and each sodium channel primer/probe set were constructed using serial dilutions of control brain cDNA. Standards and unknowns were amplified in quadruplets. Standard curves for the sodium channel primer/probe sets and endogenous control (rRNA) were constructed from respective mean critical threshold (C_T) values; the equation describing the curve was derived using Sequence Detector software v1.6.3 (Applied Biosystems).

Primers and probes for the sodium channel targets were designed using Primer Express software (Applied Biosystems) according to the specifications of the TaqMan protocol (Winer et al, 1999). Sequences are as follows: Nav1.1 forward 5'-TCCTGGAGGGTGTTTTAGATGC-3', reverse 5'-AAAGATTTTCCCAGAAGTCCTGAG-3', probe FAM-CTGGGCATTTCTGTCCCTGTTTCGACT-TAMRA; Nav1.2 forward 5'-CATCAAGTCCCTCCGAACGTTA-3', reverse 5'-GGCAGACCAGAAGTACGTTTCATT-3', probe FAM-CCTTATCCCGATTTGAAGGAATGAGGGTTG-TAMRA; Nav1.6 forward 5'-AGTAACCCTCCAGAATGGTCCAA-3', reverse 5'-GTCTAACCAGTTCCACGGGTCT-3', probe FAM-AATCATCGCAAGAGGTTTCTGCATAGACGG-TAMRA. Target specificity was confirmed by nucleotide BLAST search. Primers and probes for sodium channels were synthesized and purified by Applied Biosystems. Primers for the sodium channels and 18S rRNA were used at a final concentration of 900 and 50 nM, respectively, whereas the probes were used at a final concentration of 200 nM. The primer-probe combinations were not limiting at these concentrations. Amplification was done in a 25- μ l final volume, under the following cycling conditions: 10 min at 50 °C and then 40 cycles of 95 °C, 15 s, followed by 60 °C, 1 min. An ABI Prism 7700 (Applied Biosystems) was used to run the PCR reaction and data was recorded using Sequence Detector v1.6.3. Sodium channels and 18S rRNA templates were amplified in separate wells. The amount of mRNA in different regions of cortex was reported as the ratio of mRNA in the epileptic rats divided by mRNA in the control rats.

Immunocytochemistry

Rats were anesthetized with ketamine/xylazine (80/5 mg/kg i.p.) and then underwent intracardiac perfusion with 0.01 M PBS followed by a 4% solution of cold buffered paraformaldehyde. Brains from 4-month WAG/Rij and Wistar rats were postfixed and cryoprotected in 30% sucrose in 1 M phosphate buffer solution (PBS), and coronal sections (10 μ m) of the cerebral hemispheres, including all regions studied by quantitative PCR, were cut. Slices were mounted onto slides and incubated in blocking solution (5% normal goat serum and 1% BSA in PBS) containing 0.1% Triton X-100 and 0.02% sodium azide at room temperature for 30 min, then incubated with subtype-specific antibodies to sodium channel α -subunits Nav1.1 (residues 465–481, 1:100 dilution, Alomone, Jerusalem), Nav1.2 (residues 467–485, 1:100 dilution, Alomone), Nav1.6 (residues 1042–1061, 1:100, Alomone), and a phospho-CREB antibody (1:50, Cell Signaling Technology, Beverly, MA) overnight at 4 °C. Slides were washed in PBS and incubated with biotinylated goat anti-rabbit serum (1:1000, Sigma) in blocking solution for 3 h, then washed in PBS and incubated in avidin–HRP (1:1000, Sigma) in blocking solution for 3 h. Slides were washed in PBS and exposed to heavy metal enhanced 3,3'-diaminobenzidine·4HCl in 1 \times peroxide substrate buffer (Pierce, Rockford, IL) for 7 min.

Data analysis

A Nikon Eclipse E800 light microscope was used for sample observation, and quantitative microdensitometry of immunostaining signals was obtained using IPLab v3.0 Image Processing software (Scanalytics., Fairfax, VA). Signal intensities were determined by outlining individual cortical neurons, and IPLab integrated densitometry

functions were used to calculate mean signal intensities for the selected areas. Results from identical regions and layers of cortex in WAG/Rij (epileptic) rats were compared to Wistar (control) rats processed in parallel and differences were assessed by non-paired *t*-tests. Immunopositivity was quantified by averaging multiple counts within a defined area ($1.9 \times 10^4 \mu\text{m}^2$) within layers II–IV. For counts of immunopositive neurons, cells that displayed a signal of >50% above background were scored as positive.

For the EEG data in *Figure 7* and the quantitative PCR data in *Figure 9*, differences were analyzed using ANOVA with post hoc Fisher's least significant difference analysis with Bonferroni adjustment. An alpha level of 0.05 was used as a threshold for statistical significance.

IV. Results

WAG/Rij vs. control EEG data

Figure 7, A-D, shows typical 60-second recordings from a Wistar control, and two-, four-, and six-month old WAG/Rij animals. *Figure 7-E* shows a typical 7-11 Hz SWD on an expanded timescale.

In this study, WAG/Rij rats had recordable seizures as early as 2-months of life, and the percent time spent in SWD increased with increasing age as shown in *Figure 7-F*. Percent time spent in SWD depends on both the duration of each SWD and the frequency of SWDs (number SWDs per minute). A certain percentage (~15%) of Wistar control rats also had seizures. These epileptic Wistar rats were not used either in EEG recordings or in RNA/immunocytochemistry data. Only those Wistar rats that exhibited no seizures clinically and had no SWD on EEG were used as control animals.

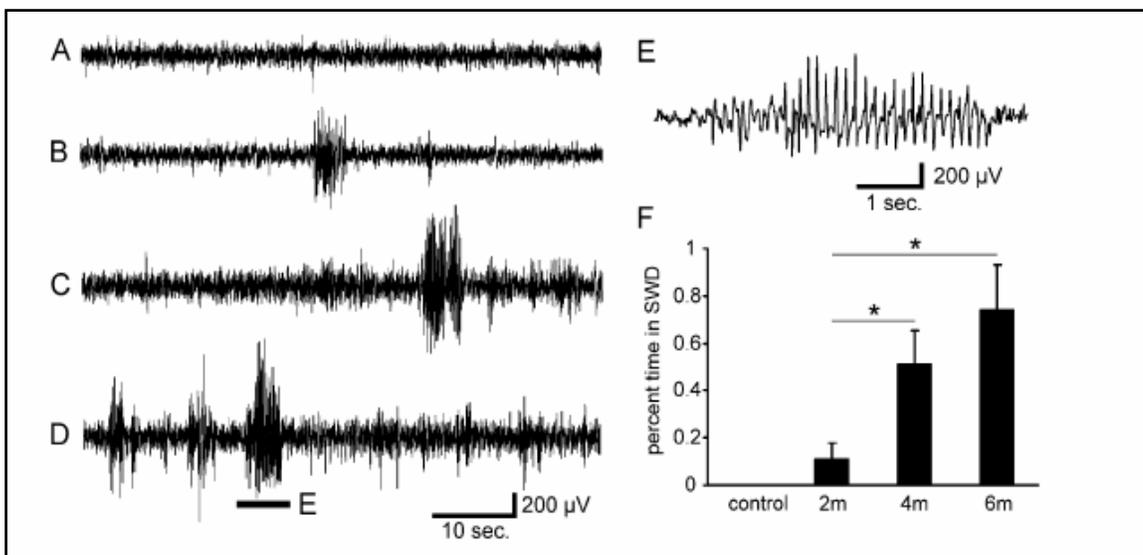
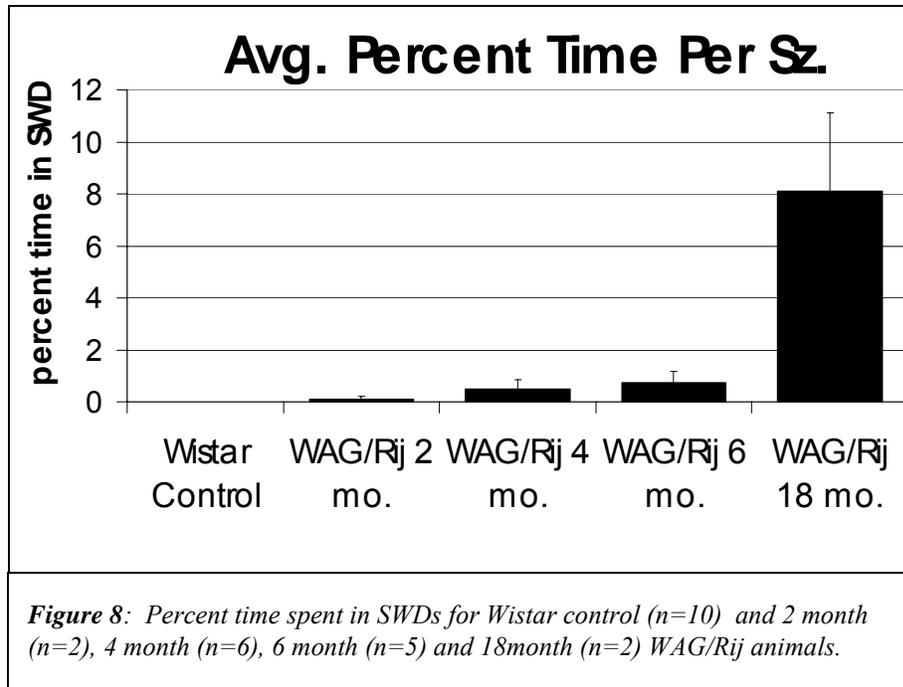


Figure 7: Typical 60-second EEG recordings from (A) a Wistar control, and (B) 2-month, (C) 4-month, and (D) 6-month old WAG/Rij animals. (E) is the magnified spike-wave discharge shown in (D). (F) shows the Wistar control animals (n=10) with 0% time in SWD, and the increase in % time in SWD as the WAG/Rij animals increase in age [WAG/Rij at 2 (n=2), 4 (n=6), and 6(n=5) months]. Data is plotted as mean SWD±S.E, *= $p < 0.05$ where 4 and 6 months were each compared to 2 month old animals.

As noted previously, all WAG/Rij rats in this study had more than one seizure during the total six hours of recording. In addition, the percent time spent in SWD increased with increasing age as shown in both *Figure 7-F* (excluding 18-month data) and *Figure 8*.

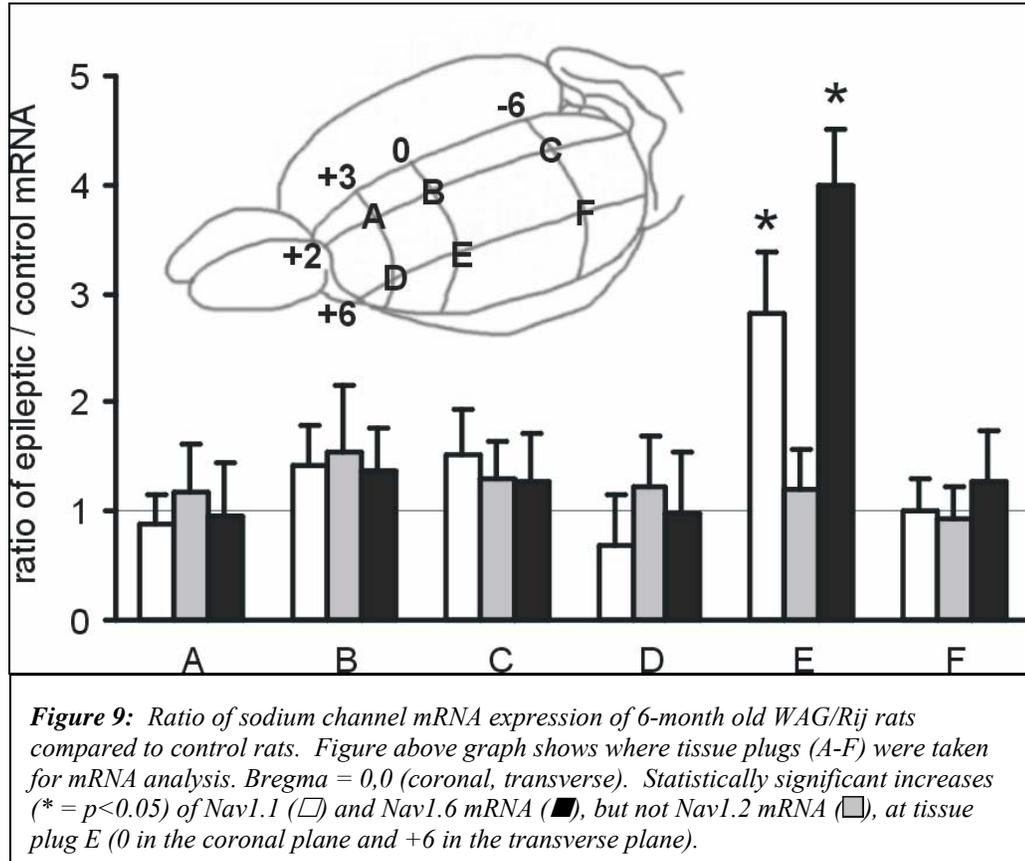
As shown in *Figure 8*, the WAG/Rij animals spent $0.11 \pm 0.070\%$ (mean \pm S.E.) of the time in SWDs at 2 months of age, $0.51 \pm 0.141\%$ at 4 months, $0.74 \pm 0.188\%$ at 6 months, and $8.1 \pm 3.01\%$ at 18 months. As shown in *Figure 7-F*, the difference of percent time in SWDs between 2- and 4-month, as well as between 2- and 6-month WAG/Rij rats, was statistically significant ($p < 0.05$). The difference between the 4- and 6-month animals was not statistically significant.



With increasing age, both seizure frequency and seizure duration rose. WAG/Rij rats had a seizure frequency of 0.023 ± 0.017 seizures/minute at 2 months of age, 0.19 ± 0.08 sz/min. at 4 months, 0.14 ± 0.06 sz/min. at 6 months, and 0.68 ± 0.26 sz/min at 18 months. Average seizure durations for these WAG/Rij rats were 2.79 ± 1.17 s. at 2 months of age, 2.61 ± 1.67 s at 4 months, 3.14 ± 1.83 s at 6 months, and 7.30 ± 4.15 s at 18 months old.

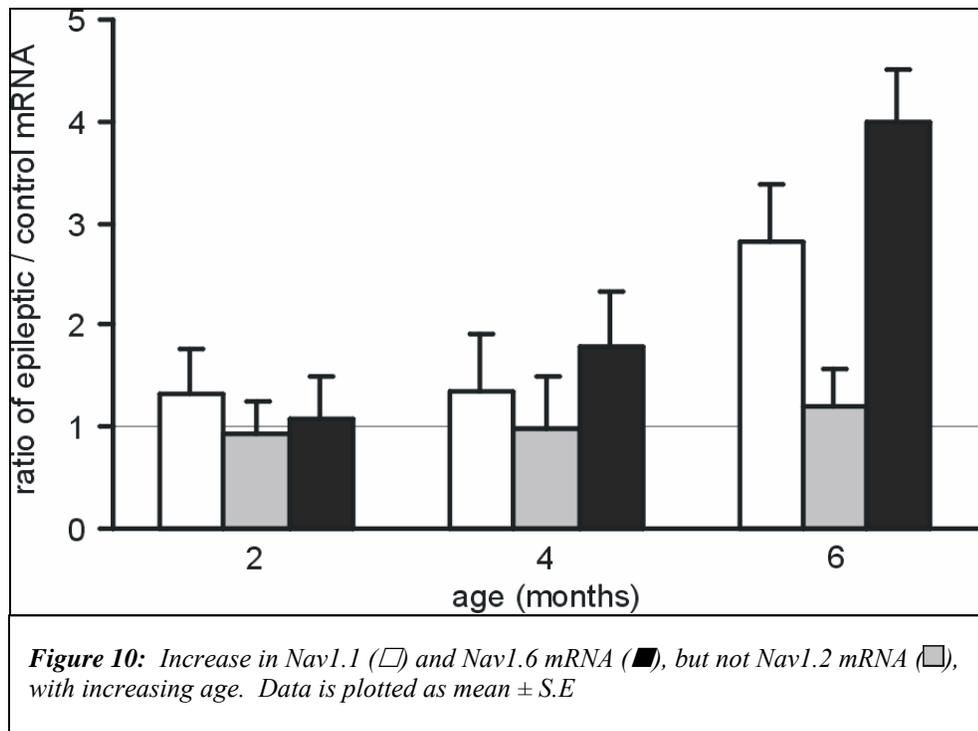
Sodium Channel mRNA in WAG/Rij rats and Controls

Sodium channels $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$ were all found to be present in the cortices of both control and epileptic animals. $Na_v1.3$ was expressed at very low levels in both animals while $Na_v1.8$ was not detected in either epileptic or control animals.



As shown in *Figure 9*, six tissue plugs labeled A-F were taken from both left and right hemispheres of each control and epileptic rat. *Figure 9* demonstrates that both Nav1.1 and Nav 1.6 are significantly up-regulated at AP +6 and ML 0 (bregma), roughly corresponding to the region of seizure onset in WAG/Rij animals found by Meeren et al (2002).

As WAG/Rij animals age, their levels of Nav 1.1 and Nav 1.6 (at tissue plug E in *Figure 9*) increased as shown in *Figure 10*. Nav 1.2 remained stable over the three age groups.



Sodium Channel Expression in WAG/Rij and Controls

Figure 11 shows representative cortical slices of both control and WAG/Rij animals, immunostained for Nav1.1, Nav1.2, Nav1.6, and phospho-CREB, a marker of transcription activation. Qualitatively, there is an upregulation of both Nav1.1 and Nav1.6 in layers II-IV of the cortex at bregma in the coronal plane and +6 in the transverse plane. Other parts of cortex, and other layers within the cortex did not exhibit any marked difference in the expression of these sodium channels between control and WAG/Rij animals (data not shown). Also, there was no difference between control and WAG/Rij animals in Nav1.2 expression at any location within the cortex sampled.

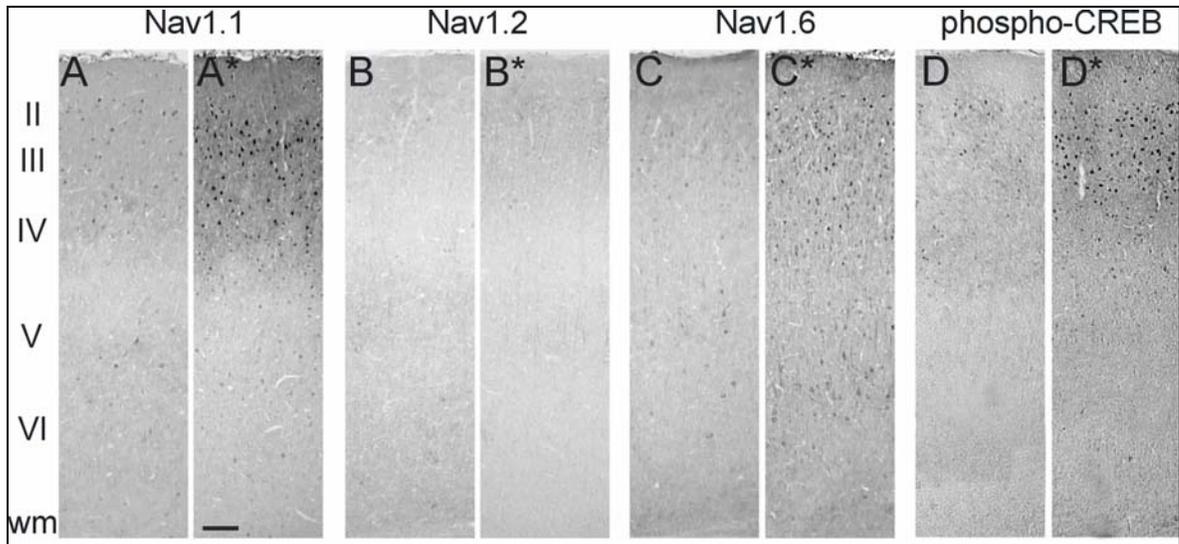


Figure 11: Coronal sections of cortex at bregma and +6 in the transverse plane. Sodium channel protein Nav1.1, Nav1.2, and Nav1.6 exhibited by immunocytochemistry in different layers of cortex, II-VI, for control Wistar and WAG/Rij (*) animals. Phospho-CREB = a marker of transcription activation. wm = white matter. scale bar = 100 μ m.

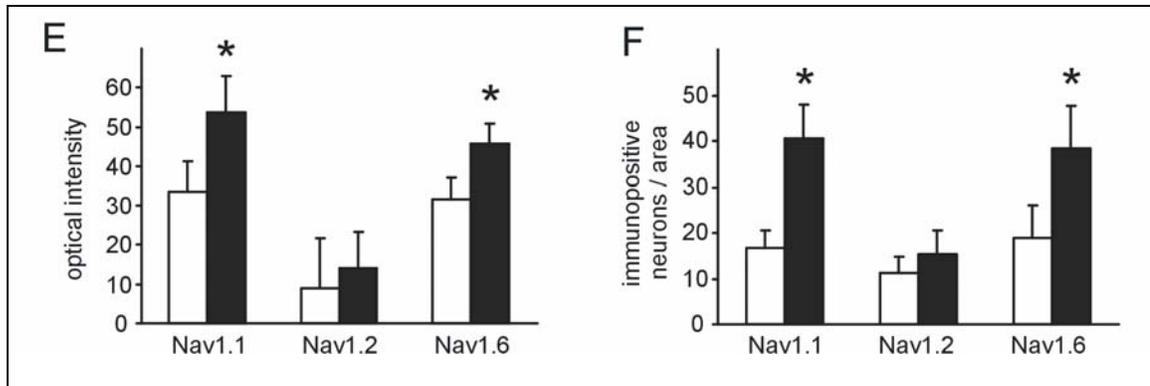


Figure 12: Increase in Nav1.1 and Nav1.6 expression in layers II-IV of cortex at ML=0, AP=+6. E, Optical intensity quantification shows increased (* = $p < 0.05$) Nav1.1 and Nav1.6 in epileptic animals (■) compared to controls (□). F, Quantification of immunopositive neurons in epileptic (■) and control (□) animals shows a significant increase (* = $p < 0.05$) in the number of neurons/area positive for Nav1.1 and Nav1.6.

This qualitative data demonstrates that Nav1.1 and Nav1.6 are increased in epileptic animals. This data was quantified by optical intensity quantification and quantification of immunopositive neurons, shown in Figure 12, E and F. The increase

in $\text{Na}_v1.1$ and $\text{Na}_v1.6$ in epileptic rats vs. controls was statistically significant both in optical intensity and immunopositive staining per unit area.

V. Discussion

The recent evidence that spike-wave discharges in the WAG/Rij rat model of absence epilepsy originate from a single cortical focus (Meeren et al., 2002) suggest that a focal cortical defect may be the cause of SWDs in this particular rodent model. Our findings suggest that two voltage-gated sodium channel subunits, $\text{Na}_v1.1$ and $\text{Na}_v1.6$, are upregulated in the WAG/Rij animal vs. control at this cortical electrographic focus. Furthermore, immunocytochemistry of the cortex shows an increase in $\text{Na}_v1.1$ and $\text{Na}_v1.6$ in cortical layers II-IV.

What does the upregulation of $\text{Na}_v1.1$ and $\text{Na}_v1.6$ imply? From our study, it is unclear whether the increase in $\text{Na}_v1.1$ and $\text{Na}_v1.6$ in epileptic rats is the cause or effect of the WAG/Rij SWDs. Although it seems plausible that an increase in sodium channels in a focal area of the brain could lead to spontaneous SWDs, it is also possible that the seizures themselves cause an increase in sodium channels in the peri-oral region of the somatosensory cortex. Further studies of other ion channels in the cortex, as well as sodium and other ion channels in subcortical structures, is needed for further insight into this question.

The next question is: Does an increase in sodium channels make an animal (or human) more susceptible to seizures? We do know that sodium channels are responsible for the initiation and spread of action potentials along a neuron. Presumably an increased

amount of sodium channels could lower the seizure threshold by bringing the membrane potential of neurons closer to depolarization.

As discussed in the Introduction, many sodium channel mutations have been linked with epilepsy syndromes. In fact, more than 150 mutations in the sodium channel gene SCN1A, coding for the protein Nav1.1, have been found in various human epilepsy syndromes as of August 2005. Variants of another sodium channel gene SCN2A, coding for the protein Nav1.6, have been linked to human patients with seizures (Meisler and Kearney 2005).

As noted earlier, the WAG/Rij model does not perfectly mirror human absence epilepsy. Whereas approximately 70% of human absence remits by puberty, SWDs persist in WAG/Rij animals. In fact, as WAG/Rij animals age, the severity of their epilepsy worsens. In our study, older WAG/Rij animals spent a larger percentage of time in SWD versus younger animals. In parallel with this, the older WAG/Rij animals also had an increased amount of $Na_v1.1$ and $Na_v1.6$ mRNA at the presumed seizure focus versus their younger counterparts (see *Figure 10*). This result indicates that increasing amounts of $Na_v1.1$ and $Na_v1.6$ in the peri-oral region of the somatosensory cortex portend worsening epilepsy, whether because more of these channels lower the seizure threshold or because more seizures cause more sodium channels to be present in this area of cortex.

Immunocytochemistry of cortical slices revealed an upregulation of $Na_v1.1$ and $Na_v1.6$ in cortical layers II-IV. Cortical layers II and III involve mainly cortical-cortical connections; layer IV receives inputs from the thalamus (Kandel, Schwartz, and Jessell, 2000).

If the dysregulation of sodium channels in the somatosensory cortex is in fact the cause of seizure genesis in WAG/Rij rats, it is somewhat surprising that there was no dysregulation of $Na_v1.1$ or $Na_v1.6$ in layer VI of the cortex, as it is this layer that sends outputs from the cortex to thalamic structures. This is perhaps evidence against the fact that increased $Na_v1.1$ and $Na_v1.6$ are the primary defect leading to SWDs in the WAG/Rij rat. Again, further studies are required to elucidate this, and to see if other ion channelopathies are present as well.

Our lab is currently looking at the effects of ethosuximide on sodium channel composition in the cortex. In this as yet unpublished experiment, there were 15 epileptic and 16 control rats. One-half of the epileptic group were placed on water, while the other were given an ethosuximide solution at 300 mg/kg/d starting shortly after birth.

WAG/Rij animals on ethosuximide had a very low seizure frequency comparable to the control animals, while those on water had a much higher seizure frequency. The results show the epileptic animals on ethosuximide shortly after birth had a normalization of sodium channel expression comparable to that of the control rats, while the epileptic rats on water alone continued to have an increased expression of $Na_v1.1$ and $Na_v1.6$. This data suggests that ethosuximide alters the sodium channel composition when administered early in the life of the epileptic animal.

It may turn out that many different channel defects and many different ion channels can cause the different forms of absence epilepsy. This and other studies of its kind could, in the end, lead to ion channel subunit-specific therapies. This is of course enticing, as a targeted therapy could hypothetically control seizure activity without the untoward side effects. In addition, someday it may be possible to do a thorough genetic

analysis of an individual to see if they have a channelopathy. If a channelopathy were found, prophylactic therapy could be initiated to both prevent seizures as well as the behavioral and intellectual consequences of a seizure disorder.

VI. References

- Aminoff, M.J. (2001). Neurology and General Medicine. 3rd Edition. Philadelphia, Pennsylvania: Churchill Livingstone.
- Aronica, E., B. Yankaya, et al. (2001). Induction of neonatal sodium channel II and III alpha-isoform mRNAs in neurons and microglia after status epilepticus in the rat hippocampus. *European Journal of Neuroscience*. 13(6): 1261-6.
- Bartolomei, F., M. Gastaldi, et al. (1997). Changes in the mRNAs encoding subtypes I, II and III sodium channel alpha subunits following kainate-induced seizures in rat brain. *Journal of Neurocytology*. 26(10): 667-78.
- Blumenfeld H, McCormick D A. (2000) Corticothalamic Inputs Control the Pattern of Activity Generated in Thalamocortical Networks. *The Journal of Neuroscience* 20(13):5153-5162.
- Blumenfeld, H. (2003). "From Molecules to Networks: Cortical/Subcortical Interactions in the Pathophysiology of Idiopathic Generalized Epilepsy." *Epilepsia* 44(s2): 7-15.
- Burr, W., Korner, E., and Stefan, H. (1991). Circadian distribution of generalized spike-wave activity in relation to sleep. *Epilepsy Res. Suppl.* 2: 121-35.
- Chang, BS, Lowenstein, DH. (2003). Epilepsy. *N Engl J Med* 349:1257.
- Coenen, A. M. and E. L. Van Luijtelaaar (1987). The WAG/Rij rat model for absence epilepsy: age and sex factors. *Epilepsy Research* 1(5): 297-301.
- Coenen, A. M. and E. L. Van Luijtelaaar (2003). Genetic Animal Models for Absence Epilepsy: A Review of the WAG/Rij Strain of Rats. *Behavior Genetics* 3(6): 635-656.
- Commission of Classification and Terminology of the International League Against Epilepsy. (1981). Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia* 22:489-501.

Commission on Classification and Terminology of the International League Against Epilepsy. (1989). Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 30:389-99.

Coulter DA, Huguenard JR, Prince DA. (1989). Specific petit mal anticonvulsants reduce calcium currents in thalamic neurons. *Neuroscience Letters*. 98(1):74-8.

Coulter DA. (1997) Antiepileptic drug cellular mechanisms of action: where does lamotrigine fit in? *J Child Neurol* 12(Suppl 1):S2-9.

Crunelli, V. and N. Leresche (2002). Childhood absence epilepsy: genes, channels, neurons and networks. *Nature Reviews Neuroscience* 3(5): 371-82.

Czapinski P, Blaszczyk B, Czuczwar SJ. (2005). Mechanisms of action of antiepileptic drugs. *Current Topics in Medicinal Chemistry*. 5(1): 3-14.

Danober L., Deransart C., Depaulis A., Vergnes M., Marescaux C. (1998). Pathophysiological Mechanisms of Genetic Absence Epilepsy in the Rat. *Progress in Neurobiology* 55: 27-57.

Delmas P, Raggenbass M and Gola M. (1997). Low-threshold Na⁺ currents: a new family of receptor-operated inward currents in mammalian nerve cells. *Brain Res Brain Res Rev* 25: 246-254.

Drinkenberg, W.H.I.M., Coenen, A.M.L., Vossen, J.M.H. and van Luijtelaaar, E.L.J.M. (1991). Spike-wave discharges and sleep-wake states in rats with absence epilepsy. *Epilepsy Res*. 9:218-224.

Drinkenburg, W.H.I.M., Coenen, A.M.L., Vossen, J.M.H., and van Luijtelaaar, E.L.J.M. (1995). Sleep deprivation and spike-wave discharges in epileptic rats. *Sleep* 18:252-256.

Engel J Jr. (2001). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 42(6):796-803.

Engel Jr. J, Pedley TA. (1997). Epilepsy: A Comprehensive Textbook. Lippincott-Raven Publishers, Philadelphia PA.

Escayg A. et al. (2001). A novel SCN1A mutation associated with generalized epilepsy with febrile seizures plus – and prevalence of variants in patients with epilepsy. *Am. J. Hum. Genet.* 68: 866-873.

Fadiman, A. (1997). The Spirit Catches You and You Fall Down. New York: Farrar, Strauss, and Giroux.

- Feucht M et al. (1999). Possible association between childhood absence epilepsy and the gene encoding GABRB3. *Biol. Psychiatry* 46: 997-1002.
- Futatsugi, Y. and J. J. Riviello, Jr. (1998). Mechanisms of generalized absence epilepsy. *Brain Dev* 20: 75-79.
- Gastaldi, M., A. Robaglia-Schlupp, et al. (1998). mRNA coding for voltage-gated sodium channel beta2 subunit in rat central nervous system: cellular distribution and changes following kainate-induced seizures. *Neuroscience Letters*. 249(1): 53-6.
- Goldin, A. L. (2001). Resurgence of sodium channel research. *Annual Review of Physiology*. 63: 871-94.
- Goldin, A. L. (2002). Evolution of voltage-gated Na(+) channels. *Journal of Experimental Biology*. 205(Pt 5): 575-84.
- Goldin, A. L., R. L. Barchi, et al. (2000). Nomenclature of voltage-gated sodium channels. *Neuron*. 28(2): 365-8.
- ILAE (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia* 22:489-501.
- Jouveneau A et al. (2001). Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet*. 358: 801-807.
- Kandel ER, Schwartz JH, Jessell TM (eds.). (2000). Principles of Neural Science, 4th Ed. McGraw-Hill, New York.
- Klein JP. Khera DS. Nersesyan H. Kimchi EY. Waxman SG. Blumenfeld H. (2004). Dysregulation of sodium channel expression in cortical neurons in a rodent model of absence epilepsy. *Brain Research*. 1000(1-2):102-9.
- Kotagal, P. and Luders, H. (1999). The Epilepsies: Etiologies and Prevention. London: Academic Press, San Diego.
- Leresche N, Parri H R, et al. (1998). On the action of the anti-absence drug ethosuximide in the rat and cat thalamus. *Journal of Neuroscience* 18(13): 4842-53.
- Macdonald RL. Kelly KM. (1995). Antiepileptic drug mechanisms of action. *Epilepsia*. 36 Suppl 2: S2-12.
- Manning JP, Richards DA, Bowery NG. (2003). Pharmacology of absence epilepsy. *Trends Pharmacol Sci* 24(10):542-9.

- Marini C et al. (2004). Genetic Architecture of Idiopathic Generalized Epilepsy: Clinical Genetic Analysis of 55 multiplex families. *Epilepsia* 45(5): 467-478.
- Meeren H, Pijn J P M, Van Luijtelaar E L J M, da Silva F H L. (2002). Cortical Focus Drives Widespread Corticothalamic Networks during Spontaneous Absence Seizures in Rats. *The Journal of Neuroscience* 22(4): 1480-1495.
- Nersesyan H, Hyder F, Rothman D, McCormick D, Blumenfeld H. (2002). BOLD fMRI and electrophysiological recordings of spike-wave seizures in WAG/Rij rats. *Epilepsia* 43(suppl. 7): 272.
- Niespodziany I, Klitgaard H, Margineanu DG. Is the persistent sodium current a specific target of anti-absence drugs? (2004). *Neuroreport* 15(6):1049-52.
- Panayiotopoulos, CP. (2004). International League Against Epilepsy. Typical Absence Seizures. <<http://www.ilae-epilepsy.org/>>.
- Richards DA, Manning JP, Barnes D, Rombola L, Bowery NG, Caccia S, Leresche N, Crunelli V. (2003). Targeting thalamic nuclei is not sufficient for the full anti-absence action of ethosuximide in a rat model of absence epilepsy. *Epilepsy Research* 54(2-3):97-107.
- Rodin E, Ancheta O. (1987). Cerebral electrical fields during petit mal absences. *Electroencephalogr Clin Neurophysiol* 66: 457-66.
- Rosenberg, R. N. (2003). Atlas of Clinical Neurology. Philadelphia, Current Medicine, Inc.
- Sander T et al. (1998). The gene encoding the $\alpha 1A$ -voltage-dependent calcium channel (CACN1A4) is not a candidate for causing common subtypes of idiopathic generalized epilepsy. *Epilepsy Res.* 29: 115-122.
- Sander T et al. (2000). Genetic variation of the human μ -opioid receptor and susceptibility to idiopathic absence epilepsy. *Epilepsy Res.* 39: 57-61.
- Steinlein O et al. (1997). Possible association of a silent polymorphism in the neuronal nicotinic acetylcholine receptor subunit $\alpha 4$ with common idiopathic generalized epilepsies. *Am. J. Med. Genet.* 74: 445-449.
- Steinlein O et al. (1999). The voltage gated potassium channel KCNQ2 and idiopathic generalized epilepsy. *Neuroreport* 10: 1163-1166.
- Su H., Alroy G., Kirson E.D., and Yaari Y. (2001). Extracellular Calcium Modulates Persistent Sodium Current-Dependent Burst-Firing in Hippocampal Pyramidal Neurons. *The Journal of Neuroscience* 21(12): 4173-4182.

- Taylor CP and Meldrum BS. (1997). Na⁺ channels as targets for neuroprotective drugs.
- Taylor CP, Narasimhan LS. (1997). Sodium channels and therapy of central nervous system diseases. *Advances in Pharmacology* 39:47-98.
- Taylor CP, Meldrum BS. (1995). Na⁺ channels as targets for neuroprotective drugs. *Trends in Pharmacological Sciences* 16(9):309-16.
- Taylor CP. Na⁺ currents that fail to inactivate. (1993). *Trends Neurosci.* 16: 455-460.
- Taverna S, Mantegazza M, et al. (1998). Valproate selectively reduces the persistent fraction of Na⁺ current in neocortical neurons. *Epilepsy Research.* 32(1-2): 304-8.
- Turnbull J, Lohi H, Kearney JA, Rouleau GA, Delgado-Escueta AV, Meisler MH, Cosette P, Minassian BA. (2005). Sacred disease secrets revealed: the genetics of human epilepsy. *Hum. Molec. Genet.* 14(7): 2491-2500.
- Vega-Saenz de Miera E, Rudy B, Sugimori M, Llinas R. (1997). Molecular characterization of the sodium channel subunits expressed in mammalian cerebellar Purkinje cells. *Proc. Natl. Acad. Sci. USA* 94: 7059-64.
- Wallace RH et al. (2001). Mutant GABA_A receptor γ 2-subunit in childhood absence epilepsy and febrile seizures. *Nature Genetics* 28: 49-52.
- Williams D. (1953) A study of thalamic and cortical rhythms in petit mal. *Brain*, 76:50-69.
- Wilson JV and R. GH (1990). Translation and analysis of a cuneiform text forming part of a Babylonian treatise on epilepsy. *Medical History* 34(2): 185-198.
- Winer J., Jung C.K., Shackel I. and Williams P.M.. (1999). Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. *Anal. Biochem.* 270: 41-49.
- Wyllie, E. (1993). The Treatment of Epilepsy: Principles and Practice. Malvern, PA, Lea&Febiger.