Psychoneuroendocrinology (2014) 39, 225—236

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Scn8a voltage-gated sodium channel mutation alters seizure and anxiety responses to acute stress

Nikki T. Sawyer [a], Ligia A. Papale [a], Jessica Eliason [a], Gretchen N. Neigh [b,c], Andrew Escayg [a,*]

[a] *Department of Human Genetics, Emory University, Atlanta, GA, USA*
[b] *Department of Physiology, Emory University, Atlanta, GA, USA*
[c] *Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA, USA*

Received 5 August 2013; received in revised form 16 September 2013; accepted 17 September 2013

KEYWORDS
Scn8a;
Stress;
Epilepsy;
Anxiety;
Absence;
Seizures

Summary Stress is known to trigger seizures in patients with epilepsy, highlighting the physiological stress response as a possible therapeutic target for epilepsy treatment. Nevertheless, little is currently known about how a genetic predisposition to epilepsy interacts with the stress response to influence seizure outcome. To address this question, we examined the effect of acute stress on seizure outcome in mice with mutations in the voltage-gated sodium channel (VGSC) gene *Scn8a*. *Scn8a* mutants display spontaneous spike-wave discharges (SWDs) characteristic of absence epilepsy. We saw that the baseline frequency of SWDs in *Scn8a* mutants correlates closely with the diurnal activity of the hypothalamic—pituitary—adrenal (HPA) axis, with a peak in seizure activity occurring at around the same time as the peak in corticosterone (1700—1900 h). A 20-min acute restraint stress administered in the morning increases the frequency of spontaneous SWDs immediately following the stressor. Seizure frequency then returns to baseline levels within 3 h after stressor exposure, but the subsequent evening peak in seizure frequency is delayed and broadened, changes that persist into the next evening and are accompanied by long-lasting changes in HPA axis activity. *Scn8a* mutants also show increased anxiety-like behavior in mildly stressful situations. A 20-min acute restraint stress can also increase the severity and duration of chemically induced seizures in *Scn8a* mutants, changes that differ from wild-type littermates. Overall, our data show that a voltage-gated sodium channel mutation can alter the behavioral response to stress and can interact with the stress response to alter seizure outcome.

* Corresponding author at: Department of Human Genetics, Emory University, 615 Michael Street, Whitehead Biomedical Research Building, Suite 301, Atlanta, GA 30322, USA. Tel.: +1 404 712 8328; fax: +1 404 727 3949.
E-mail address: aescayg@emory.edu (A. Escayg).

0306-4530/$ — see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.psyneuen.2013.09.018

1. Introduction

Seizures are paroxysmal events, suggesting the presence of endogenous or environmental triggering factors. Epilepsy patients self-report stress as being the most common trigger for seizure onset (Spatt et al., 1998; Haut et al., 2003, 2007; Nakken et al., 2005), an assertion further supported by controlled studies that establish a relationship between stress and increased seizure frequency (Feldman and Paul, 1976; Swinkels et al., 1998). In at least one study, the exacerbating effects of stress on seizure incidence was especially apparent in patients with typical absence seizures (Bosnjak et al., 2002), a subtype of epilepsy characterized by a brief loss of awareness associated with bursts of generalized synchronous spike-wave discharges (SWDs). Although stress and seizures are consistently linked, the physiological mechanisms that connect stress and seizure incidence or severity are not fully understood.

The acute stress response involves activation of the hypothalamic—pituitary—adrenal (HPA) axis, which results in the sequential release of corticotropin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), and corticosterone (CORT). Receptors for both CRF and CORT can be found in numerous brain areas, such as the hippocampus and amygdala, suggesting these stress mediators can directly influence neuronal activity and excitability in limbic regions that are also important for seizure activity (Joels and Baram, 2009). In addition to stress, anxiety levels predict changes in seizure frequency (Thapar et al., 2009), and hyperemotionality may underlie the majority of behavioral problems in epilepsy (Frucht et al., 2000). Furthermore, patients with epilepsy have higher incidences of depression, anxiety, and even psychosis compared to the general population (Garcia-Morales et al., 2008). HPA axis function modulates activity in limbic regions, and dysfunction of the HPA axis is implicated in a number of affective disorders. Therefore, it has been hypothesized that the HPA axis could mediate both stress-induced seizures and behavioral alterations in seizure models (Heinrichs, 2010). Given the bidirectional relationship between hyperemotionality, stress, and epilepsy, a genetic mouse model of epilepsy would provide a unique opportunity for investigating the stress response and affective-like behaviors and may provide important insight into the pathophysiological effects of stress on seizure incidence and severity.

A *de novo* gain-of-function point mutation in *SCN8A* was recently identified in a individual who exhibited generalized seizures and sudden unexplained death in epilepsy (SUDEP) (Veeramah et al., 2012). The *SCN8A* gene encodes the α-subunit of the $Na_v1.6$ voltage-gated sodium channel (VGSC). VGSCs are transmembrane complexes that facilitate the initiation and propagation of transient depolarizing currents and electrical signaling between cells in the nervous system. $Na_v1.6$ is the most widely expressed VGSC in the central nervous system, including areas involved in the stress response such as the hippocampus and hypothalamus (Schaller et al., 1995; Krzemien et al., 2000). $Na_v1.6$ plays a major role in the transmission of subthreshold currents, namely persistent current and resurgent current (Raman et al., 1997), and the electrophysiological properties of $Na_v1.6$ make these channels especially suited for the sustained repetitive firing of neurons (Van Wart and Matthews, 2006), a key feature in many neuronal circuits. In addition to its role in seizure activity, a 2-bp deletion in *SCN8A* was found to co-segregate with motor and cognitive deficits in a small pedigree comprising six affected members (Trudeau et al., 2006). Furthermore, findings from two association studies point to possible roles for *SCN8A* in emotional instability, suicide, and bipolar disorder (Wasserman et al., 2005; Wang et al., 2008).

Today there are several mouse models of *Scn8a* dysfunction. Null mutants, such as the $Scn8a^{med}$ mouse, are the most severe, with homozygous $Scn8a^{med/med}$ mice showing muscle weakness, progressive paralysis, and premature death (Burgess et al., 1995). In contrast, heterozygous $Scn8a^{med/+}$ mice exhibit normal gross motor behavior and a normal lifespan. Interestingly, the $Scn8a^{med/+}$ mice have increased resistance to chemically induced seizures (Martin et al., 2007), but exhibit frequent SWDs characteristic of absence epilepsy (Supplementary Fig. 1A) (Papale et al., 2009). Other *Scn8a* mutants, such as $Scn8a^{med\text{-}jo}$ and $Scn8a^{8J}$, also display spontaneous SWDs (Papale et al., 2009), and greater resistance to chemically induced seizures and hippocampal kindling have been seen in $Scn8a^{med\text{-}jo}$ and $Scn8a^{tg}$ mutants, respectively (Martin et al., 2007; Blumenfeld et al., 2009). Increases in both anxiety-like and depressive-like behaviors have been reported in the $Scn8a^{tg}$ mutant (McKinney et al., 2008), while our lab previously found only minor changes in anxiety-like behavior in the $Scn8a^{med\text{-}jo}$ mutant (Papale et al., 2010). *Scn8a* mutants, therefore, provide the opportunity to investigate the consequences of altered sodium channel function on the stress response and behavior, as well as the interaction between stress and spontaneous and induced seizures.

The goal of this study was to investigate the interaction between acute stress and epilepsy using the $Scn8a^{med/+}$ mouse to answer these four questions: (i) Does acute stress alter the frequency of spontaneous absence seizures? (ii) Does acute stress affect seizure thresholds? (iii) Does sodium channel dysfunction affect HPA axis function? and (iv) Does sodium channel dysfunction alter anxiety-like or depressive-like behaviors?

Supplementary material related to this article can be found in the online version at http://dx.doi.org/10.1016/j.psyneuen.2013.09.018.

2. Methods and materials

2.1. Subjects

$Scn8a^{med/+}$ mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and maintained on the C3HeB/FeJ background. Genotyping was performed on tail DNA using The Jackson Laboratory protocol. Male mice, 3—4 months old, were used for all experiments. Wild-type (WT) littermates were used as controls for all experiments. Mice were group-housed after weaning in ventilated cages under uniform conditions with a 12/12 h light/dark cycle with lights on at 0700 h and lights off at 1900 h. Food and water were available *ad libitum*. All behavioral tests and all stressors were administered between the hours of 0800 h and 1100 h to minimize variation due to circadian factors and were performed in rooms with lighting of approximately 1000 lux. All experiments were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).