

Research Article

Elevated extracellular potassium ion concentrations suppress hippocampal oscillations in a mouse model of Dravet syndrome *in-vitro*

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Keywords:

Dravet syndrome, artificial cerebrospinal fluid (ACSF), *Scn1a* mutant mouse, depolarization block.

ABSTRACT

Background: Hippocampal hyperexcitability and seizure-like events have been consistently demonstrated in hippocampal slice preparations perfused with ≥ 5 mM high $[K^+]_o$ artificial cerebrospinal fluid (ACSF). Accordingly, high $[K^+]_o$ ACSF has been effectively employed as ionic model of seizure for *in vitro* experiments, but then, how reliable is this model when employed for *in-vitro* studies of brain tissues with dysregulated K^+ homeostasis? To address this question, we examined how elevations of $[K^+]_o$ affect hippocampal oscillations in *Scn1a* mutant mouse, a mouse model of Dravet syndrome, a devastating genetic-epilepsy associated with gliosis, a major cause of dysregulated K^+ homeostasis in epileptic brain. **Methods:** To this end, performing local field potential (LFP) recordings from hippocampi of P30 to P38 *Scn1a* mutant mice (*Scn1a*^{-/-}) and wild-type littermates (*Scn1a*^{+/+}), maintained on a C57BL/6 genetic background, in brain slice preparations in normal and high K^+ conditions, we studied the effect of 4 mM and 5 mM high $[K^+]_o$ ACSF(s) on hippocampal oscillations. **Results:** Hippocampal hyperexcitability was observed only in *Scn1a*^{+/+} but not in *Scn1a*^{-/-} mice. In *Scn1a*^{-/-} mice, spontaneous hippocampal hyperexcitability was observed in normal ACSF but was significantly suppressed by 4 mM and 5 mM high $[K^+]_o$ ACSF(s). **Conclusion:** In conclusion, these findings, for the first time, provide evidence of spontaneous hippocampal activity in *Scn1a*^{-/-} mice older than P30 which may be potentially used as a target for screening anti-epileptic approaches, beneficial for the treatment of DS. Elevated $[K^+]_o$ -induced depolarization block of neuronal action potentials is involved in epileptic brain tissues modulated in elevated $[K^+]_o$. This mechanism underlies the suppressing effect of high $[K^+]_o$ ACSF on hippocampal oscillations in *Scn1a*^{-/-} mice *in vitro*. Future studies employing the high K^+ ionic model for studies of epileptic brain tissues are required to determine how K^+ homeostasis is handled by neurons and glial cells in epileptic brain tissues.

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INTRODUCTION

Elevations of extracellular potassium-ion concentration $[K^+]_o$ in hippocampal slice preparations, promotes epileptiform activity by depressing K^+ efflux in a positive feed-back manner (Durand *et al.*, 2010; Gerson

et al., 2012; Sibille *et al.*, 2015). Although, elevated $[K^+]_o$ is tightly regulated by K^+ homeostatic mechanisms (Cheung *et al.*, 2015; Gerson *et al.*, 2012), hippocampal hyperexcitability and seizure-like events has been consistently demonstrated in hippocampal slice preparations perfused with ≥ 5 mM high $[K^+]_o$ artificial cerebrospinal fluid (ACSF) (Jensen and Yaari, 1997; Borck and Jefferys, 1999; Gerson *et al.*, 2012). Accordingly, high $[K^+]_o$ ACSF has been effectively employed as ionic model of seizure for *in vitro* experiments (Gerson *et al.*, 2012), but then, how reliable is this model when employed for *in-vitro* studies of brain tissues with dysregulated K^+ homeostasis?

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To address this question, we tested the effect of high $[K^+]$ ACSF on hippocampal oscillations in *Scn1a* mutant mouse, a mouse model of Dravet syndrome (DS). DS is a devastating infantile-onset genetic-epilepsy, which largely results from heterozygous missense or truncating *de novo* loss-of-function mutations in the *SCN1A* gene that encodes for the α -subunit of the voltage-gated sodium channel, Nav1.1 (Catterall *et al.*, 2010; Marini *et al.*, 2011). Notably, DS is associated with gliosis (Catarino *et al.*, 2011; Guerrini *et al.*, 2011), a well-recognized cause of dysregulated K^+ homeostasis in epileptic brain (Devinsky, *et al.*, 2013; Cheung *et al.*, 2015). The *Scn1a* mutant mouse replicates many of the phenotypic features of DS patients' (Yu *et al.*, 2006; Oakley *et al.*, 2009; Catterall *et al.*, 2010; Cheah *et al.*, 2013) including cognitive impairments and increased risk of premature death (Cheah *et al.*, 2013), in addition, the *Scn1a* mutant mouse show recurrent spontaneous seizures after P32 (Oakley *et al.*, 2009), a leading cause of gliosis and dysregulated glial functions in epileptic brain (Durand *et al.*, 2010; Devinsky, *et al.*, 2013; Cheung *et al.*, 2015), making it an appropriate model for the study.

Hippocampal oscillations in the *Scn1a* mutant mice have not been studied under the high $[K^+]$ model. Preliminary studies in our laboratory suggest that 4 mM high $[K^+]$ ACSF was sufficient for induction of hippocampal hyperexcitability in wild-type littermates of *Scn1a* mutant mice but not in the *Scn1a* mutant mice *in-vitro*. To this end, we tested the effect of 5 mM high $[K^+]$ ACSF(s) on hippocampal oscillations in *Scn1a* mutant mice.

MATERIALS AND METHODS

Animals

Heterozygous *Scn1a* knockout mice (*Scn1a*^{+/-}) and their wild-type littermates (*Scn1a*^{+/+}) in the age-range of P30 to P38, maintained on a C57BL/6 genetic background, were used for the study. The *Scn1a*^{+/-} mice have age-dependent increase in susceptibility to temperature-induced seizures but not spontaneous seizures, only observed in mice older than P32 (Oakley *et al.*, 2009). Given the age-dependency of spontaneous seizures in this model, P30 to P38 *Scn1a*^{+/-} mice are expected to show spontaneous hippocampal activity which has been associated with dysregulated K^+ homeostasis in epileptic brain (Cheung *et al.*, 2015).

Scn1a^{+/-} mice, generated by targeted deletion of the last exon 25 encoding domain IV from the S3 to S6 segment and the entire C-terminal tail of Nav1.1 channel (Yu *et al.*, 2006) were generously provided by Professor Franck Kalume of the Department of Neurosurgery, University of Washington, Seattle, WA, USA. Study mice were generated by breeding *Scn1a*^{+/-}

males with *Scn1a*^{+/-} females and *Scn1a*^{+/-} mice were determined by genotyping using a 4-oligonucleotide multiplex PCR of genomic DNA samples isolated from mouse tails (Wu *et al.*, 1995). The wild-type (WT) band (291 bp) was amplified by FHY209 (5'-CGAATCCAGATGGAAGAGCGGTTTCATGGCT-3') and FHY210 (5'-ACAAGCTGCTATGGACATTGTCAGGTCAGT-3'), and the mutant band (493 bp) was amplified by Neo5 (5'-AGGATCTCCTGTCATCTCACCTTGCTCCTG-3') and Neo3 (5'-AAGAACTCGTCAAGAAGGCGATAGAAGGCG-3'). The two primer sets were mixed in a single PCR using the protocol: 2 min. denaturation at 94 °C followed by 30 cycles of 20 s at 95 °C, 20 s at 66 °C, and 30 s at 71 °C. PCR products were analyzed by agarose gel electrophoresis. Mice were reared on 12 hour light/dark cycles with access to food and water *ad libitum*. All experiments were performed in accordance with animal protocols, approved by the Institutional Animal Care and Use Committee of the University of Washington, Seattle, WA, USA.

Hippocampal slice preparations

The *Scn1a*^{+/-} and *Scn1a*^{+/+} mice in the age range P30 to P38 were anesthetized with inhaled Isoflurane and then decapitated. The brain was quickly and gently extracted and immediately placed on 200 mL of slushed cutting solution containing (in mM): 87 NaCl, 25 NaHCO₃, 2.5 KCl, 0.5 CaCl₂, 7 MgCl₂, 1.25 NaH₂PO₄, 25 Glucose and 75 Sucrose. Coronal sections (350 μ m thickness) of the hippocampus were cut in cold cutting solution using a Vibratome and were incubated for (45-60) minutes submerged in a holding chamber filled with normal artificial cerebrospinal fluid (nACSF) containing (in mM): 125 NaCl, 26 NaHCO₃, 3 KCl, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, and 10 glucose (Jiruska *et al.*, 2010), aerated with 95 % O₂-5 % CO₂ mixture (PH: 7.35, room temperature: ~25 °C). After incubation, hippocampal slices were transferred from the holding chamber and submerged in the bath chamber of a Kerr Tissue Recording System (Kerr Scientific Instruments, Christchurch, New Zealand).

Electrophysiology

In the submerged chamber, hippocampal slice was perfused with nACSF aerated with 95 % O₂-5 % CO₂ mixture, at a flow-velocity of 5 ml/min and room temperature ~25 °C for approximately 10 min to stabilize. Hippocampal hyperexcitability was induced by switching perfusion from nACSF to high $[K^+]$ ACSF which differ from the nACSF by a higher concentration of KCl (4 mM or 5 mM) aerated with 95 % O₂-5% CO₂ mixture (Jiruska *et al.*, 2010).

Extracellular field potential activity was recorded according to Voss *et al.* (2012a, 2012b). A 50- μ m

Teflon-coated silver electrode was positioned in the pyramidal layer of CA1 area of the hippocampus under a digital microscope. A silver/silver-chloride reference electrode was located at the bottom of the bath chamber. Recordings were conducted in a Faraday cage to minimize electrical background noise. The signal was acquired at 20 KHz, amplified at 100 X (Kerr Scientific Instruments), and band-filtered between 1 and 100 Hz using LabChart 7.0 software (PowerLab, AD Instruments, Inc., Colorado Springs, CO).

Data and statistical analysis

The peak-analysis module for AD Instruments LabChart pro 7.0 was employed for the analysis of electrical signals, (5-10) min. traces of network oscillations at the end of each perfusion was considered

a more suitable representative trace for the analysis given the time required for wash-out of the preceding perfusion from the submerged chamber. To increase the signal-to-noise ratio, the peak-analysis of frequency and amplitude was performed above the threshold of background noise calculated as: $m + 2SD$, where “m” is the average peak of background noise and “SD” is the standard deviation. The amplitude of oscillations (in mV) was calculated as: $m \times (1000/100)$, where ‘m’ is the average peak of oscillations (in Volts) and ‘(1000/100)’ is the correction factor for pre-amplification (X100) of electrical signals. Data obtained were compared using Student’s *t*-tests. Results were reported as $m \pm SEM$ and $P < 0.05$ was considered statistically significant.

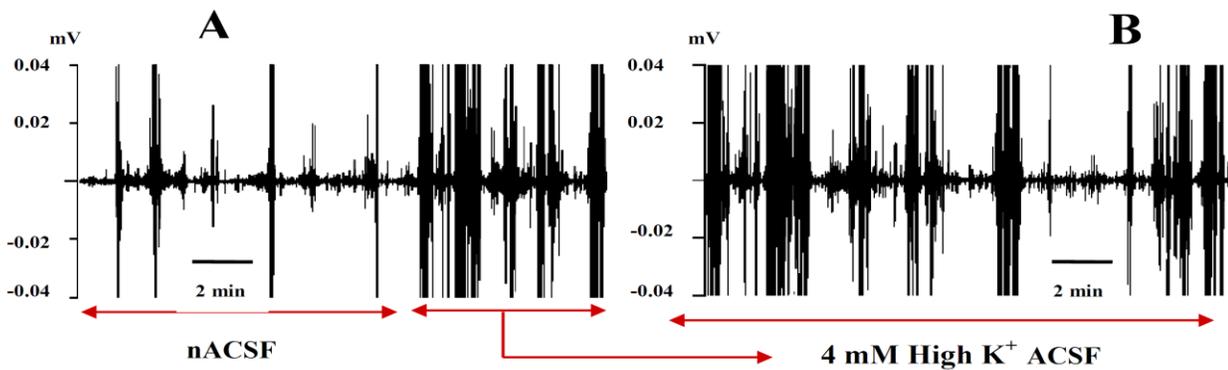


Fig. 1: Representative traces of hippocampal oscillations in *Scn1a*^{+/+} mice. 15 min representative traces in (A) nACSF, (B) 4 mM high [K⁺] ACSF.

RESULTS

Effect of elevated [K⁺]_o on hippocampal oscillations in *Scn1a*^{+/+} and *Scn1a*^{+/-} mice

The frequency, but not the amplitude, of hippocampal oscillations was significantly ($P < 0.05$) higher in 4 mM high [K⁺] ACSF compared with nACSF in *Scn1a*^{+/+} mice (frequencies: nACSF: 0.12 ± 0.04 Hz, 4 mM high [K⁺] ACSF: 0.48 ± 0.14 Hz, $n = 12$ slices, $P = 0.04$) (Figures 1A, 1B and 3B). Amplitudes: nACSF: 0.03 ± 0.01 mV, 4 mM high [K⁺] ACSF: 0.07 ± 0.03 mV, $n = 12$ slices, $P = 0.1266$; paired *t*-tests for all).

In contrast, in the *Scn1a*^{+/-} mice, the frequencies of hippocampal oscillations were significantly ($P < 0.05$) suppressed in 4 mM high [K⁺] ACSF (frequencies: nACSF: 0.50 ± 0.19 Hz, 4 mM high [K⁺]: 0.12 ± 0.03 Hz, $P = 0.04$, amplitudes: nACSF: 0.23 ± 0.07 mV, 4 mM high [K⁺] ACSF: 0.02 ± 0.01 mV, $P = 0.03$, $n = 13$ slices) and in 5 mM high [K⁺] ACSF (frequencies: Return to nACSF: 0.63 ± 0.17 Hz, 5 mM high [K⁺]: 0.10 ± 0.05 Hz, $P = 0.04$, $n = 7$ slices, amplitudes: return to nACSF: 0.07 ± 0.03 mV, 5 mM high [K⁺]

ACSF: 0.02 ± 0.003 mV, $P = 0.16$, $n = 7$ slices, paired *t*-tests for all) (Figures 2A, 2B and 3A).

Hippocampal oscillations in nACSF in the *Scn1a*^{+/-} mice

Spontaneous hippocampal activity, characterized by aperiodic burst of oscillatory activities lasting for 5 to 32 seconds was observed in the *Scn1a*^{+/-} mice in nACSF. The frequency and the amplitude of hippocampal oscillations in nACSF in the *Scn1a*^{+/-} mice were significantly ($P < 0.05$) higher compared with the frequency and the amplitude of oscillations in nACSF in the *Scn1a*^{+/+} mice, however, there was no significant difference compared with the frequency of oscillation in 4 mM high [K⁺] ACSF in the *Scn1a*^{+/+} mice (frequencies in nACSF: *Scn1a*^{+/-}: 0.50 ± 0.19 Hz, $n = 13$ slices, *Scn1a*^{+/+}: 0.12 ± 0.04 Hz, $n = 12$ slices; amplitudes in nACSF: *Scn1a*^{+/-}: 0.23 ± 0.07 mV, *Scn1a*^{+/+}: 0.03 ± 0.01 mV; frequency in 4 mM high [K⁺] ACSF: *Scn1a*^{+/-} 0.48 ± 0.14 Hz vs. frequencies in nACSF: *Scn1a*^{+/-}: 0.50 ± 0.19 Hz all unpaired *t*-tests) (Figures 3C and 3D).

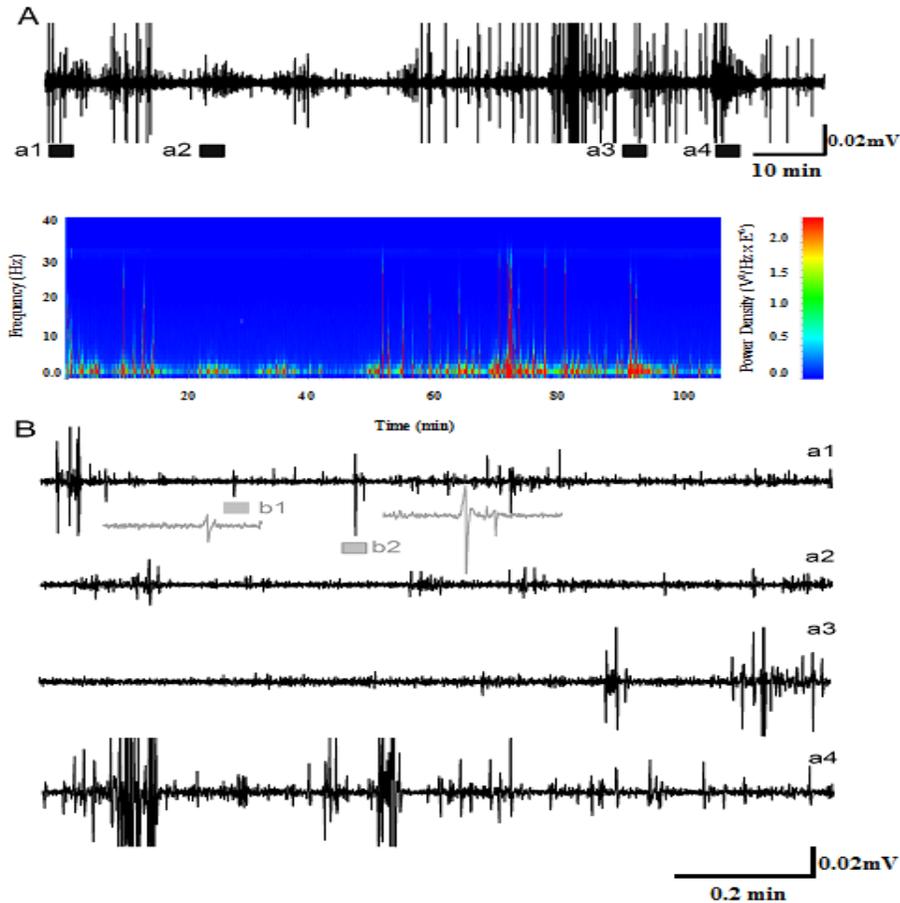


Fig. 2: Representative traces of hippocampal oscillations in *Scn1a*^{+/+} mice: (A) 1 hr.: 50 min representative trace of oscillations in nACSF and in 4 mM and 5 mM high [K⁺] ACSF(s) (upper panel), corresponding activity pattern as power density of oscillatory frequencies (lower panel). (B) Magnified sections of the recordings in “A” showing sections of recordings in four conditions; nACSF (a1), 4 mM high [K⁺] ACSF (a2), 5 mM high [K⁺] ACSF (a3) and return to nACSF (a4), b1 and b2 are magnified sections of a1.

DISCUSSION

Effect of elevated [K⁺]_o on hippocampal oscillations in Scn1a^{+/+} mice

Potassium ion currents including K⁺ leakage current, delayed rectifier K⁺ current, A-type K⁺ current, muscarinic-sensitive K⁺ current, slow Ca²⁺-activated K⁺ current and fast Ca²⁺-activated K⁺ current constitutes important inhibitory mechanisms of neuronal electrical activity which is contingent upon the balance of intra- and extra-cellular K⁺ concentrations. In high [K⁺]_o, the balance is distorted in a way that results in depression of these K⁺ currents in a positive feed-back manner, creating a favourable condition for transition to ictal hyperexcitable state and epileptiform bursts in neurones (Hahn and Durand, 2001; Durand *et al.*, 2010; Gerson *et al.*, 2012). Indeed, a transition from interictal to ictal discharges has been consistently demonstrated in hippocampal slice preparations perfused with ≥ 5 mM high [K⁺] ACSF (Jensen and Yaari, 1997; Borck and Jefferys, 1999; Gerson *et al.*, 2012). In the present

study, the effect of 4 mM high [K⁺] ACSF was tested. The frequency, but not the amplitude, of network oscillations in hippocampal CA1 area in *Scn1a*^{+/+} mice was significantly increased in 4 mM high [K⁺] ACSF (Figures 1A, 1B and 3B). The results suggest that 4 mM high [K⁺] ACSF is sufficient for induction of hippocampal hyperexcitability in *Scn1a*^{+/+} mice.

Notably, in the C57BL/6 inbred mouse strain used for the present study, the frequencies but not the amplitudes of the hippocampal oscillations was a better indicator of oscillatory response to challenges, as the amplitudes of the oscillation did not change significantly ($P = 0.1266$) in 4 mM high [K⁺] ACSF. In a study on strain-related variations in the response of the amplitude of hippocampal oscillations to carbachol-induced increase in oscillations in eight inbred mouse strains, including 129S1SvImJ, A/J, Balb/cByJ, C3H/HeJ, C57BL6/J, DBA/2J, FVB/NJ, and NOD/LtJ. The Balb/cByJ mice had the highest oscillatory amplitude while the C57BL6/J mice had the lowest

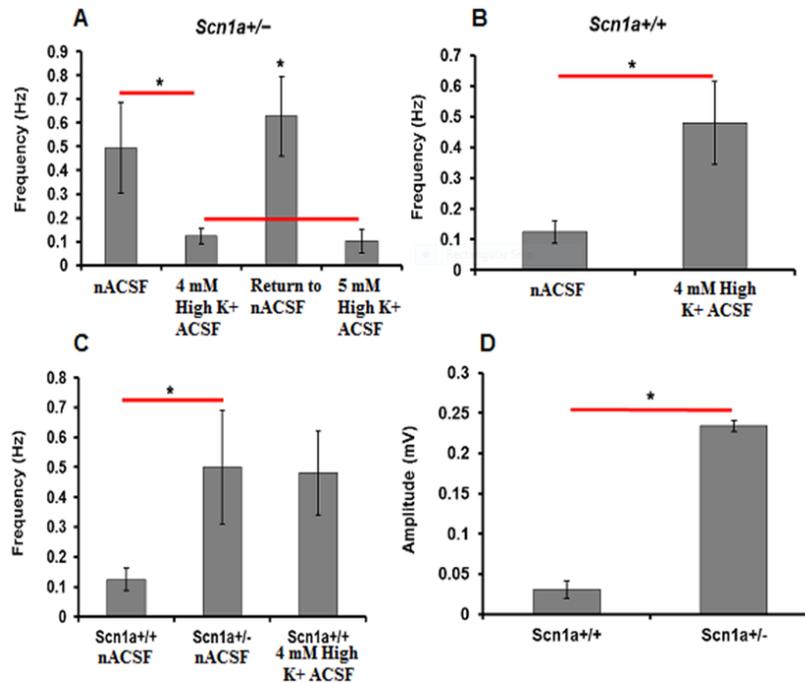


Fig. 3: Effect of high [K⁺] ACSF on hippocampal oscillations in *Scn1a*^{+/+} and *Scn1a*^{+/-} mice. (A) Frequencies of hippocampal oscillations in nACSF and, 4 mM and 5 mM [K⁺] ACSF(s) in *Scn1a*^{+/-} mice. (B) Frequencies of hippocampal oscillations in nACSF and 4 mM [K⁺] ACSF in *Scn1a*^{+/+} mice. (C) Frequencies of oscillations in nACSF and 4 mM [K⁺] ACSF in *Scn1a*^{+/+} mice compared with oscillations in nACSF in the *Scn1a*^{+/-} mice. (D) Amplitudes of oscillations in nACSF in *Scn1a*^{+/+} and *Scn1a*^{+/-} mice **P*<0.05.

amplitude (Jansen *et al.*, 2009). It is not unlikely that the insignificance in the response of oscillatory amplitude to high [K⁺] ACSF in the present study was due to peculiar attributes of the C57BL/6 inbred mouse strain. Indeed, genes influencing the amplitude of hippocampal oscillations have been identified in the BXD mouse strain, derived from an inter-cross of C57BL/6J and DBA/2J inbred mouse-strains (Jansen *et al.*, 2011).

Hippocampal oscillations in the *Scn1a*^{+/-} mice in normal ACSF

Recent studies in *Scn1a*^{+/-} mice suggest that the hippocampus is hyperexcitable, but non-stimulated hippocampal slices in normal ACSF do not show spontaneous activity (Liautard *et al.*, 2013; Gu *et al.*, 2014). The present study, however, provides evidence of spontaneous hippocampal activity in normal ACSF in *Scn1a*^{+/-} mice older than P30 *in-vitro* (Figures 3C and 3D) which is consistent with the afebrile spontaneous seizures only observed in *Scn1a*^{+/-} mice older than P32 *in vivo* (Oakley *et al.*, 2009). However, the discrepancy in the results could be due to differences in the age of animals studied and distinct experimental settings.

Typically, in the *Scn1a*^{+/-} mice, febrile seizures occur after P19, whereas, afebrile spontaneous seizures occur only in mice older than P32 (Oakley *et al.*, 2009). This age-dependency of seizure-onset has been attributed to

the functional effect of *Scn1a* gene mutations and the differential developmental expressions of Nav1.1 and Nav1.3 channels, preferentially localized in the cell bodies of neurones (Yu *et al.*, 2006; Kalume *et al.*, 2007; Cheah *et al.*, 2013). In rodents, during the second and third postnatal weeks; that is, between P10 and P21, the neuronal expressions of Nav1.3 decline and Nav1.1 increase to reach its full level of expression and contribute most significantly to neuronal function after P21 (Gordon *et al.*, 1987; Beckh *et al.*, 1989; Westenbroek *et al.*, 1992). On the contrary, in the *Scn1a*^{+/-} mice, there is a selective loss of function and reduced expression of Nav1.1 channels in GABAergic inhibitory interneurons during the third postnatal week, resulting in loss of GABAergic inhibition and increased susceptibility to afebrile spontaneous seizures after around P30 (Yu *et al.*, 2006; Kalume *et al.*, 2007; Oakley *et al.*, 2009; Cheah *et al.*, 2013). The *Scn1a*^{+/-} mice in the present study were within the age-range for afebrile spontaneous seizures; that is > P30, whereas Gu *et al.* (2014) used P16 to P22 *Scn1a*^{+/-} mice, within the age range for febrile seizures, but below the age for afebrile spontaneous seizures. Most likely, non-stimulated hippocampal slices in *Scn1a*^{+/-} mice younger than P30 would not show spontaneous activity given the age-dependency of spontaneous seizures in this model.

Liautard *et al.* (2013) used P30–P36 *Scn1a*^{+/-} mice, within the age range for afebrile spontaneous seizures, but also did not observe spontaneous activity in non-

stimulated hippocampal slices. Difference in experimental settings, may at least in part, provide explanation for the discrepancy in results. Liautard *et al.* (2013) used an interface recording chamber, superfused with nACSF kept at 35 °C; whereas, in the present study, a submerged recording chamber was used and field potential recordings were performed at room temperature (~25 °C). Most cellular processes are known to be strongly temperature-dependent. Temperature has complex, non-linear effects on brain tissue, and it has been suggested that the extension of conclusions drawn from *in vitro* data obtained at temperatures less than or greater than physiological temperatures (36-37 °C) should be done with utmost caution (Kim and Connors, 2012). It is possible that the different recording temperatures and brain slice chambers used are responsible for the discrepancy in results.

Hippocampal oscillations in the Scn1a^{+/-} mice in high [K⁺] ACSF

In contrast to the 4 mM high [K⁺] ACSF induced hippocampal hyperexcitability in *Scn1a^{+/+}* mice, the findings of the present study for the first time, showed that hippocampal oscillations were rather suppressed in 4 mM and 5 mM high [K⁺] ACSF(s) in *Scn1a^{+/-}* mice (Figures 2A, 2B and 3A). A plausible explanation is that, spontaneous neuronal activity-dependent elevations of [K⁺]_o promotes epileptiform activity by depressing K⁺ efflux in a positive feed-back manner (Durand *et al.*, 2010; Gerson *et al.*, 2012; Sibille *et al.*, 2015) but under a high [K⁺] ACSF condition that overwhelms K⁺-clearance mechanisms or dysregulated glial functions and K⁺ homeostasis, the epileptiform activity can be suppressed through depolarisation block of neuronal activity, which has been demonstrated in experimental and computer modeling studies (Hahn *et al.*, 2001; Bikson *et al.*, 2003; Durand *et al.*, 2010; Gerson *et al.*, 2012; Sibille *et al.*, 2015). Depolarisation block occurs when persistent membrane depolarisation due to excess build-up of [K⁺]_o results in tonic inactivation of Na⁺ channels such that action potentials cannot be initiated (Bikson *et al.*, 2003; Durand *et al.*, 2010). Although the actual [K⁺]_o threshold for induction of neuronal depolarisation block is still unsettled, depolarisation block of hippocampal hyperexcitability in ≤ 5 mM high [K⁺] ACSF has not been previously reported, therefore, the implications of possible dysregulated glial functions and K⁺ homeostasis could be considered in elucidating the mechanisms of the suppressing effect of high [K⁺] ACSF on hippocampal oscillations in the *Scn1a^{+/-}* mice. Typically, during activity-dependent elevations of [K⁺]_o, extracellular K⁺ clearance by glial cells, astrocytes in particular, is conducted either by net K⁺ uptake, involving the activity of co-transporters (Na⁺-

K⁺-ATPase or Na⁺-K⁺-2Cl⁻), as well as K⁺ and Cl⁻ channels, or by K⁺ spatial buffering, that allows for uptake and redistribution of K⁺ from areas of high to low [K⁺]_o (Walz, 2000; Kofuji and Newman, 2004; Cheung *et al.*, 2015). Several studies have shown that dysregulated glial functions leads to hyperexcitability, synchrony and excess build-up of [K⁺]_o (Durand *et al.*, 2010; Devinsky, *et al.*, 2013; Cheung *et al.*, 2015). Dysfunction of glial cells is a prominent feature of the human epileptic brain (Devinsky, *et al.*, 2013; Cheung *et al.*, 2015) as well as the brains of both paediatric and adult patients with DS (Catarino *et al.*, 2011; Guerrini *et al.*, 2011). It is associated with alterations in astrocytic expressions of membrane K⁺ and Na⁺ channels, pro-epileptic changes in the regulation of [K⁺]_o and excess build-up of [K⁺]_o, which could lead sustained inactivation of persistent sodium current consistent with induction of neuronal depolarisation block (Durand *et al.*, 2010; Cheung *et al.*, 2015). Although, it is not known if dysfunction of glial cells is also a prominent feature of the *Scn1a^{+/-}* mice, but recurrent spontaneous seizures, as observed in *Scn1a^{+/-}* mice older than P30 (Oakley *et al.*, 2009), has been associated with dysregulated glial functions in epileptic brain (Durand *et al.*, 2010; Devinsky, *et al.*, 2013; Cheung *et al.*, 2015), and the implication for K⁺ homeostasis in *Scn1a^{+/-}* mice cannot be disregarded. Therefore, with the likelihood of dysregulated glial functions in the *Scn1a^{+/-}* mice, perfusion with a high [K⁺] ACSF could result in an excess build-up of [K⁺]_o, induction of depolarisation block and suppression of hippocampal hyperexcitability in the *Scn1a^{+/-}* mice.

In conclusion, these findings, for the first time, provides evidence of spontaneous hippocampal activity in *Scn1a^{+/-}* mice older than P30 which may be potentially used as a target for screening anti-epileptic approaches, beneficial for the treatment of DS. Elevated [K⁺]_o induced depolarization block of neuronal action potentials is involved in epileptic brain tissues modulated in elevated [K⁺]_o. This mechanism underlies the suppressing effect of high [K⁺] ACSF on hippocampal oscillations in *Scn1a^{+/-}* mice *in vitro*. Future studies employing the high K⁺ ionic model for studies of epileptic brain tissues are required to determine how K⁺ homeostasis is handled by neurones and glial cells in epileptic brain tissues.

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