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BRIEF COMMUNICATION:

Mapt deletion fails to rescue premature lethality in two models of sodium channel epilepsy

Running head: *Mapt* deletion in *Scn1b* null and *Scn8a* EIEE13 mice

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Abstract

Deletion of *Mapt*, encoding the microtubule-binding protein Tau, prevents disease in multiple genetic models of hyperexcitability. To investigate whether the effect of Tau depletion is

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generalizable across multiple sodium channel gene-linked models of epilepsy, we examined the *Scn1b*$^{-/-}$ mouse model of Dravet syndrome, and the *Scn8a*$^{N1768D/+}$ model of Early Infantile Epileptic Encephalopathy. Both models display severe seizures and early mortality. We found no prolongation of survival between *Scn1b*$^{-/-}$,*Mapt*$^{+/+}$, *Scn1b*$^{-/-}$,*Mapt*$^{+/-}$, or *Scn1b*$^{-/-}$,*Mapt*$^{-/-}$ mice or between *Scn8a*$^{N1768D/+}$,*Mapt*$^{+/+}$, *Scn8a*$^{N1768D/+}$,*Mapt*$^{+/-}$, or *Scn8a*$^{N1768D/+}$,*Mapt*$^{-/-}$ mice. Thus, the effect of *Mapt* deletion on mortality in epileptic encephalopathy models is gene specific and provides further mechanistic insight.

Introduction

Deletion of *Mapt*, encoding the microtubule-binding protein Tau, has been shown to attenuate hyperexcitability and prevent disease in a mouse model of Alzheimer's Disease with epilepsy [1], the *Kcna1*$^{-/-}$ mouse model of temporal lobe epilepsy [2], the *Scn1a*^{R1407X} mouse model of Dravet syndrome (DS) [3], and bang-sensitive *Drosophila* mutants [2]. As a result of this work, Tau has been proposed to be a viable target for the development of novel anti-epileptic therapeutic agents. To investigate whether the effect of Tau depletion is generalizable across additional gene models of epilepsy with premature lethality, we conducted a similar experiment using two different models of sodium channel gene-linked epileptic encephalopathy: the *Scn1b*$^{-/-}$ mouse model of DS [4] and the *Scn8a*$^{N1768D/+}$ model of Early Infantile Epileptic Encephalopathy (EIEE13) [5].

Scn1b encodes the β1 and β1B subunits of voltage-gated sodium channels [6]. Homozygous *Scn1b*$^{-/-}$ mice are underweight, have cardiac defects, develop severe seizures at approximately postnatal day (P) 10, and 100% die by approximately P21. *SCN1B* is the only known genetic link to DS that is due to recessive inheritance [7, 8]. The limited number of reported *SCN1B*-linked DS cases show seizure onset in the first months of life, dramatic cognitive and motor delays, microcephaly, generalized wasting, severe kyphoscoliosis, central hypotonia, and spastic quadriplegia [7, 8]. *SCN8A* encodes $Na_v1.6$, a pore-forming α subunit of the voltage-gated sodium channel. Heterozygous missense mutations of *SCN8A* have been identified in more than 150 individuals with *SCN8A*-EIEE13 many of which exhibit gain-of-function features [9]. A mouse model expressing the patient mutation p.Asn1758Asp (N1768D) exhibits seizure onset at 2 to 5 months of age [5, 10]. Death is usually observed within one week of seizure onset [11]. Since *SCN1B*-linked Dravet syndrome and *SCN8A*-linked EIEE13 are both resistant to multiple anti-

epileptic drugs, there is a major need for the development of novel therapeutics for these devastating epilepsy syndromes.

Methods

Animals: All procedures were performed in accordance with the guidelines of the National Institutes of Health, as approved by the Animal Care and Use Committee of the University of Michigan and Baylor College of Medicine.

Scn1b$^{+/-}$ mice, congenic for over 20 generations (N>20) on the C57Bl/6J background, were generated as described [4]. *Scn1b*$^{+/-}$ mice were crossed with *Mapt*$^{+/-}$ mice (JAX stock #007251,*B6.129X1-Mapt*^{tm1Hnd}*/J)* [12] to generate *Scn1b*$^{+/-}$,*Mapt*$^{+/-}$ mice, which were then bred to generate *Scn1b*$^{-/-}$,*Mapt*$^{+/+}$, *Scn1b*$^{-/-}$,*Mapt*$^{+/-}$, and *Scn1b*$^{-/-}$,*Mapt*$^{-/-}$ mice for analysis. *Scn8a*$^{N1768D/+}$ mice [13] were backcrossed to strain C57BL/6J for six generations (N6) and crossed with C57BL/6J.*Mapt*$^{-/-}$ mice. F2 mice with genotypes *Scn8a*$^{N1768D/+}$,*Mapt*$^{+/+}$, *Scn8a*$^{N1768D/+}$,*Mapt*$^{+/-}$ and *Scn8a*$^{N1768D/+}$,*Mapt*$^{-/-}$ were used for analysis of survival. Additional *Scn8a*$^{N1768D/+}$,*Mapt*$^{-/-}$mice were obtained by crossing F1 mice with *Mapt*$^{-/-}$ mice. Additional *Scn8a*$^{N1768D/+}$,*Mapt*$^{+/+}$ were collected from generations N6 to N9 of the backcross to strain C57BL/6J. Male and female mice were used for all experiments. Mouse survival was monitored twice daily by individuals blinded to genotype. For the *Scn1b* mice, half of the animals were bred and analyzed at the University of Michigan and half at Baylor College of Medicine. There were no differences in the results and thus the data were pooled.

PCR analysis of mouse tail DNA: DNA was prepared from mouse tail biopsies at postnatal day (P) 10-14 using standard methods [4].

For *Scn1b,Mapt* mice: Two sets of primers were used in genotyping: *Mapt* primers: *Mapt*$^{-/-}$ forward 5'-GCC AGA GGC CAC TTG TGT AG-3'; reverse 5'-ATT CAA CCC CCT CGA ATT TT-3'; *Mapt*$^{+/+}$: forward 5' AAT GGA AGA CCA TGC TGG AG 3'; reverse 5'-ATT CAA CCC CCT CGA ATT TT-3'. *Scn1b* primers: *Scn1b*$^{-/-}$: forward 5'- AGA GAG AAT GGA GAA TCA AGC CAT AG-3'; reverse 5'-GCT ACT TCC ATT TGT CAC GTC CTG CAC-3'; *Scn1b*$^{+/+}$: forward 5'-CTT CTT TGA TCC CTC ACT GTC CG -3'; reverse 5'-AGG TGG ATC TTC TTG ACG ACG CTG-3'. The two primer sets were mixed and used together in a single PCR performed according to the following protocol: an initial denaturation step at 95℃ for 2 min, followed by 30-35 cycles of denaturation at 94℃ for 30 s, annealing at 60℃ for 30 s, and elongation at 71℃ for 1 min 20 s, and a final step at 72℃ for 7 min. The results were analyzed by agarose gel electrophoresis.

For *Scn8a*^{N1768D},*Mapt* mice, PCR primers 5'-TACTGCTGCCAATCCTGAAC-3' and 5'-CAAAGTCGGCCAGCTTACA-3' were used to amplify a 306 bp product. An initial denaturation at 95℃ for 2 min was followed by 35 cycles of 95℃ for 45 s, 60℃ for 30 s, 72℃ for 40 s. The reaction was then digested with HincII restriction enzyme. The amplicon is resistant to HincII when amplified from the WT allele. When amplified from the mutant allele the presence of a HincII RE site results in digestion to a 146 bp and a 160 bp fragment. Genotyping of the *Mapt*^{tmHnd} allele was based on the protocol published on the Jackson Laboratory website (stock number 007251). Primers 5'- AATGGAAGACCATGCTGGAG-3', 5'-ATTCAACCCCCTCGAATTTT-3' and 5'-GCCAGAGGCCACTTGTGTAG-3' were used in a touchdown PCR. After initial denaturation at 95℃ for 2 min, 10 cycles of 95℃ for 30 s, 65℃ (decreasing by 0.5℃ per cycle) for 30 s, 72℃ 30 s, was followed by 28 cycles of 95℃ for 30 s, 60℃ for 30 s, 72℃ for 30 s. Amplification of the WT allele results in a 269 bp product, while the *Mapt*^{tmHnd} allele gives a 190 bp product.

Statistical analysis: Mouse survival was analyzed by Kaplan-Meir Log Rank (Mantel-Cox).

Results

In contrast to *Kcna1*$^{-/-}$ and *Scn1a*^{R1407X} mice, *Mapt* deletion had no observable effects on survival of *Scn1b* DS mice. *Scn1b*$^{-/-}$,*Mapt*$^{+/+}$, *Scn1b*$^{-/-}$,*Mapt*$^{+/-}$, and *Scn1b*$^{-/-}$,*Mapt*$^{-/-}$ mice have overlapping survival curves (Fig. 1). Although affected mice were too young and too small for electroencephalographic monitoring prior to their death in the third postnatal week, all three groups of animals showed similar and clearly visible behavioral seizures up until death. In addition, the time of death was not significantly different between genotypes.

Deletion of *Mapt* alleles also had no effect on the survival of *Scn8a*$^{N1768D/+}$ mice. Comparison of survival during a ten-month observation period did not detect any difference between *Scn8a*$^{N1768D/+}$ mutant mice with the compound genotypes of *Mapt*$^{+/+}$, *Mapt*$^{+/-}$ and *Mapt*$^{-/-}$ (Fig. 2).

Discussion

We report that the effects of *Mapt* deletion on genetic models of neural hyperexcitability are not uniform across all mouse models of epileptic ion channelopathy. Mice bearing the null mutation in *Scn1b*, a regulatory subunit of voltage-gated neuronal and cardiac sodium channels, show epilepsy even in the absence of Tau protein, and loss of Tau does not prevent or delay premature lethality in this model. This is likely explained by the increased molecular complexity of *Scn1b* interactions with ion channel pore-forming subunits. The mouse phenotype of *SCN1B*-linked DS [4, 8] is more severe than models of *SCN1A*-linked DS [14, 15], and may involve more subclasses of neurons and brain regions. β1/β1B subunits associate with and modulate the voltage-gating properties of all sodium channel and some potassium channel α subunits [6]. β1 and β1B are multi-functional, with non-conducting functions resembling those of immunoglobulin superfamily cell adhesion molecules [6]. In addition to brain, *Scn1b* is expressed in heart, where it contributes to the regulation of cardiomyocyte excitability and excitation-contraction coupling[16, 17]. *SCN1B* mutations are linked to cardiac arrhythmia in humans and *Scn1b* $^{-/-}$ mice have prolonged QT and RR intervals on the ECG[18]. *Scn1b* is also expressed in pancreatic beta cells. *Scn1b*$^{-/-}$ mice display a metabolic hypoglycemic phenotype due to abnormal insulin and glucagon release, likely contributing to failure to thrive and early neurologic hypofunction[19]. Thus, *SCN1B*-linked mortality may relate to combined downstream excitability disturbances in brain, heart, and neuroendocrine cells, not all of which can be rescued by loss of Tau, and the human disease may be more challenging in terms of therapeutic development.

SCN8A gain-of-function mutations are associated with EIEE13, with onset ranging from prenatal life to one year of age. The functional effects of patient mutations in this pore-forming subunit include premature channel opening and delayed channel inactivation [9]. Elevated neuronal firing rates have been observed in hippocampal [20] and cortical [21] neurons from mice carrying the patient mutation *SCN8A*-N1768D, which is located in the last transmembrane segment of the channel. $Na_v1.6$ is concentrated at the axon initial segment (AIS), where it mediates action potential initiation in neurons throughout the CNS and PNS. The lack of effect of *Mapt* deletion on survival of mice with the N1768D mutation suggests that Tau may not be involved in the mechanism of $Na_v1.6$ localization to the AIS. *SCN8A* is also expressed at low abundance in cardiac myocytes, which exhibit arrhythmic contractions and altered calcium handling in *Scn8a*$^{N1768D/+}$ mice [22]. The role of cardiac arrhythmia in premature lethality of this mouse model remains unclear. At the cellular level, the effects of gain-of-function mutations of *SCN8A* are quite distinct from the loss-of-function mutations of *SCN1A* responsible for Dravet syndrome.

How this difference in mechanism leads to divergent responses to *Mapt* deletion is a question for the future.

The mechanism of sudden unexpected death in epilepsy (SUDEP) in epileptic encephalopathy is not known, although spreading depolarization to the brainstem, respiratory compromise, autonomic dysfunction, and cardiac arrhythmias have been implicated [22-27]. *Mapt* deletion, which has been shown to prolong life in the Kv1.1 null model of SUDEP, also restores the normal brainstem threshold for spreading depression [23], possibly implicating *Mapt* in SUDEP mechanisms. Nevertheless, our results provide the first indication that targeting Tau will not provide general protection against premature lethality among all genetic channelopathies, which may require development of gene-specific therapies for individual subtypes of epileptic encephalopathy.

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Author Contributions: CC generated the *Scn1b*$^{+/-}$ mouse line, supervised breeding, and developed genotyping assays. CA and JKH monitored mouse survival, analyzed data, and prepared figures for *Scn1b* mice. RB-S carried out crosses, monitored survival, analyzed data and prepared figures for *Scn8a* mice. LLI, JLN, and MHM oversaw the experiments and wrote the manuscript.

Conflicts of Interest

None

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Figure Legends

Fig. 1. *Mapt* deletion does not affect survival of *Scn1b*$^{-/-}$ mice. Kaplan-Meir analysis shows that *Mapt* deletion does not alter survival in *Scn1b*$^{-/-}$ mice (*Scn1b*$^{-/-}$, *Mapt*$^{+/+}$: n=11 ;*Scn1b*$^{-/-}$, *Mapt*$^{+/-}$: n=26 ; *Scn1b*$^{-/-}$, *Mapt*$^{-/-}$: n=11 ; Kaplan-Meir log rank). χ^2=1.063, p>0.05. Log-rank (Mantel-Cox) Chi square was calculated in GraphPad Prism assuming 2 degrees of freedom.

Fig. 2. *Mapt* deletion does not affect survival of *Scn8a* EIEE13 mice. The survival of *Scn8a*$^{N1768D/+}$ mice was unaffected by their *Mapt* genotype (*Scn8a*$^{N1768D/+}$,*Mapt*$^{+/+}$: n=217; *Scn8a*$^{N1768D/+}$,*Mapt*$^{+/-}$: n=54 ; *Scn8a*$^{N1768D/+}$,*Mapt*$^{-/-}$ *:* n=36 ; Kaplan-Meir log rank). χ^2=5.968, p>0.05. Log-rank (Mantel-Cox) Chi square was calculated in GraphPad Prism assuming 2 degrees of freedom.