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Tau Loss Attenuates Neuronal Network Hyperexcitability in Mouse and *Drosophila* Genetic Models of Epilepsy

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Neuronal network hyperexcitability underlies the pathogenesis of seizures and is a component of some degenerative neurological disorders such as Alzheimer's disease (AD). Recently, the microtubule-binding protein tau has been implicated in the regulation of network synchronization. Genetic removal of *Mapt*, the gene encoding tau, in AD models overexpressing amyloid-β (Aβ) decreases hyperexcitability and normalizes the excitation/inhibition imbalance. Whether this effect of tau removal is specific to Aβ mouse models remains to be determined. Here, we examined tau as an excitability modifier in the non-AD nervous system using genetic deletion of tau in mouse and *Drosophila* models of hyperexcitability. *Kcna1*$^{-/-}$ mice lack Kv1.1-delayed rectifier currents and exhibit severe spontaneous seizures, early lethality, and megencephaly. Young *Kcna1*$^{-/-}$ mice retained wild-type levels of Aβ, tau, and tau phospho-Thr^{231}. Decreasing tau in *Kcna1*$^{-/-}$ mice reduced hyperexcitability and alleviated seizure-related comorbidities. Tau reduction decreased *Kcna1*$^{-/-}$ video-EEG recorded seizure frequency and duration as well as normalized *Kcna1*$^{-/-}$ hippocampal network hyperexcitability *in vitro*. Additionally, tau reduction increased *Kcna1*$^{-/-}$ survival and prevented megencephaly and hippocampal hypertrophy, as determined by MRI. Bang-sensitive *Drosophila* mutants display paralysis and seizures in response to mechanical stimulation, providing a complementary excitability assay for epistatic interactions. We found that tau reduction significantly decreased seizure sensitivity in two independent bang-sensitive mutant models, *kcc* and *eas*. Our results indicate that tau plays a general role in regulating intrinsic neuronal network hyperexcitability independently of Aβ overexpression and suggest that reducing tau function could be a viable target for therapeutic intervention in seizure disorders and antiepileptogenesis.

Introduction

The microtubule-binding protein tau is implicated in cytoskeletal and intracellular trafficking functions including microtubule stability, transport, and signal transduction (Dixit et al., 2008; Wang and Liu, 2008; Ittner et al., 2010). This multifaceted protein figures prominently in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD) and frontotemporal dementia, where abnormally phosphorylated tau proteins aggregate to form neurofibrillary tangles (Goedert and Spillantini, 2006; Morris et al., 2011).

Although the underlying molecular mechanisms are unclear, recent studies have implicated tau in the regulation of excitability and synchronization of neuronal networks in AD mouse models. Genetic removal of tau decreases interictal spiking and spontaneous seizures in the J20 human amyloid precursor protein (hAPP) AD mouse, which overexpresses amyloid-β (Aβ) (Roberson et al., 2011). In this model, tau knockout normalizes the inhibitory/excitatory imbalance and rescues both the early lethality and cognitive dysfunction (Roberson et al., 2007, 2011). Tau protein is also linked to Aβ-induced axonal transport deficits and synaptic long term potentiation alterations in hAPP AD mice, both of which are rescued by tau knockout (Vossel et al., 2010; Shipton et al., 2011). Expression of *ApoE4*, an AD risk factor allele, in mice also leads to network hyperexcitability (Hunter et al., 2012). ApoE4 mice exhibit GABAergic interneuron loss accompanied by learning and memory deficits due to high levels of toxic hyperphosphorylated tau that are rescued by tau reduction (Li et al., 2009; Andrews-Zwilling et al., 2010).

While the interaction between tau and network excitability is robust in AD models with Aβ or tau pathology, the ability of tau to modulate neuronal excitability in models without aberrant amyloid expression or tau hyperphosphorylation remains unknown. Tau knock-out mice themselves show decreased seizure severity following activation by convulsants (Roberson et al., 2007; Ittner et al., 2010). If tau removal plays a more general role in limiting excessive network firing, the interaction could be a viable target for treatment of other central excitability disorders.

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To address this possibility, we examined the effect of genetically decreased tau expression in mouse and *Drosophila* ion channelopathy mutants. The *Kcna1*$^{-/-}$ mouse is a model of temporal lobe epilepsy bearing a null allele for the α subunit of the Kv1.1 voltage-gated potassium channel (Smart et al., 1998). Humans with loss-of-function mutations in this gene have hyperexcitability phenotypes including epilepsy, myokymia, and episodic ataxia (Adelman et al., 1995; Zuberi et al., 1999; Liguori et al., 2001). *Kcna1*$^{-/-}$ mice exhibit severe spontaneous seizures beginning in the third week of life that are accompanied by early lethality and megencephaly, making this model a robust test for the effects of tau on hyperexcitability (Smart et al., 1998; Glasscock et al., 2007; Persson et al., 2007). Bang-sensitive (BS) *Drosophila* mutants display behavioral seizure susceptibility following mechanical stimulation that is characterized by a period of paralysis followed by seizure-like limbshaking movements. BS mutants represent a genetically tractable model system for evaluating modifiers of human seizure disorders (Pavlidis et al., 1994; Kuebler and Tanouye, 2000; Kuebler et al., 2001; Parker et al., 2011).

Materials and Methods

Animals. Double-mutant mice were produced carrying various combinations of *Kcna1*− and *Tau*− alleles. *Kcna1*− mice carry a null mutation in the *Kcna1* gene on chromosome 6 (Smart et al., 1998). *Tau*− mice contain a targeted knock-out mutation in the *Mapt* gene on chromosome 11 (Dawson et al., 2001). Heterozygous F1 mice (*Kcna1*$^{+/-}$*Tau*$^{+/-}$), derived by crossing heterozygous *Kcna1*$^{-/+}$ mice (Tac:N:NIHS-BC) with homozygous *Tau*$^{-/-}$ (C57BL/6) mice, were intercrossed to produce F2 double mutants of mixed Black Swiss (BlSw, Tac:N:NIHS-BC) and C57BL/6 background. Mice of either sex were used for all experiments unless otherwise stated. Mice were housed at 22°C with a 12 h light/dark cycle and fed *ad libitum*. All procedures were performed in accordance with the guidelines of the National Institute of Health, as approved by the Animal Care and Use Committee of Baylor College of Medicine.

Genotyping. Genomic DNA was isolated from tail clips using DirectPCR Lysis Reagent (Viagen Biotech). Genotype was determined using PCR amplification for specific alleles as described previously for *Kcna1*- (Smart et al., 1998) and *Tau*- (Dawson et al., 2001). PCR products were separated on a 1.5% agarose gel by electrophoresis.

In vivo *video-electroencephalography recordings. Kcna1*$^{-/-}$ mice bred with varying tau alleles (*Tau*$^{+/+}$, *Tau*$^{+/-}$, and *Tau*$^{-/-}$) were anesthetized by Avertin and surgically implanted with bilateral silver wire electrodes (0.005 inch diameter) attached to a microminiature connector. Electroencephalography (EEG) wires were inserted into the subdural space over the temporal cortex through cranial burr holes. Mice were allowed to recover for 24 or more hours before analysis. EEG and behavioral activity in freely moving mice were analyzed using simultaneous video-EEG monitoring (Haramonie software version 6.1c, Stellate Systems). All EEG signals were filtered using a 0.3 Hz high-pass filter, 70 Hz low-pass filter, and 60 Hz notch filter. Eight mice of each genotype were monitored at 4–6 weeks of age and were each evaluated for a total of 9 or more hours. Mice were recorded in one or more 3–9 h monitoring sessions over several days to mitigate the effect of seizure clustering. Seizure activity defined by EEG waveform and corresponding video-recorded behavior was quantified by visual inspection.

Hippocampal slice preparation and electrophysiology. Transverse hippocampal slices (300 μm thickness) from double-mutant mice of genotypes *Kcna1*$^{+/+}$*Tau*$^{+/+}$, *Kcna1*$^{+/+}$*Tau*$^{-/-}$, *Kcna1*$^{-/-}$*Tau*$^{+/+}$, and *Kcna1*$^{-/-}$*Tau*$^{-/-}$ (6–11 weeks old) were prepared using a vibratome. Slices were sectioned in cutting solution containing (in mM) 100 sucrose, 30 NaCl, 3 KCl, 0.5 $CaCl_2$, 28 $NaHCO_3$, 7 $MgCl_2$, 1.4 NaH_2PO_4, and 11 D-glucose and were constantly gassed with 95%O_2/5%CO_2 to maintain a constant pH of 7.4. After incubation in artificial CSF (ACSF) at 32°C for 1 h, slices were transferred into a submerged recording chamber for electrophysiological recordings. Brain slices were constantly perfused with ACSF containing (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 25 $NaHCO_3$, 1 MgCl, 1.25 NaH_2PO_4, and 11 D-glucose. Recording chamber temperature was controlled at 32−33°C. Recording pipettes (4–6 Mohm) were pulled from borosilicate glass and filled with 2 M NaCl. CA3 pyramidal neurons have been previously shown to exhibit altered *in vitro* network excitability in *Kcna1*$^{-/-}$ mice (Smart et al., 1998; Lopantsev et al., 2003; Glasscock et al., 2007). Therefore, field recordings were made from the CA3 pyramidal somata identified visually in the stratum pyramidale using a Getting Instruments Model 5A amplifier, digitized by a Digidata 1322A, and collected using Clampex (Molecular Devices). A low-pass filter was set at 5 kHz. Slices were perfused with ACSF containing 7.5 mM KCl (Glasscock et al., 2007) and began synchronously discharging within a period of 15–25 min. To calculate the burst frequency, slices were allowed to equilibrate to 7.5 mM KCl for an additional 5–15 min and the burst frequency was then calculated over a 5 min period. The burst duration was defined as the interval between baseline crossings and analyzed as the average of 10 bursts per slice. Data analysis was performed using Clampfit (Molecular Devices) and Origin 7.5 (OriginLab).

Three-dimensional magnetic resonance imaging and brain volumetry. MRI of the brain was performed on 12-week-old male mice of genotypes *Kcna1*$^{-/-}$*Tau*$^{+/+}$, *Kcna1*$^{-/-}$*Tau*$^{-/-}$, *Kcna1*$^{+/+}$*Tau*$^{+/+}$, and *Kcna1*$^{+/+}$*Tau*$^{-/-}$ ($n = 3$). Mice were overdosed with isofluorane, placed in a 50ml conical tube, and all imaged identically within 1 h. To mitigate postmortem delay, mice were cooled to 20°C during imaging. MRI images were obtained using a 9.4T, 21 cm bore horizontal scanner with a 35 mm volume resonator (Bruker, BioSpin). The imaging parameters used to obtain three-dimensional (3D) rapid acquisition with relaxation enhancement (RARE) images of the mouse brain were as follows: TR = 2000 ms; TE = 11.713 ms; effective TE = 46.85 ms; FOV = 25 mm^3; matrix = 256 × 256 × 164; RARE Factor = 8; number of averages = 5; total scan time = 14 h 34 m 40 s. Images were obtained using Paravision software version 4 (Bruker, BioSpin).

MRI images were analyzed while blinded to genotype using Amira 3.1 software (Visage Imaging). The hippocampal border was segmented manually in both coronal and sagittal planes and the hippocampus volume measurement for each mouse was computed as the average of the volumes in the two planes. The forebrain volume was segmented in the sagittal plane.

Fly stocks and behavioral testing. Drosophila melanogaster were maintained on standard media at 25−26°C. The BS strains used were *easily shocked* (*eas*), which encodes an ethanolamine kinase, and *kazachoc* (*kcc*), which encodes a K^+/Cl^- cotransporter, and were obtained from M. Tanouye at University of California, Berkley, Berkley, CA (Pavlidis et al., 1994; Hekmat-Scafe et al., 2006). The BS alleles used in this study were *eas*^{PC80} and *kcc*^{DHS1}. Tau P-element (*tau*^{EP3203}) and deficiency (*Df(3R) MR22*) alleles were obtained from Bloomington Stock Center and D. St. Johnston at University of Cambridge, Cambridge, UK, respectively (Doerflinger et al., 2003). Male and female *kcc* flies and male *eas* flies were analyzed for bang sensitivity 1–2 d posteclosion and not exposed to CO_2 within 2 h preceding testing. To test, ≤10 flies were placed in a clean vial and allowed to rest for 30 min. Flies were vortexed (VWR) at maximum strength for 10 s and visually monitored for the presence of paralysis and seizure in each fly.

Western blotting. Kcna1$^{-/-}$ and *Kcna1*$^{+/+}$ forebrain hemisphere samples (4.5 weeks old) were extracted and flash frozen in liquid nitrogen. Samples were homogenized on ice using a Tissue Tearor (VWR) in lysis buffer with phosphatase inhibitors (20 mM Tris, pH 7.5, 138 mM NaCl, 3 mM KCl, 1% Tx-100, 1 mM EGTA, 2 mM EDTA, 1 mM Benzamidine, 5 μg/ml Aprotinin, 5 μg/ml Leupeptin, 5 μg/ml Pepstatin A, 1 mM PMSF, 1 mM DTT, 50 mM NaF, 1 mM Na_3VO_4) and protein concentration determined by Pierce BCA Protein Assay Kit (Thermo Scientific). Twenty micrograms of protein were separated on 12% Tris-Glycine-SDS polyacrylamide gels (Thermo Scientific) and transferred to nitrocellulose membrane. Membranes were blocked overnight at 4°C in 5% milk in TBST, and incubated with primary antibodies including mouse anti-Tau (Tau-5, 1:1000, Millipore), rabbit anti-Tau phospho-Threonine 231 (Tau-pT231, 1:1000, Millipore), and mouse anti-GAPDH (6C5, 1:5000, Advanced ImmunoChemical) for 2 h at room temperature. Membranes were then rinsed in TBST and incubated with appropriate HRP-conjugated secondary antibody, either donkey anti-mouse or goat anti-

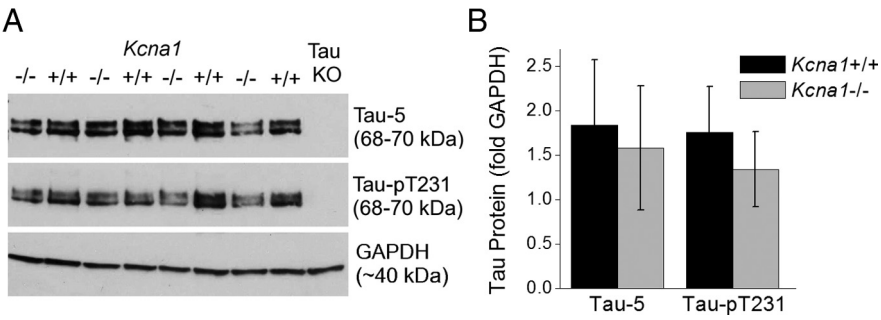

Figure 1. Tau protein levels and phosphorylation at Thr^{231} in 4.5-week-old *Kcna1*$^{-/-}$ mouse forebrain do not significantly differ from *Kcna1*$^{+/+}$ mice. ***A***, Representative Western blots of total tau (Tau-5), tau phospo-Thr^{231} (Tau-pT231), and GAPDH-loading control for *Kcna1*$^{-/-}$ and *Kcna1*$^{+/+}$ mice, as well as a tau knock-out, which showed no tau or tau phospho-Thr^{231} staining. ***B***, Quantification of Tau-5 and Tau-pT231 normalized to GAPDH showed no significant difference in total tau or tau phospho-Thr^{231} levels between *Kcna1*$^{-/-}$ and *Kcna1*$^{+/+}$ mice. $p > 0.05$; one-way ANOVA; $n = 8$; error bars represent SD.

rabbit (1:10,000, Santa Cruz Biotechnology), for 1 h at room temperature. Protein was detected using SuperSignal chemiluminescent substrate (Pierce Thermo Scientific) and quantified by ImageJ (NIH). Membranes were stained sequentially for Tau-5 and GAPDH, stripped in stripping buffer (2% SDS, 100 mM β-mercaptoethanol, 50 mM Tris, pH 6.8) at 50°C for 30 min, and stained for Tau-pT231. Results were replicated three times to ensure accuracy.

Aβ ELISA. Forebrain hemispheres were sonicated in 0.2% diethylamine (DEA) in 50 mM NaCl with protease inhibitor (Sigma) at a volume of 1 ml per 100 mg of tissue. Samples were spun at 100,000 × *g* (53,000 rpm) for 30 min at 4°C and supernatant collected, neutralized in 0.5 M Tris-HCl, pH 6.8, and analyzed by ELISA as previously described (Kawarabayashi et al., 2001). The pellet was then sonicated in 70% formic acid, centrifuged, and supernatant analyzed by ELISA. Mouse Aβ levels were analyzed in *Kcna1*$^{-/-}$ and *Kcna1*$^{+/+}$ mice using capture antibody BNT77, which specifically recognized rodent Aβ11–28, and BA27 and BC05 antibodies to detect Aβ_{40} and Aβ_{42}, respectively.

Statistical analysis. Data were analyzed for statistical significance using SPSS 16.0 (IBM). Survival analysis was completed by Kaplan–Meier log rank (Mantel–Cox). One-way ANOVA, with Tukey *post hoc* when necessary, was used to compare tau protein expression, abnormal EEG discharge duration, hippocampal volume, and forebrain volume between genotypes. Comparisons of seizure frequency between genotypes were made using the nonparametric Kruskal–Wallis test. Electrophysiology data were analyzed by one-way ANOVA with Bonferroni *post hoc* and Aβ level comparisons made by *t* test (two-tailed) using GraphPad Prism 5.0 (Graphpad Software). All analysis of *Drosophila* results was completed by χ^2 analysis. Error bars represent SD unless otherwise stated with the exception of *in vitro* electrophysiology results, which are reported as SEM.

Results

Young Kv1.1-deficient mice maintain wild-type Aβ, tau, and tau phospho-Thr^{231} protein levels in the forebrain

Since electrically induced seizures are known to acutely stimulate Aβ secretion, and kainic acid injections induce tau phosphorylation in mice, we first evaluated the *Kcna1*$^{-/-}$ brain for evidence of AD-like molecular pathology (Cirrito et al., 2005; Crespo-Biel et al., 2007; Liang et al., 2009). To determine whether chronic spontaneous seizures in young *Kcna1*$^{-/-}$ mice alter Aβ levels or tau levels and phosphorylation, 4.5-week-old *Kcna1*$^{-/-}$ and *Kcna1*$^{+/+}$ ($n = 8$) littermate mouse forebrain samples were analyzed by ELISA for Aβ_{40} and Aβ_{42} as well as Western blot for total tau (Tau-5) and tau phosphorylated at threonine 231 (Tau-pT231). ELISA analysis showed that soluble, DEA-extracted Aβ_{40}, Aβ_{42}, and total Aβ levels in young *Kcna1*$^{-/-}$ mice were not significantly different from *Kcna1*$^{+/+}$ wild-type controls (Aβ_{40}: *Kcna1*$^{-/-}$: 108.44 ± 8.26 pmol, *Kcna1*$^{+/+}$: 96.91 ± 14.84 pmol, $p = 0.08$; Aβ_{42}: *Kcna1*$^{-/-}$: 27.07 ± 3.05 pmol, *Kcna1*$^{+/+}$: 24.28 ± 3.60 pmol, $p = 0.12$; total Aβ: *Kcna1*$^{-/-}$: 135.51 ± 11.22 pmol, *Kcna1*$^{+/+}$: 121.19 ± 18.37 pmol, $p = 0.08$, $n = 8$). There was also no difference in the ratio of Aβ_{40}/Aβ_{42} between *Kcna1*$^{-/-}$ and wild-type mice (*Kcna1*$^{-/-}$: 4.02 ± 0.18, *Kcna1*$^{+/+}$: 3.99 ± 0.13, $p = 0.69$, $n = 8$). No aggregated Aβ was detected in formic acid isolated samples from mice of either genotype.

Next, we analyzed Tau levels and phosphorylation at threonine 231. We selected this phosphorylation site since Thr^{231} phosphorylation is increased within 3 d after kainic acid-induced status epilepticus in mice, but the effect of spontaneous seizures on this site is not currently known (Crespo-Biel et al., 2007). Additionally, tau phosphorylation at Thr^{231} occurs early in the pre-NFT stage of human AD pathogenesis, and a high level of tau phospho-Thr^{231} in patients with mild cognitive impairment correlates with subsequent development of AD (Augustinack et al., 2002; Buerger et al., 2002), making it an informative biomarker to test for early changes in tau phosphorylation status in young Kv1.1-null mice. Western blot analysis (Fig. 1*A*) showed that *Kcna1*$^{-/-}$ total tau and tau phospho-Thr^{231} levels, normalized to GAPDH, were not significantly different from those in wild-type *Kcna1*$^{+/+}$ mice (Fig. 1*B*; Tau-5: *Kcna1*$^{+/+}$: 1.84 ± 0.74, *Kcna1*$^{-/-}$: 1.58 ± 0.70, $p = 0.49$; Tau-pT231: *Kcna1*$^{+/+}$: 1.76 ± 0.52, *Kcna1*$^{-/-}$: 1.34 ± 0.42, $p = 0.10$, $n = 8$). The ratio of tau phospho-Thr^{231} to total tau also did not significantly differ between *Kcna1*$^{-/-}$ and *Kcna1*$^{+/+}$ mice (*Kcna1*$^{+/+}$: 1.06 ± 0.32, *Kcna1*$^{-/-}$: 0.92 ± 0.23, $p = 0.33$, $n = 8$). These results demonstrate that hyperexcitability and spontaneous epileptic seizures in the forebrain of young Kv1.1-null mice do not appreciably affect Aβ and tau levels, or induce sustained abnormal tau phosphorylation at Thr^{231}. Thus, young *Kcna1*$^{-/-}$ mice do not display signs of AD-like molecular pathology despite the occurrence of spontaneous seizures.

Decreasing tau reduces hyperexcitability in Kv1.1-deficient mice

To determine whether tau loss affects hyperexcitability and spontaneous seizures we recorded *Kcna1*$^{-/-}$*Tau* double-mutant male and female mice (4–6 weeks old) with *in vivo* video-EEG for 9 or more hours. Electrocorticograms of *Kcna1*$^{-/-}$ mice showed frequent and severe spontaneous seizures characterized by abnormal, generalized, high-frequency hypersynchronous EEG discharges (200–500 mV amplitude) with abrupt cessation, followed by a period of EEG amplitude depression (Fig. 2*A*). Seizures in *Kcna1*$^{-/-}$ mice occurred up to 1.27 times an hour with an average of 0.51 ± 0.19 seizures/h (Fig. 2*B*; ±SEM). Decreasing tau by heterozygous and homozygous tau knock out significantly decreased seizure frequency in *Kcna1*$^{-/-}$ mice to 0 and 0.03 ± 0.03 seizures/h, respectively (Fig. 2*B*; ±SEM; *Kcna1*$^{-/-}$*Tau*$^{+/+}$ vs *Kcna1*$^{-/-}$*Tau*$^{+/-}$: $p = 0.011$; *Kcna1*$^{-/-}$*Tau*$^{+/+}$ vs *Kcna1*$^{-/-}$*Tau*$^{-/-}$: $p = 0.035$, $n = 8$). None of the 8 *Kcna1*$^{-/-}$*Tau*$^{+/-}$ mice and only 1 of 8 *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice displayed seizures during recording, compared with 5 of 8 *Kcna1*$^{-/-}$*Tau*$^{+/+}$ mice (Fig. 2*B*). Individual *Kcna1*$^{-/-}$*Tau*$^{+/+}$ mice showed a variable range of seizure frequency that was not due to sex or litter. The range was most likely due to phenotypic variation of the *Kcna1*$^{-/-}$ model within a mixed background (C57BL/6/BlSw). Importantly, this variability was not

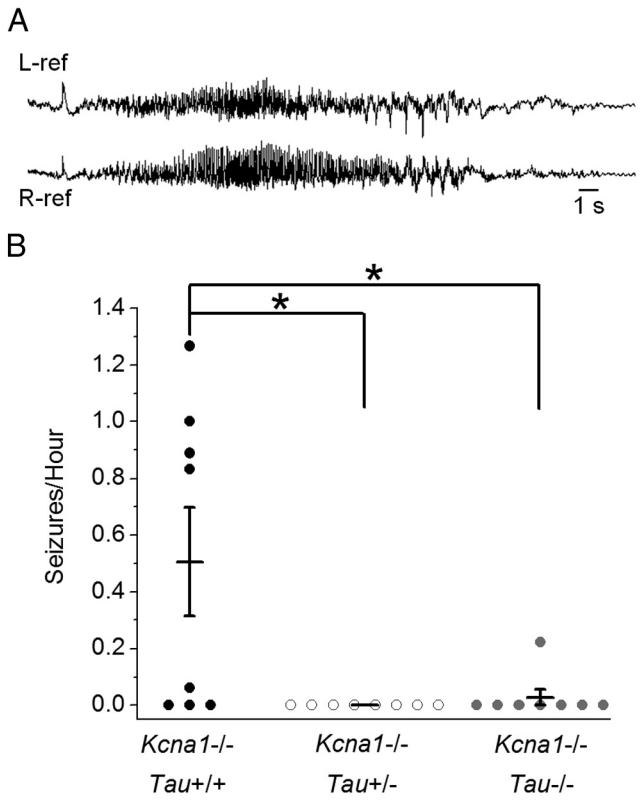

Figure 2. Tau reduction significantly decreases seizure frequency in *Kcna1*$^{-/-}$ mice. ***A***, Representative spontaneous cortical seizure recorded bilaterally in a *Kcna1*$^{-/-}$ mouse during chronic *in vivo* EEG monitoring. ***B***, Analysis of seizures/h in double-mutant mice recorded for 9 or more hours. Both tau reduction and loss significantly decreased seizure frequency in *Kcna1*$^{-/-}$*Tau*$^{+/-}$ ($n = 8$) and *Kcna1*$^{-/-}$*Tau*$^{-/-}$ ($n = 8$) double mutants compared with *Kcna1*$^{-/-}$*Tau*$^{+/+}$ ($n = 8$). Seizures were observed in 5/8 *Kcna1*$^{-/-}$*Tau*$^{+/+}$ mice, 0/8 *Kcna1*$^{-/-}$*Tau*$^{+/-}$ mice, and only 1/8 *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice. Total deletion of tau reduced the average seizure frequency by >94%. *$p < 0.05$; Kruskal–Wallis; error bars represent SEM.

observed in *Kcna1*$^{-/-}$*Tau*$^{+/-}$ or *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice, which both had significantly decreased seizure frequency compared with *Kcna1*$^{-/-}$*Tau*$^{+/+}$.

In addition to decreasing the number of behavioral seizures in *Kcna1*$^{-/-}$ mice, tau loss also significantly decreased the duration of abnormal electrographic cortical discharge activity by ~60%. This measure includes *Kcna1*$^{-/-}$ seizures (Fig. 2*A*) as well as abnormal spike bursts that consist of repetitive, distinct spikes, and/or spike-wave complexes occurring at a maximum frequency of 3–9 Hz. These aberrant electrographic discharges in *Kcna1*$^{-/-}$*Tau*$^{+/+}$ mice averaged 43 ± 32 s in duration ($n = 54$) compared with *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice, which averaged only 18 ± 8 s ($n = 11$, $p = 0.013$). Together, these results demonstrate that decreasing tau dosage, even to heterozygous levels, can decrease cortical hyperexcitability in *Kcna1*$^{-/-}$ seizure mutants.

Kcna1$^{-/-}$ mice have altered *in vitro* network excitability in the CA3 pyramidal region of the hippocampus when exposed to elevated potassium levels (Smart et al., 1998; Lopantsev et al., 2003; Glasscock et al., 2007). To further examine the effect of tau loss on hyperexcitability, we analyzed *in vitro* hippocampal network excitability in the CA3 pyramidal region of *Kcna1Tau* double mutants. When extracellular K^+ was raised from 2.5 mM to 7.5 mM, we observed spontaneous discharges that stabilized at frequencies after 5–15 min in all slices, but frequency and duration of bursts varied by genotype. *Kcna1*$^{-/-}$*Tau*$^{+/+}$ burst, on average, almost three times faster than *Kcna1*$^{+/+}$*Tau*$^{+/+}$ wild-type controls (Fig. 3*A*,*B*; *Kcna1*$^{-/-}$*Tau*$^{+/+}$: 0.52 ± 0.04 Hz, $n = 22$, *Kcna1*$^{+/+}$*Tau*$^{+/+}$: 0.17 ± 0.02 Hz, $n = 17$, $p < 0.0001$). Conversely, burst duration was significantly decreased in *Kcna1*$^{-/-}$*Tau*$^{+/+}$ mice compared with *Kcna1*$^{+/+}$*Tau*$^{+/+}$ controls (Fig. 3*C*,*D*; *Kcna1*$^{-/-}$*Tau*$^{+/+}$: 94.4 ± 5.0 ms, $n = 22$, *Kcna1*$^{+/+}$*Tau*$^{+/+}$: 118.6 ± 5.4 ms, $n = 17$, $p < 0.05$).

Tau loss significantly decreased burst frequency in *Kcna1*$^{-/-}$ mice and *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice were not significantly different from *Kcna1*$^{+/+}$*Tau*$^{+/+}$ controls. Tau loss in *Kcna1*$^{-/-}$ mice reduced burst frequency to wild-type levels (Fig. 3*A*,*B*; *Kcna1*$^{-/-}$*Tau*$^{-/-}$: 0.26 ± 0.03 Hz, $n = 19$, vs *Kcna1*$^{-/-}$*Tau*$^{+/+}$: $p < 0.0001$, vs *Kcna1*$^{+/+}$*Tau*$^{+/+}$: $p > 0.05$). Interestingly, tau loss did not alter burst frequency in wild-type slices (Fig. 3*A*,*B*; *Kcna1*$^{+/+}$*Tau*$^{-/-}$: 0.18 ± 0.02 Hz, $n = 15$, vs *Kcna1*$^{+/+}$*Tau*$^{+/+}$: $p > 0.05$, vs *Kcna1*$^{-/-}$*Tau*$^{-/-}$: $p > 0.05$). Similarly, burst duration in *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice is significantly increased compared with *Kcna1*$^{-/-}$*Tau*$^{+/+}$ and not significantly different from *Kcna1*$^{+/+}$*Tau*$^{+/+}$ controls. Loss of tau rescued burst duration in *Kcna1*$^{-/-}$ mice to wild-type levels (Fig. 3*C*,*D*; *Kcna1*$^{-/-}$*Tau*$^{-/-}$: 134.8 ± 8.1 ms, $n = 19$, vs *Kcna1*$^{-/-}$*Tau*$^{+/+}$: $p < 0.0001$, vs *Kcna1*$^{+/+}$*Tau*$^{+/+}$: $p > 0.05$). *Kcna1*$^{+/+}$*Tau*$^{-/-}$ burst duration was also not significantly different from *Kcna1*$^{+/+}$*Tau*$^{+/+}$ controls or *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice (Fig. 3*C*,*D*; *Kcna1*$^{+/+}$*Tau*$^{-/-}$: 123.8 ± 5.8 ms, $n = 15$, vs *Kcna*1$^{+/+}$*Tau*$^{+/+}$: $p > 0.05$, vs *Kcna1*$^{-/-}$*Tau*$^{-/-}$: $p > 0.05$). These results demonstrate that tau loss can prevent *in vitro* hippocampal network hyperexcitability in the epileptic *Kcna1*$^{-/-}$ brain, but does not alter wild-type hippocampal excitability.

Decreasing tau dosage increases survival in Kv1.1-deficient mice

Kv1.1-null mice die prematurely and are an established model of sudden unexpected death in epilepsy (SUDEP) (Glasscock et al., 2010, 2012). Since tau loss in *Kcna1*$^{-/-}$ mice decreased cortical hyperexcitability and seizures, we examined whether tau loss could prolong lifespan in *Kcna1*$^{-/-}$ mice by daily monitoring of *Kcna1*$^{-/-}$*Tau* double-mutant survival. *Kcna1*$^{-/-}$*Tau*$^{+/+}$ mice ($n = 27$) died prematurely beginning in the third week of life with only 30% surviving to 10 weeks (Fig. 4). However, when tau dosage is decreased by heterozygous tau deletion, *Kcna1*$^{-/-}$*Tau*$^{+/-}$ mice ($n = 37$) had a significant twofold increase in survival, with 59% alive at 10 weeks of age (Fig. 4; $p = 0.013$). *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice ($n = 23$) exhibited an even more striking decrease in mortality rate, with 74% surviving until 10 weeks (Fig. 4; $p = 0.003$). This increase in survival was also demonstrated in video-EEG monitoring studies, where episodes of status epilepticus followed by death were observed in *Kcna1*$^{-/-}$*Tau*$^{+/+}$ and *Kcna1*$^{-/-}$*Tau*$^{+/-}$ mice but not *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice while undergoing video-EEG recording. These results support a robust role for tau loss in suppressing central hyperexcitability, and demonstrate the positive effect of decreasing tau dosage, even by 50%, on seizures and premature lethality.

Tau loss prevents megencephaly in Kv1.1-deficient mice

In addition to seizures and early lethality, *Kcna1*$^{-/-}$ mice exhibit abnormal enlargement of the hippocampus and ventral cortex (Persson et al., 2007). To determine whether tau loss can influence megencephaly in *Kcna1*$^{-/-}$ mice, double-mutant mouse brains were imaged by MRI and analyzed for total hippocampal and forebrain volume (Fig. 5). At 12 weeks of age, *Kcna1*$^{-/-}$ mice had noticeably larger hippocampal and forebrain volumes that were significantly increased relative to *Kcna1*$^{+/+}$*Tau*$^{+/+}$ controls by 43.3 and 33.1%, respectively (Fig. 5*Q*,*R*; hippocampus:

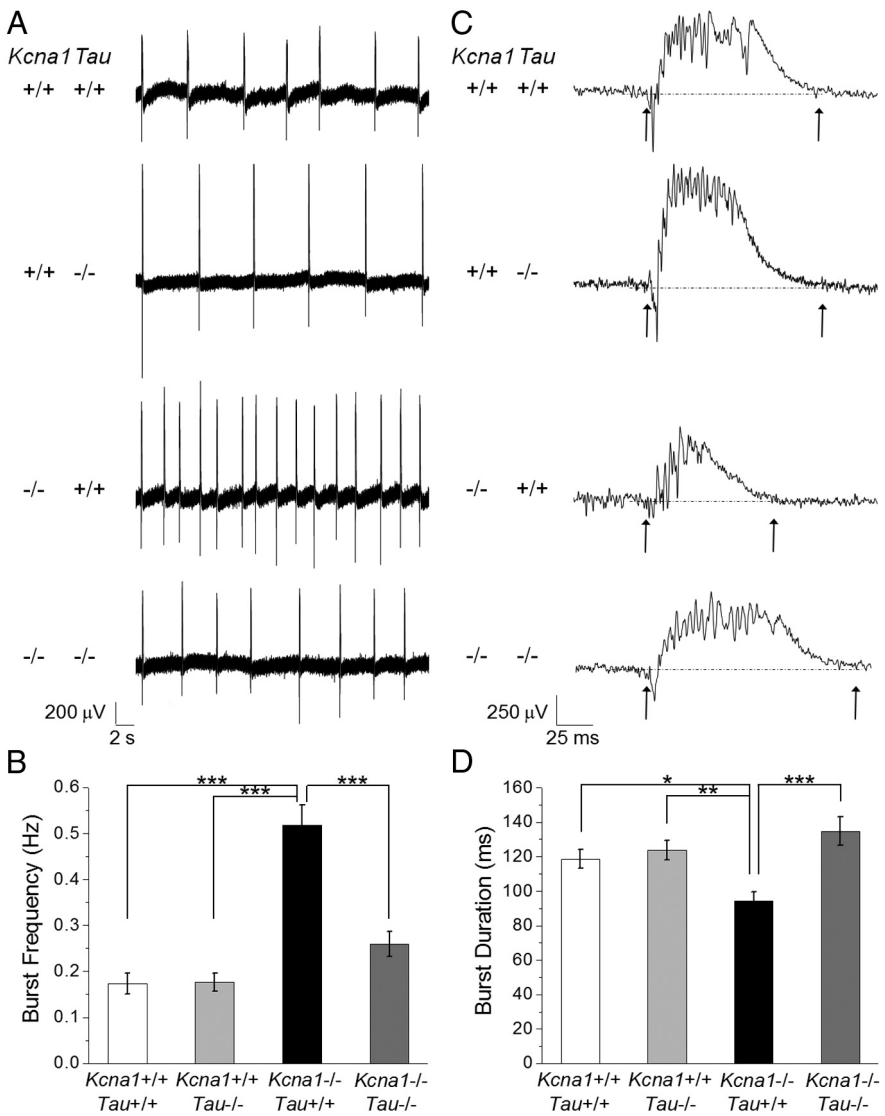

Figure 3. Tau loss decreases network hyperexcitability in *Kcna1* $^{-/-}$ hippocampal slices exposed to increased extracellular K^+ levels. ***A***, Spontaneous discharges in CA3 pyramidal cells were observed in 6–11 week-old mouse brain slices when K^+ was raised from 2.5 to 7.5 mM. Representative 30 s traces illustrate differences in burst frequency between genotypes. ***B***, Analysis of burst frequency during 5 min periods of spontaneous bursting per slice. *Kcna1* $^{-/-}$*Tau* $^{+/+}$ hippocampus ($n = 22$) had significantly increased burst frequency compared with *Kcna1* $^{+/+}$*Tau* $^{+/+}$ wild-type controls ($n = 17$), while *Kcna1* $^{-/-}$*Tau* $^{-/-}$ slices ($n = 19$) were not significantly different from *Kcna1* $^{+/+}$*Tau* $^{+/+}$. *Kcna1* $^{+/+}$*Tau* $^{-/-}$ burst frequency ($n = 15$) was also indistinguishable from wild type or *Kcna1* $^{-/-}$*Tau* $^{-/-}$ double mutants. ***C***, Representative 200 ms traces illustrate burst duration differences between genotypes, as defined by baseline crossings (arrows). ***D***, Quantification of burst duration in 10 spontaneous bursts per brain slice. *Kcna1* $^{-/-}$*Tau* $^{+/+}$ hippocampus ($n = 22$) generated significantly shorter bursts than *Kcna1* $^{+/+}$*Tau* $^{+/+}$ ($n = 17$), *Kcna1* $^{+/+}$*Tau* $^{-/-}$ ($n = 15$), and *Kcna1* $^{-/-}$*Tau* $^{-/-}$ slices ($n = 19$), which were not significantly different. Loss of tau normalized *Kcna1* $^{-/-}$ spontaneous burst frequency and duration to wild-type levels. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$; one-way ANOVA; error bars represent SEM.

Kcna1 $^{-/-}$*Tau* $^{+/+}$: 31.65 ± 1.42 mm^3, *Kcna1* $^{+/+}$*Tau* $^{+/+}$: 22.08 ± 2.48 mm^3, $p = 0.001$; forebrain: *Kcna1* $^{-/-}$*Tau* $^{+/+}$: 379.86 ± 13.24 mm^3, *Kcna1* $^{+/+}$*Tau* $^{+/+}$: 285.38 ± 27.64 mm^3, $p = 0.001$, $n = 3$). Removal of tau resulted in a significant decrease in both hippocampal and forebrain volume by 25.7 and 22.4%, respectively, for *Kcna1* $^{-/-}$*Tau* $^{-/-}$ mice compared with *Kcna1* $^{-/-}$*Tau* $^{+/+}$ (Fig. 5*Q*,*R*; *Kcna1* $^{-/-}$*Tau* $^{-/-}$: hippocampus: 23.53 ± 2.01 mm^3, $p = 0.003$; forebrain: 294.59 ± 20.88 mm^3, $p = 0.003$, $n = 3$). *Kcna1* $^{-/-}$*Tau* $^{-/-}$ hippocampal and forebrain volumes did not differ from *Kcna1* $^{+/+}$*Tau* $^{-/-}$ or *Kcna1* $^{+/+}$*Tau* $^{+/+}$ controls (Fig. 5*Q*,*R*; hippocampus: *Kcna1* $^{-/-}$*Tau* $^{-/-}$ vs *Kcna1* $^{+/+}$*Tau* $^{-/-}$: $p = 0.43$; *Kcna1* $^{-/-}$*Tau* $^{-/-}$ vs *Kcna1* $^{+/+}$*Tau* $^{+/+}$: $p = 0.78$; forebrain: *Kcna1* $^{-/-}$*Tau* $^{-/-}$ vs *Kcna1* $^{+/+}$*Tau* $^{-/-}$: $p = 0.49$; *Kcna1* $^{-/-}$*Tau* $^{-/-}$ vs *Kcna1* $^{+/+}$*Tau* $^{+/+}$: $p = 0.93$, $n = 3$). Loss of tau rescued *Kcna1* $^{-/-}$ megencephaly and decreased brain volume to wild-type levels. This observed decrease in volume was not due to tau loss alone, since hippocampal and forebrain volumes in *Kcna1* $^{+/+}$*Tau* $^{-/-}$ mice did not differ from those of *Kcna1* $^{+/+}$*Tau* $^{+/+}$ wild-type controls (Fig. 5*Q*,*R*; *Kcna1* $^{+/+}$*Tau* $^{-/-}$: hippocampus: 21.07 ± 1.30 mm^3, $p = 0.91$; forebrain: 271.35 ± 10.87 mm^3, $p = 0.81$, $n = 3$). Light microscopic examination of cresyl violet-stained sections revealed that tau loss had no discernible neurocytological effects on regional brain structure or organization, including cortical or hippocampal lamination (data not shown). These findings suggest that tau loss not only decreases network hyperexcitability, but also prevents megencephaly associated with epilepsy in the Kv1.1 model.

Reducing tau decreases hyperexcitability in bang-sensitive *Drosophila* mutants

BS *Drosophila* mutants define a class of functional excitability phenotypes that display behavioral seizure susceptibility following mechanical or electrical shock. Upon stimulation, these mutants exhibit intense activation (limbshaking) followed by a period of paralysis and a second period of hyperexcitability characterized by uncoordinated seizure-like movements (Pavlidis et al., 1994), and are an established model system for human seizure disorders (Kuebler and Tanouye, 2000; Kuebler et al., 2001; Song and Tanouye, 2008). To determine whether tau reduction is sufficient to decrease hyperexcitability in *Drosophila* BS mutants, double-mutant flies generated by decreasing tau gene expression in several different BS models were tested for paralysis and seizure phenotypes following 10 s of a vortex stimulus. *Drosophila* with homozygous tau deficiency are nonviable. Therefore, to reduce tau levels in *Drosophila*, two previously reported alleles were used. The first, a P-element disruption of the *tau* gene, *tau*^{EP3203}, has a P-element insertion in the first intron of the gene (Doerflinger et al., 2003). The second is a tau deficiency allele, *Df(3R)MR22* (*MR22*), which has a 62 kb deletion caused by recombination of EP3203, removing most of the tau locus and resulting in homozygous lethality (Doerflinger et al., 2003).

Kcc BS *Drosophila* (*kcc* ^{DHS1}) carry a partial loss-of-function mutation in the K^+/Cl^- cotransporter gene *kazachoc* (Hekmat-Scafe et al., 2006). When tau was reduced in *kcc* flies by *tau*^{EP3203}, the percentage of flies that exhibited bang-sensitive behavior and paralysis was significantly decreased by 40% with homozygous *tau*^{EP3203} and 34% with *tau*^{EP3203}/+ (Fig. 6; *kcc*;+/+: 47% BS, *kcc*;*tau*^{EP3203}: 28% BS, *kcc*;*tau*^{EP3203}/+: 31% BS, *kcc*;+/+ vs *kcc*;*tau*^{EP3203}: $p < 0.01$, *kcc*;+/+ vs *kcc*;*tau*^{EP3203}/+: $p < 0.05$, n≥87).

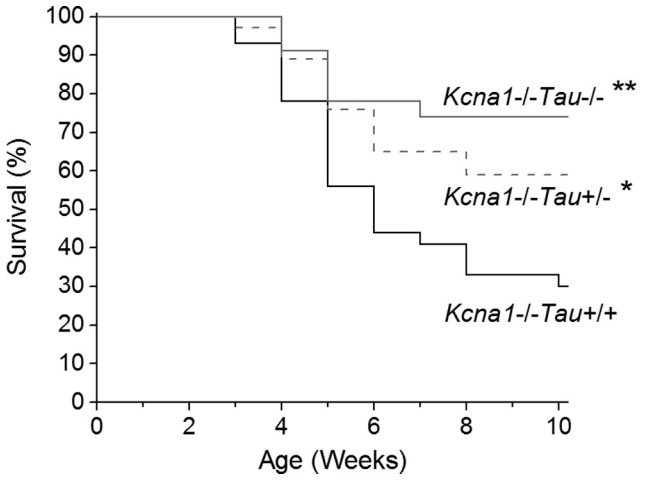

Figure 4. Decreasing tau dosage significantly increases survival of *Kcna1*$^{-/-}$ mice. Kaplan–Meier survival curves show that *Kcna1*$^{-/-}$*Tau*$^{+/+}$ mice ($n = 27$) exhibited severe early lethality beginning in the third week of life, with only 30% surviving to 10 weeks of age. Decreasing tau dosage in *Kcna1*$^{-/-}$ mice by heterozygous ($n = 37$) and homozygous ($n = 23$) tau knock-out significantly increased survival, with 59 and 74% survival, respectively, to 10 weeks of age. *$p = 0.013$, **$p = 0.003$, all significant with respect to *Kcna1*$^{-/-}$*Tau*$^{+/+}$; Kaplan–Meier log rank test.

Additionally, we decreased tau levels in the more seizure-sensitive *eas* mutant, which displays hyperexcitability due to reduced ethanolamine kinase activity, using the deficiency allele *MR22* (Pavlidis et al., 1994; Doerflinger et al., 2003). Tau reduction by *MR22* significantly decreased bang sensitivity in *eas Drosophila* by 13% in *eas;MR22/+* and 15% in *eas;MR22/tau*^{EP3203} flies (Fig. 6; *eas;+/+*: 97% BS, *eas;MR22/+*: 84% BS, *eas;MR22/tau*^{EP3203}: 82% BS, $p < 0.001$, $n \geq 98$). Tau reduction therefore decreased bang sensitivity in multiple *Drosophila* models, suggesting a broader role of tau in regulation of hyperexcitability due to a variety of molecular pathologies.

Discussion

The removal of tau and consequent suppression of epilepsy in AD mouse models established a critical epistatic role for tau in regulating cortical network excitability (Ittner et al., 2010; Roberson et al., 2011). Here, we demonstrate that the beneficial effects of tau loss on aberrant synchronization extend beyond the prevention of Aβ-induced network excitability to encompass hyperexcitability phenotypes due to other, non-AD related causes. Genetic reduction of tau dosage, even to heterozygous levels, significantly decreased spontaneous seizure frequency and duration in the *Kcna1*$^{-/-}$ mouse model of temporal lobe epilepsy. Tau knock-out also decreased *Kcna1*$^{-/-}$ hippocampal network hyperexcitability in response to elevated K^+ levels *in vitro*. In addition to attenuating hyperexcitability, tau loss decreased the early brain and vagal nerve-driven lethality in this model (Glasscock et al., 2010, 2012) and prevented Kv1.1-related megencephaly, with hippocampal and forebrain volumes decreased to wild-type levels. These findings demonstrate a robust and beneficial effect of tau removal on hyperexcitability-related brain phenotypes in the absence of AD-related molecular pathology. As further confirmation, we also found a decrease in experimentally evoked hyperexcitability behaviors in *Drosophila kcc* and *eas* BS mutants when tau levels were reduced, consistent with findings that tau loss decreases chemically evoked seizure severity in mice (Roberson et al., 2007; Ittner et al., 2010).

Aβ, tau, and network excitability in young *Kcna1*$^{-/-}$ mice

Hyperphosphorylation of tau at Thr^{231} is an early event in the progression of tau phosphorylation and NFT production in the human AD brain (Augustinack et al., 2002). Although only one of many tau phosphorylation sites, Thr^{231} phosphorylation is increased in response to kainic acid-induced seizures, and is thus a suitable indicator of tau phosphorylation in our spontaneous seizure mouse model (Crespo-Biel et al., 2007). Additionally, electrically induced synaptic activity and seizures stimulates Aβ secretion (Cirrito et al., 2005). Despite chronic spontaneous seizures, young *Kcna1*$^{-/-}$ mice had no changes in forebrain Aβ production or tau levels and phosphorylation at Thr^{231}. Therefore, rescue of the *Kcna1*$^{-/-}$ hyperexcitability phenotype by tau loss in 4–6 week-old mice is unlikely due to prevention of a neurotoxic hyperphosphorylated tau species, as seen in AD ApoE4 knockin mice, but rather to a loss or reduction of an intrinsic molecular function of tau (Andrews-Zwilling et al., 2010). Transgenic hAPP mice, including the J20 model, also lack hyperphosphorylated tau yet are protected from hyperexcitability by tau loss, supporting the possibility that tau regulates excess excitability in developing or adult brain (Roberson et al., 2007, 2011; Ittner et al., 2010).

Tau loss alleviates downstream seizure-induced comorbidities

Longevity

Tau removal increases survival in two hAPP transgenic mouse strains as well as kainic acid injection seizure models (Roberson et al., 2007; Ittner et al., 2010; Roberson et al., 2011). Our results show that tau loss is similarly effective in a genetic model of epilepsy. Even heterozygous reduction of tau was sufficient to significantly increase survival in the *Kcna1*$^{-/-}$ mutant, as it did in hAPP AD models. In contrast, tau deletion has no effect on survival of SOD1 mutants, a model of ALS with excitotoxic neurodegeneration, indicating that tau loss does not confer resistance to premature mortality in all neurological disorders (Roberson et al., 2011).

Megencephaly

A dramatic enlargement of the hippocampus and ventral cortex in *Kcna1*$^{-/-}$ BALB.C3HeB mice is detectable by 12 weeks of age (Persson et al., 2007). We confirmed this pathology in *Kcna1*$^{-/-}$ mutants on a different genetic background (mixed C57BL/6/BlSw), where we found a 43 and 33% increase in hippocampal and forebrain volume, respectively, compared with wild-type mice. Since the original *megenchephaly* (*Mceph*) mouse model containing a spontaneous *Kcna1* truncation mutation shows a comparable increase in brain volume, the megencephaly phenotype is likely caused by loss of *Kcna1* function (Persson et al., 2007). The *Mceph* mutation increases neuronal and astrocytic proliferation, leading to slow postnatal brain enlargement (Almgren et al., 2007; Yang et al., 2012). This hyperplasia can be suppressed by early treatment with the antiepileptic drug carbamazepine (Lavebratt et al., 2006). The mechanism linking impaired Kv1.1 current, seizures, and the multifactorial megencephaly phenotype has not been fully explored; however, recent findings indicate that Kv1.1 currents can play a cell autonomous role in precursor cell neurogenesis (Yang et al., 2012).

We found that tau loss prevented Kv1.1 megencephaly, with both hippocampal and forebrain volume decreased in 12-week-old *Kcna1*$^{-/-}$*Tau*$^{-/-}$ mice to wild-type levels. This was not due to the simple absence of tau, since hippocampal and forebrain volume in pure tau knock-out mice were indistinguishable from

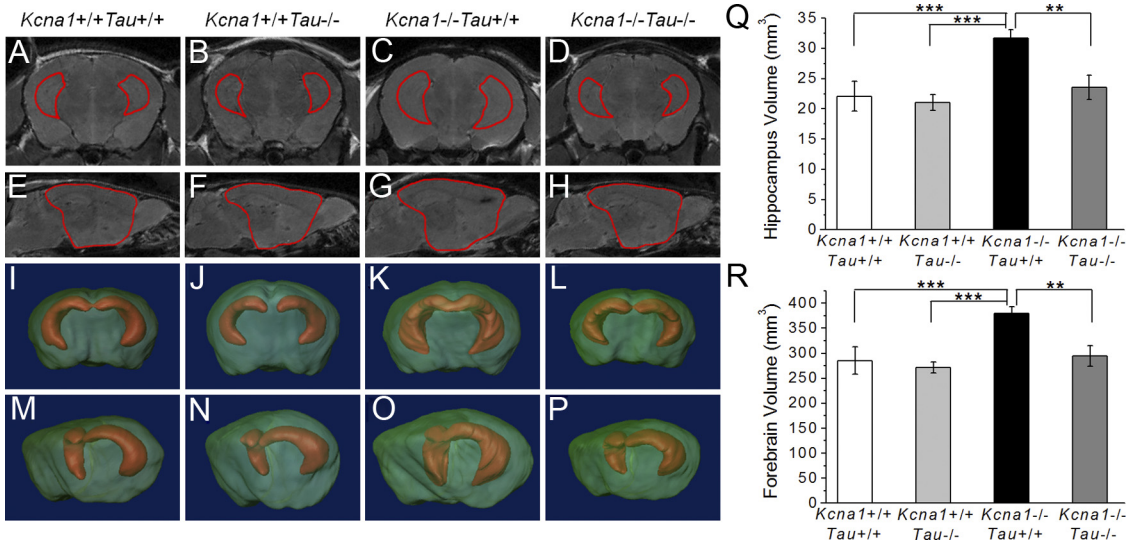

Figure 5. ***A–H***, Tau loss prevents megencephaly in *Kcna1* $^{-/-}$ mouse brain. Left, MRI images of *Kcna1* $^{+/+}$*Tau* $^{+/+}$, *Kcna1* $^{+/+}$*Tau* $^{-/-}$, *Kcna1* $^{-/-}$*Tau* $^{+/+}$, and *Kcna1* $^{-/-}$*Tau* $^{-/-}$ mouse brain were manually segmented and hippocampus (***A–D***) and forebrain (***E–H***) borders drawn to allow 3D reconstruction. ***I–P***, 3D reconstructions shown in both coronal (***I–L***) and partial sagittal (***M–P***) views demonstrate the enlargement of the hippocampus and forebrain in *Kcna1* $^{-/-}$*Tau* $^{+/+}$ (***K***, ***O***) mice relative to other genotypes. ***Q***, ***R***, Quantification of hippocampus (***Q***) and forebrain (***R***) volume in double-mutant mice. *Kcna1* $^{-/-}$*Tau* $^{-/-}$ mice had significantly decreased hippocampus and forebrain volume compared with the megencephalic *Kcna1* $^{-/-}$*Tau* $^{+/+}$. Loss of tau in *Kcna1* $^{-/-}$ mice decreased hippocampus and forebrain volume to wild-type levels. $^{**}p = 0.003$, $^{***}p = 0.001$; one-way ANOVA; $n = 3$; error bars represent SD.

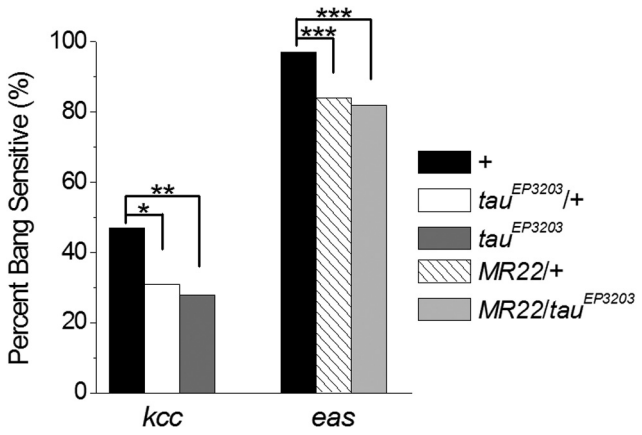

Figure 6. Tau reduction significantly decreases hyperexcitability in bang-sensitive *Drosophila* mutants *kcc* and *eas*. Quantification of the percentage of flies that were bang sensitive in response to a vortex stimulus. Decreasing tau dosage by the *tau*^{EP3203} allele significantly decreased bang sensitivity in *kcc* mutants by 34% in *kcc;tau*^{EP3203}/+ and 40% in *kcc;tau*^{EP3203} compared with *kcc* flies with wild-type tau ($n \geq 87$). Decreasing tau by the deficiency allele *MR22* significantly decreased bang sensitivity in *eas* mutants by 13% (*eas;MR22*/+) and 15% (*eas;MR22/tau*^{EP3203}) compared with *eas* flies with wild-type tau ($n \geq 98$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; χ^2; genotypes: *kcc*^{DHS1}, *kcc*^{DHS1}*;tau*^{EP3203}/+, *kcc*^{DHS1}*;tau*^{EP3203}, *eas*^{PC80}/Y, *eas*^{PC80}/Y*;Df(3R)MR22*/+, *eas*^{PC80}/Y*;Df(3R)MR22/tau*^{EP3203}.

wild type at 12 weeks of age. A previous report also shows that *Tau* $^{-/-}$ mice exhibit wild-type brain size at 6 months of age, although beyond one year brain atrophy is observed (Lei et al., 2012). Based on these lines of evidence, early rescue of megencephaly in our model is likely due to the decreased seizures and hyperexcitability in developing *Kcna1* $^{-/-}$*Tau* $^{-/-}$ mice.

Tau reduction decreases hyperexcitability in *Drosophila* seizure models

We have further shown that partial tau reduction is sufficient to significantly ameliorate the BS phenotype in two different *Drosophila* hyperexcitability mutants. *Kcc* encodes the fly K^+/Cl^- cotransporter, and a partial loss-of-function allele increases seizure susceptibility due to reduction of the Cl^- gradient and impaired GABAergic transmission (Hekmat-Scafe et al., 2010). Loss of *KCC2*, the mammalian homolog of *kcc*, in mice also results in seizures and hyperexcitability (Woo et al., 2002). Truncation of the *eas* gene reduces ethanolamine kinase activity, interfering with metabolism of phosphatidylethanolamine, the predominant membrane lipid, which may alter neuroblast development in the *Drosophila* mushroom body (Pavlidis et al., 1994; Pascual et al., 2005). The ability of tau reduction to decrease seizure susceptibility in all three mutant models demonstrates that tau is a genetic modifier of hyperexcitability that is not specific to a single pathway or molecular mechanism of excitability.

Is tau a possible therapeutic target in epilepsy?

While our results reflect the absence of tau during nervous system development rather than reversal of a preexisting seizure disorder, the robust effect of even partial genetic tau reduction on hyperexcitability phenotypes supports future examination of pharmacological tau reduction as a possible strategy for early disease modification in epilepsy. Although the potentially adverse effects of tau loss have not been fully evaluated, they so far appear to be limited. Despite delays in neuronal migration and process length in culture, synaptic connectivity in *Tau* $^{-/-}$ mouse brain is not apparently altered, as evident by normal synaptophysin and GAP-43 distribution as well as normal GFAP staining patterns (Dawson et al., 2001). In addition, we saw no visible differences in cellular lamination patterns in cresyl violet stained *Tau* $^{-/-}$ sections. *Tau* $^{-/-}$ mice also reportedly show no learning or memory deficits, as well as relatively normal hippocampal electrophysiological properties through 6 months of age (Ikegami et al., 2000; Roberson et al., 2007; Dawson et al., 2010; Ittner et al., 2010; Roberson et al., 2011; Lei et al., 2012). *Tau* $^{-/-}$ mice do show increased sIPSCs in dentate granule cells and less network bursting in disinhibited hippocampal slices, providing one candidate mechanism for decreasing network hyperexcitability in the brain (Morris et al., 2011; Roberson et al., 2011). Tau loss may also be neuroprotective, since knockout leads to defective postsynaptic localization of fyn kinase and decreased excito-

toxic damage in response to neuronal activity (Ittner et al., 2010). Some deleterious consequences of early and complete genetic tau loss were found in aged, 12-month-old *Tau*$^{-/-}$ mice including brain atrophy, iron accumulation, substantia nigra cell loss, and axonal spheroids. (Dawson et al., 2010; Lei et al., 2012).

We have shown that tau loss decreases excitability in the non-AD, epileptic brain. Interestingly, even heterozygous reduction of tau was sufficient to significantly lower seizure frequency, suggesting the potential of tau regulation as a therapeutic target. This is supported by evidence in AD mouse models where heterozygous tau loss decreases hyperexcitability and also improves cognition (Roberson et al., 2007, 2011; Ittner et al., 2010). Importantly, to our knowledge, no neurological deficits have been shown in *Tau*$^{+/-}$ mice at any age studied (Ikegami et al., 2000; Roberson et al., 2007, 2011). Additionally, pharmacological tau reduction by methylene blue, which reduces soluble tau and improves cognition in transgenic tau mice, does not impair Morris Water Maze memory recall in wild-type mice (O'Leary et al., 2010). These results suggest that while complete loss of tau throughout life is not inconsequential, there are potential therapeutic benefits of decreasing tau at an early stage of epileptogenesis.

Overall, these data reflect the genomic absence of tau throughout brain development rather than the reversal of an established seizure disorder. Further studies using developmentally delayed reduction of tau levels will be necessary to distinguish whether the observed changes in hyperexcitability are due to early, synaptic reorganization of the brain or to an ongoing intrinsic regulatory role of tau in adult neuronal firing properties and synaptic transmission.

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