Received: 2 August 2021 | Revised: 30 December 2021 | Accepted: 1 February 2022

DOI: 10.1111/epi.17192

Epilepsia®

FULL-LENGTH ORIGINAL RESEARCH

Pharmacological inhibition of STriatal-Enriched protein tyrosine Phosphatase by TC-2153 reduces hippocampal excitability and seizure propensity

Jennifer M. Walters[1,2] | **Eung Chang Kim**[2] | **Jiaren Zhang**[2] | **Han Gil Jeong**[2] | **Archit Bajaj**[2] | **Brian C. Baculis**[1,2] | **Gregory C. Tracy**[2] | **Baher Ibrahim**[1,2,3] | **Catherine A. Christian-Hinman**[1,2,3] | **Daniel A. Llano**[1,2,3] | **Graham R. Huesmann**[1,2,3,4,5] | **Hee Jung Chung**[1,2,3]

[1]Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

[2]Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

[3]Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

[4]Department of Neurology, Carle Foundation Hospital, Urbana, Illinois, USA

[5]Carle Illinois College of Medicine, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Correspondence
Hee Jung Chung, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
Email: chunghj@illinois.edu

Funding information
National Institute of Neurological Disorders and Stroke, Grant/Award Number: R01 NS083402, R01 NS097610, R01 NS100019, R01 NS105825 and R03 NS103029; National Institute on Deafness and Other Communication Disorders, Grant/Award Number: R01 DC013073 and R01 DC016599; University of Illinois, Grant/Award Number: RB21053;

Abstract

Objective: STriatal-Enriched protein tyrosine Phosphatase (STEP) is a brain-specific tyrosine phosphatase. Membrane-bound $STEP_{61}$ is the only isoform expressed in hippocampus and cortex. Genetic deletion of STEP enhances excitatory synaptic currents and long-term potentiation in the hippocampus. However, whether $STEP_{61}$ affects seizure susceptibility is unclear. Here we investigated the effects of STEP inhibitor TC-2153 on seizure propensity in a murine model displaying kainic acid (KA)–induced *status epilepticus* and its effect on hippocampal excitability.

Methods: Adult male and female C57BL/6J mice received intraperitoneal injection of either vehicle (2.8% dimethylsulfoxide [DMSO] in saline) or TC-2153 (10 mg/kg) and then either saline or KA (30 mg/kg) 3 h later before being monitored for behavioral seizures. A subset of female mice was ovariectomized (OVX). Acute hippocampal slices from Thy1-GCaMP6s mice were treated with either DMSO or TC-2153 (10 μM) for 1 h, and then incubated in artificial cerebrospinal fluid (ACSF) and potassium chloride (15 mM) for 2 min prior to live calcium imaging. Pyramidal neurons in dissociated rat hippocampal culture (DIV 8–10) were pre-treated with DMSO or TC-2153 (10 μM) for 1 h before whole-cell patch-clamp recording.

Results: TC-2153 treatment significantly reduced KA-induced seizure severity, with greater trend seen in female mice. OVX abolished this TC-2153-induced decrease in seizure severity in female mice. TC-2153 application significantly decreased overall excitability of acute hippocampal slices from both sexes. Surprisingly, TC-2153 treatment hyperpolarized resting membrane potential and decreased firing rate, sag voltage, and hyperpolarization-induced current (I_h) of cultured hippocampal pyramidal neurons.

Jennifer M. Walters and Eung Chang Kim contributed equally.

National Institute of Biomedical Imaging and Bioengineering, Grant/Award Number: R21 EB029076; Carle Illinois Collaborative Research, Grant/Award Number: 083630

Significance: This study is the first to demonstrate that pharmacological inhibition of STEP with TC-2153 decreases seizure severity and hippocampal activity in both sexes, and dampens hippocampal neuronal excitability and I_h. We propose that the antiseizure effects of TC-2153 are mediated by its unexpected action on suppressing neuronal intrinsic excitability.

KEYWORDS
excitability, kainic acid, seizures, STEP, TC-2153

1 | INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common form of focal-onset epilepsy in adults and accounts for 60% of patients with epileptic.[1] In Mesial TLE, seizures often begin in the hippocampus and progressively worsen over time. Current antiseizure drugs are ineffective for ~75% of the patients with advanced mesial TLE, leading to severe consequences including hippocampal sclerosis, high mortality rate, cognitive decline, depression, and temporal lobe resection.[1] Furthermore, dysregulation of intrinsic excitability and synaptic transmission has been widely thought to underlie hippocampal hyperactivity, which drives the development of spontaneous seizures in TLE,[2,3] underscoring a critical need to identify the underlying mechanisms and novel therapeutic targets.

Striatal-Enriched protein tyrosine Phosphatase (STEP) is a brain-specific tyrosine (Tyr) phosphatase encoded by the protein tyrosine phosphatase non-receptor type 5 (*PTPN5*) gene.[4] Among four STEP isoforms, cytosolic $STEP_{46}$ and membrane-bound $STEP_{61}$ are catalytically active and widely expressed in the brain, except the cerebellum.[4] However, only $STEP_{61}$ is expressed in the hippocampus and neocortex,[5] where it dephosphorylates *N*-methyl-D-aspartic acid receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), the key glutamate receptors that mediate fast excitatory synaptic transmission. Specifically, $STEP_{61}$ dephosphorylates glutamate ionotropic receptor NMDA type subunit 2B (GluN2B) at Tyr^{1472} and glutamate ionotropic receptor AMPA type subunit 2 (GluA2) at Tyr^{869}, Tyr^{873}, and Tyr^{876}, resulting in their internalization.[6–10] $STEP_{61}$ also dephosphorylates and inactivates protein kinases, including extracellular signal-regulated kinase 1 and 2 (ERK1/2), p38 mitogen-activated protein kinases, Src family tyrosine-protein kinase Fyn, and proline-rich tyroine kinase 2 (Pyk2).[4] Reduction of STEP increases NMDAR and AMPAR surface expression and excitatory synaptic currents,[11,12] enhances long-term potentiation,[13] and prevents the internalization of GluA2-containing AMPARs during metabotropic glutamate receptor-dependent long-term depression in the hippocampus.[9]

Key points

- Administration of TC-2153 significantly reduces seizure severity in both male and female mice.
- Ovariectomy abolishes the TC-2153-induced decrease in seizure severity observed in female mice.
- TC-2153 treatment significantly decreases overall excitability of acute hippocampal slices prepared from both sexes.
- TC-2153 application decreases intrinsic excitability and hyperpolarization-induced currents of cultured hippocampal neurons.

Activity-dependent regulation of $STEP_{61}$ also contributes to homeostatic stabilization of excitatory synapses by regulating Tyr phosphorylation of GluN2B and GluA2.[10] Thus, $STEP_{61}$ weakens excitatory synaptic strength.

Emerging evidence suggests that STEP may be a molecular target for seizure treatment. Deletion of the STEP gene *PTPN5* results in resistance to pilocarpine-induced seizures[14] and diminishes audiogenic seizures in a fragile X syndrome (FXS) mouse model.[15] This reduction in seizure propensity is puzzling, because loss of STEP would be expected to increase seizure susceptibility by potentiating excitatory synaptic strength. It is possible that genetic deletion of STEP may have compensatory effects on other related genes and/or pathways, thereby complicating the delineation of the role of STEP in seizure susceptibility. Therefore, here we tested the hypothesis that acute pharmacological inhibition of STEP increases seizure propensity.

TC-2153 (8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine hydrochloride) is a selective STEP inhibitor that forms reversible covalent bonds with the cysteine residues near the signature catalytic domain in $STEP_{46}$ and $STEP_{61}$.[16] Despite the low half-maximal inhibitory concentration (IC50) (24.6 nM), a higher concentration of TC-2153 is required to increase Tyr phosphorylation

of $STEP_{61}$ substrates in primary cortical neuronal culture (1–10 μM) and the cortex in vivo (10 mg/kg) and to reverse cognitive deficits in a 3xTg-AD mouse model of Alzheimer's disease (AD),[16] in which these mice display hippocampal hyperactivity and spontaneous seizures.[17,18] With a low level of acute toxicity (LD_{50} [Lethal Dose 50] >1000 mg/kg),[19] TC-2153 can alleviate audiogenic seizures in the FXS mouse model[15] and block pentylenetetrazol-induced convulsions,[19] although the sex dependence of the antiseizure effects of TC-2153 and the underlying mechanism were not described.

In this study, we discovered that TC-2153 dampens hippocampal activity and exerts anticonvulsant activity in both C57BL/6J male and female mice against a single systemic injection of kainic acid (KA), which induces status epilepticus (SE) arising from the hippocampus.[20] Furthermore, TC-2153 decreases action potential (AP) firing rate, sag voltage, and hyperpolarization-induced current (I_h) in hippocampal neurons, providing novel evidence that pharmacological inhibition of STEP by TC-2153 downregulates intrinsic neuronal excitability in contrast to its well-known role in synaptic transmission.

2 | MATERIALS AND METHODS

2.1 | Kainic acid–induced seizures

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana Champaign and conformed to the ARRIVE Guidelines. Both male and female *Ptpn5* homozygous knock-out mice ($Ptpn5^{-/-}$),[8] wild-type mice ($Ptpn5^{+/+}$), C57BL/6J mice (Jax.org, Stock Number: 000664) were used for seizure studies at 6- to 12-weeks-old. C57BL/6J female mice received ovariectomy surgeries at 9- to 10-weeks-old as described[21] and were used in seizure studies at 7–10 days after surgery. Behavioral seizures were induced in mice by a single intraperitoneal (i.p.) injection of saline or KA, (30 mg/kg, Abcam)[20] and monitored using a modified Racine scale for 2 h.[22] To test the effects of TC-2153, the C57BL/6J mice received KA at 3 h after i.p. injections with either vehicle control (saline containing 2.8% dimethylsulfoxide [DMSO]) or TC-2153 (10 mg/kg in saline containing 2.8% DMSO, Sigma Aldrich). This treatment was demonstrated previously to increase Tyr-phosphorylation of STEP substrates in the cortex.[16]

2.2 | Immunoblot analysis

At 3 h after injection with either vehicle control or TC-2153, the mouse hippocampi were biochemically fractionated to the supernatant and the membrane fractions (0.5 mg/mL) as described.[23] Primary dissociated hippocampal cultures were prepared from Sprague-Dawley rat embryos on embryonic day 18 and plated at 330 cells/mm^2 as described.[10] To maximally inhibit STEP, neurons at 9–10 days in vitro (DIV) were treated for 1 h with either vehicle control (0.14% DMSO) or TC-2153 (10 μM).[16] Although the pharmacokinetics of TC-2153 is unknown, Tyr-phosphorylation of $STEP_{61}$ substrates were reported previously to increase in both neuron culture upon 10 μM TC-2153 application and forebrain tissues upon its i.p. injection at 10 mg/kg,[16] suggesting that a 10 μM concentration used in the in vitro studies is equivalent or close to its brain concentration achieved by in vivo delivery. Samples were immunoblotted for $STEP_{61}$ and its substrates. Densitometric quantification was performed with ImageJ Software (National Institutes of Health).[10,23]

2.3 | GCaMP6s imaging in acute hippocampal slices

Acute coronal hippocampal slices (200 μm) were prepared from Thy1-GCaMP6s mice (Jax.org, Stock Number: 024275) at 4- to 11-weeks-old. Slices were incubated for 1 h at room temperature in basal artificial cerebrospinal fluid (ACSF) with either DMSO (0.14%) or TC-2153 (10 μM), which was shown previously to enhance hippocampal long-term potentiation (LTP)[24] similar to STEP deletion.[13] Time-lapse fluorescence images of GCaMP6s (size: 640 × 404 pixel) were acquired in ACSF for 1 min at 3.6 frame per second with 25 ms exposure time, and 3 × 3 binning under a Zeiss Axio Observer microscope. Slices were then incubated with potassium chloride (KCl, 15 mM) in ACSF for 2 min and imaged for another 1 min. Images from 10–60 s after KCl exposure were analyzed for mean fluorescence intensity (F) in dentate gyrus (DG), CA1, and CA3 using ImageJ. $\Delta F/F = (F - F_{min})/F_{min}$ was computed as described,[25] where ΔF indicates the difference between the initial intensity in ACSF and the intensity after KCl stimulation. ΔF/F was normalized to ACSF.

2.4 | Electrophysiology

At 1 h after treatment with dimethylsulfoxide (DMSO) (0.14%) or TC-2153 (10 μM), whole-cell patch-clamp recordings of evoked AP firing, sag voltage, and rebound potential were performed at 30–32°C from cultured hippocampal pyramidal neurons held at −60 millivolts (mV) in external solution containing 6-cyano-7-nitroquinoxaline-2,3-dione

(20 μM), DL-2-Amino-5-phosphonopentanoic acid (DL-AP5) (100 μM), and bicuculline (20 μM) under current clamp mode using a Multiclamp 700B amplifier, Digidata1440A, and pClamp 10.6 software (Molecular Devices).[26] Voltage clamp recording of I_h was performed with CNQX (20 μM), DL-AP5 (50 μM), bicuculline (10 μM), and Tetrodotoxin (0.5 μM) as described.[27] Clampfit 10.7 software (Molecular Devices) were used for recording analyses.[27]

2.5 | Statistical analysis

Data are reported as mean ± standard deviation (SD). Statistical analyses were performed using Origin Pro 9.5 (Origin Lab) to compare differences between means in two groups using the Student's two-tailed *t* test, and in groups of three or more using the post hoc Tukey test. For nonparametric data, the Mann-Whitney *U* test was used. Sex difference was analyzed by two-way analysis of variance (ANOVA) with sex as one factor and treatment as the other. The priori value (p) <.05 was considered statistically significant.

Detailed description of each method is provided in Supplementary Information.

3 | RESULTS

3.1 | Homozygous loss of STEP affects sensitivity to KA-induced seizure severity in an age- and sex-dependent manner

Because $STEP_{61}$ weakens excitatory synaptic strength,[7–10,13] we hypothesized that genetic deletion of *PTPN5* would increase susceptibility to acute seizures. To test this hypothesis, homozygous $PTPN5^{-/-}$ mice (STEP knockout [KO]) and their wild-type $PTPN5^{+/+}$ littermates (STEP wild-type [WT]) were treated with KA (30 mg/kg, i.p.),[20] and behavioral seizures were scored every 10 min for the first 2 h using a modified Racine scale (Figure 1).[22] Initially, we tested 6- to 7-week-old adolescent STEP WT and KO mice (P42-55) in reference to the age range that was originally investigated by Briggs et al., which combined both sexes for analysis and showed that STEP KO mice are resistant to pilocarpine-induced seizures.[14] Consistent with Briggs et al., STEP KO male mice at this age displayed a decrease in KA-induced seizure severity at nearly every time interval (Figure 1B), resulting in a lower cumulative seizure score compared to WT male mice (Figure 1C). A similar trend was observed for STEP KO female mice, but did not reach statistical significance (Figure 1B-C, Table S2). The percentage of mice that reached Stage 5 (SE) and Stage 6 (death) was also decreased in male but not female KO compared to WT mice (Figure 1D-E).

We next tested adult mice at 8 to 12 weeks of age (P56-P90) to avoid the adolescence period when puberty onset and maturation occurs and corticolimbic circuits are still developing.[28,29] In contrast to 6- to 7-week-old STEP KO male mice (Figure 1B-D), 8- to 12-week-old STEP KO male mice showed similar seizure severity to WT male mice for the first 100 min following KA injection. However, their seizure scores at 110 and 120 min post-KA injection, % SE, and % Death were higher than WT male mice (Figure 1F-I), consistent with our original hypothesis that STEP KO mice would show increased seizure susceptibility. In contrast, STEP KO female mice displayed a decreasing trend in seizure severity, the % SE, and % Death compared to WT female mice (Figure 1F-I). Significant interaction between sex and treatment was noted (Interaction: $F_{(1, 53)} = 7.69$, $p = .008$, Table S2).

3.2 | TC-2153 decreases KA-induced seizure severity in C57BL/6J mice

To test if the effects of acute pharmacological inhibition of STEP on seizure susceptibility are similar to genetic deletion of STEP, C57BL/6J mice at 8 to 12 weeks of age (P56-P90) were injected (i.p.) first with STEP inhibitor TC-2153 (10 mg/kg) or vehicle control and 3 h later with KA (30 mg/kg) to induce behavioral seizures (Figure 2A). Such treatment with TC-2153 was shown previously to increase Tyr-phosphorylation of $STEP_{61}$ substrates in the cortex including GluN2B and ERK1/2.[16] Upon TC-2153 injection, male mice displayed lower seizure scores at 50, 90, 110, and 120 min post KA injection (Figure 2B), decreasing cumulative seizure score, % SE, and % Death compared to vehicle injection (Figure 2C-E). In female mice, TC-2153 application induced a larger decrease in seizure scores at nearly every time interval, except 20–40 min (Figure 2B) and reduced cumulative seizure score, % SE, and % Death compared to vehicle controls (Figure 2C-E). However, no sex difference was observed for the effect of TC-2153 on cumulative seizure scores (Sex: $F_{(1, 48)} = 1.32$, $p = .26$, Table S2). It is important to note that TC-2153 treatment reduced the number of mice that died by KA injection from 5 to 1 in both sexes (Figure 2E). These data indicate that TC-2153 reduced KA-induced seizure severity in C57BL/6J mice.

This result was contrary to our original hypothesis that seizure susceptibility will increase by acute pharmacological inhibition of STEP. To confirm that TC-2153 was blocking STEP activity, primary rat hippocampal neuronal culture was treated with either DMSO (vehicle control) or TC-2153 (10 μM) for 1 h. Immunoblot analysis of

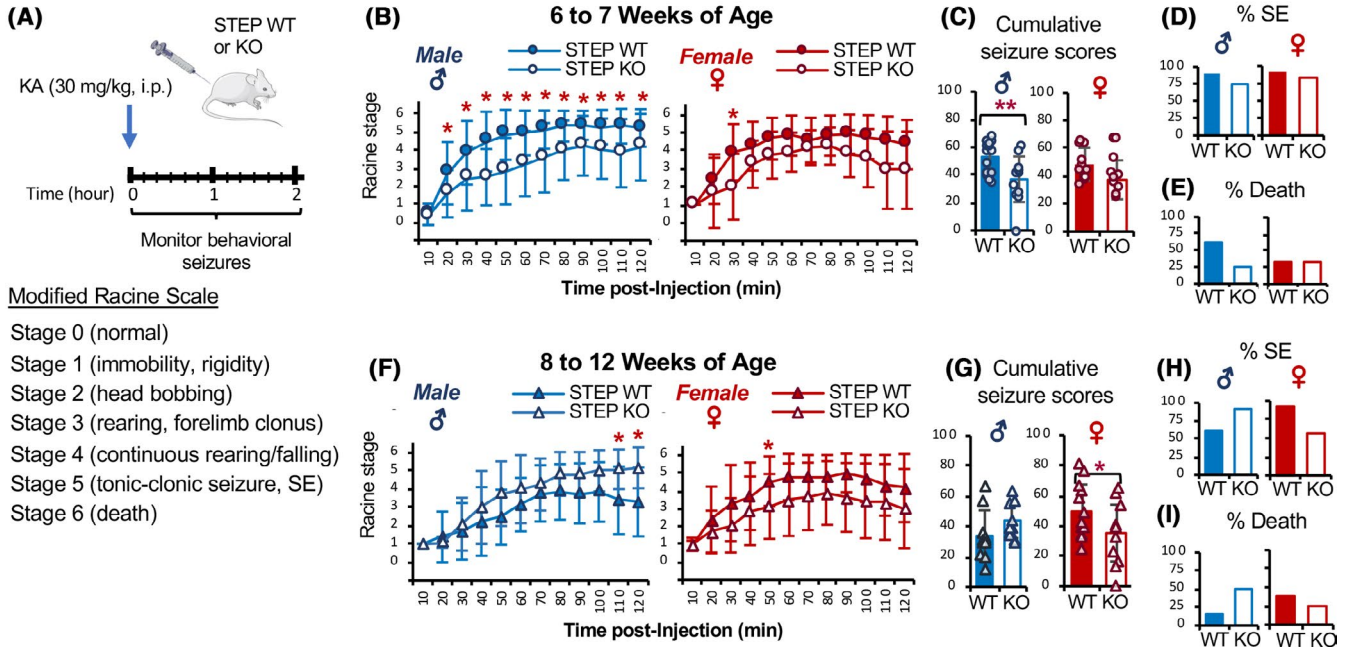

FIGURE 1 Homozygous loss of STriatal-Enriched protein tyrosine Phosphatase (STEP) affects sensitivity to kainic acid (KA)–induced seizures in an age- and sex-dependent manner. (A) Experimental schematic of KA-induced seizures in STEP knockout (KO) and wild-type (WT) mice. A modified Racine scale was used to score behavioral seizures. (B-D) KA-induced seizures in STEP WT and KO mice at age 6–7 weeks (postnatal day (P) 42-P55). (B) STEP KO male mice at age 6–7 weeks ($n = 12$) show a significant decrease in seizure scores at 20–110 minutes (min) post KA injection compared to WT male mice ($n = 21$). STEP KO female mice at age 6–7 weeks ($n = 12$) show a significant decrease in seizure scores at 30 min post KA injection compared to WT female mice ($n = 12$). Mann-Whitney *U* test results are shown (*$p < .05$). (C) Cumulative seizure scores. Two-tailed Student *t* test results are shown (*$p < .05$). (D-E) Percentage (%) of mice that achieved Stage 5 (D) and Stage 6 (E). (F-I) KA-induced seizures in STEP WT and KO mice at age 8 to 12 weeks (P56-P90). (F) STEP KO male mice at age 8–12 weeks ($n = 12$) show a significant increase in seizure scores at 110 and 120 min compared to WT male mice ($n = 13$). STEP KO female mice at age 8–12 weeks ($n = 16$) show a decreased trend in seizure propensity compared to WT female mice ($n = 13$), but this trend was not statistically significant. Mann-Whitney *U* test results are shown (*$p < .05$). (G) Cumulative seizure scores. Two-tailed Student *t* test results are shown (*$p < .05$). (H-I) Percentage (%) of mice that achieved Stage 5 (D) and Stage 6 (E). Data shown represent the mean ± SD (*$p < .05$)

$STEP_{61}$ substrates revealed that TC-2153 application significantly increased the levels of Tyr^{1472}-phosphorylated GluN2B and Tyr^{204}/Tyr^{187}-phosphorylated ERK1/2 compared to DMSO, without affecting the expression of GluN2B, ERK1/2, and $STEP_{61}$ (Figure S1A-B). Consistent with the previous reports in cultured cortical neurons,[16] these results demonstrate that TC-2153 inhibits $STEP_{61}$ activity in cultured hippocampal neurons.

To confirm that TC-2153 inhibits $STEP_{61}$ in the hippocampus in vivo, the hippocampi of C57BL/6J mice were collected at 3 h post injection with either vehicle control or TC-2153 (10 mg/kg). Unlike previous reports,[16] TC-2153 treatment did not alter hippocampal level of phosphorylated ERK1/2 in both sexes (Figure S2). However, TC-2153 application significantly increased the hippocampal level of phosphorylated GluN2B in female but not male mice, indicative of $STEP_{61}$ inhibition in female mice (Figure S2). It is interesting to note that the effect of TC-2153 on enhancing phosphorylated GluN2B in female but not male mice (Figure S2) mirrors its greater trends on reducing KA-induced seizure severity in female mice (Figure 2B-D).

3.3 | Ovariectomy abolishes the TC-2153-induced seizure suppression in female C57BL/6J mice

To investigate whether ovarian hormones were implicated in the TC-2153-induced reduction in seizure severity seen in female mice, female C57BL/6J mice received ovariectomy (OVX), which eliminates bulk circulation of ovarian-derived hormones from the system.[30] Under DMSO injection, OVX female mice reached SE more quickly than ovary-intact females following KA injection (Figure S3). Remarkably, TC-2153 injection no longer decreased KA-induced seizure severity, cumulative seizure scores, and % Death in OVX mice compared to DMSO injection (Figure 2B-C, E). Furthermore, similar numbers of OVX females reached SE regardless of treatment (vehicle:

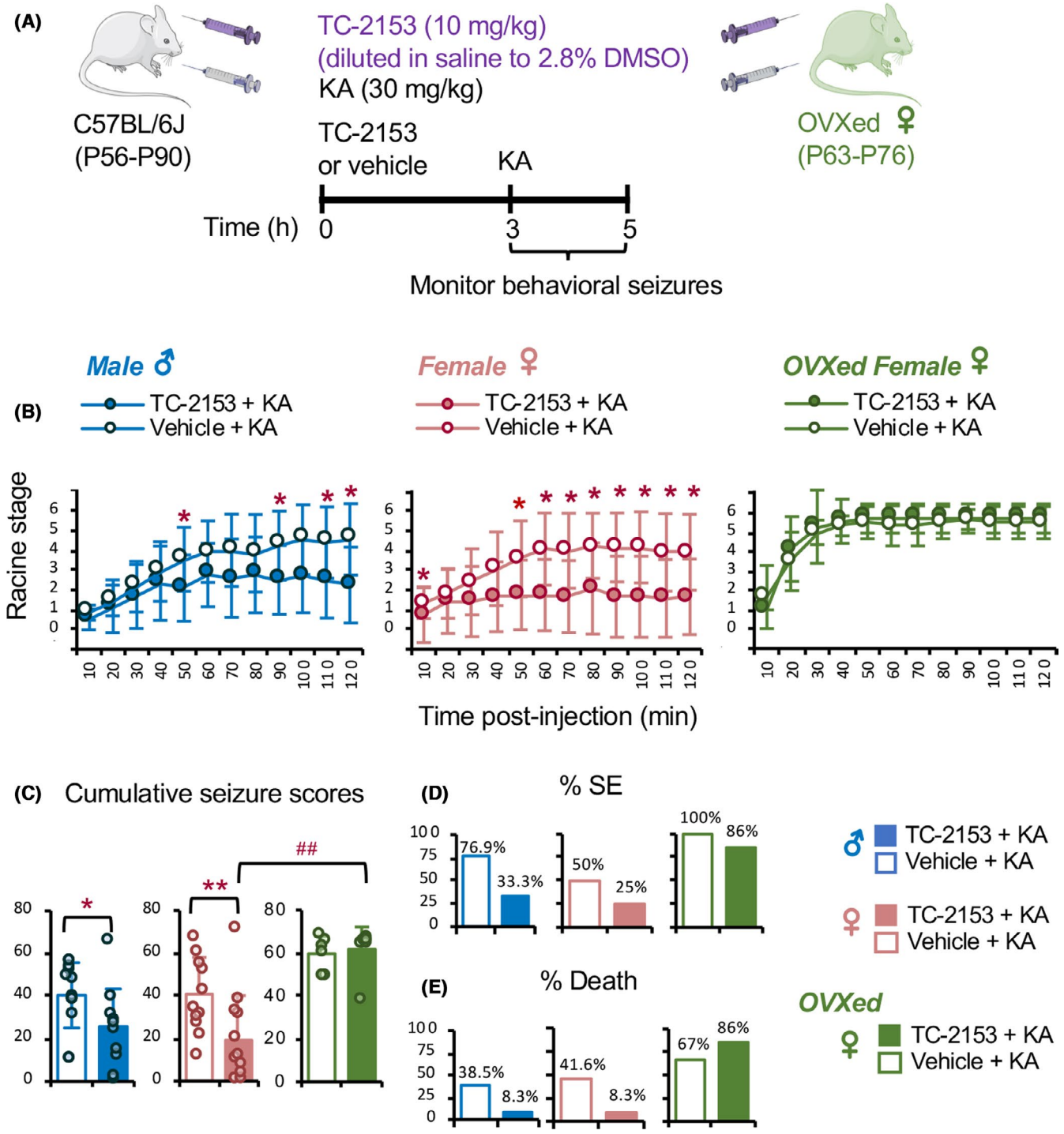

FIGURE 2 TC-2153 treatment decreases kainic acid (KA)–induced seizure severity in adult C57BL/6J mice compared to vehicle control with greater effects seen in females. (A) Experimental schematic of KA-induced seizure severity in both male and female C57BL/6J mice at 8–12 weeks (postnatal day [P] 56-90) and ovariectomized (OVX) C57BL/6J female mice at 9–10 weeks (P63-P76) at 3 h post intraperitoneal (i.p.) injection with STEP inhibitor TC-2153 (10 mg/kg in saline containing 2.8% dimethylsulfoxide [DMSO]) or vehicle control (saline containing 2.8% DMSO). Behavioral seizures were monitored using modified Racine scale. (B) TC-2153 treatment in male mice ($n = 12$) decreases severity of KA-induced seizures at 90–120 min post injection compared to vehicle treatment ($n = 13$). TC-2153 injection in female mice ($n = 12$) significantly decreases KA-induced seizure severity at nearly every time point compared to vehicle treatment ($n = 12$), whereas KA-induced seizures were similar between vehicle-injected OVX female mice ($n = 6$) and TC-2153-injected OVX female mice ($n = 7$) for the first 2 h. Mann-Whitney *U* test results are shown (*$p < .05$). (C) Cumulative seizure scores. Two-tailed Student *t* test results are shown (*$p < .05$). (D-E) Percentage (%) of mice that achieved Stage 5 (D) and Stage 6 (E). Data shown as mean ± standard deviation (SD). Table S1 shows two-way analysis of variance (ANOVA) test results with sex as one factor and treatment as the other

six of six mice, TC-2153: six of seven mice) (Figure 2D). These data indicate that OVX abolishes the TC-2153-induced suppression of seizure severity seen in intact female mice.

3.4 | TC-2153 treatment reduces the excitability of acute hippocampal slices

To investigate if TC-2153 affects hippocampal excitability, calcium imaging was performed on acute hippocampal slices prepared from mice containing genetically encoded calcium indicator GCaMP6s.[25] Acute slices were treated with either DMSO or TC-2153 (10 μM) in ACSF for 1 h prior to imaging of GCaMP6s (Figure 3A-C). Under DMSO application, KCl-mediated depolarization significantly increased calcium signals in the somatic and dendritic layers in the CA1, CA3, and DG regions from both sexes (Figure 3C-D, Table S3). In contrast, TC-2153 treatment significantly reduced the KCl-evoked calcium signals in every region (Figure 3C-D, Table S3), indicating that TC-2153 decreases the excitability of the hippocampal slices.

3.5 | TC-2153 treatment hyperpolarizes resting membrane potential and decreases intrinsic excitability in cultured hippocampal neurons

The inhibitory action of TC-2153 on the excitability of the hippocampal slices was contrary to the well-known role of $STEP_{61}$ in weakening excitatory synaptic strength in the hippocampus. Therefore, we hypothesized that TC-2153 may regulate the intrinsic excitability of hippocampal pyramidal cells. To test this, we performed whole-cell patch-clamp recording of cultured hippocampal neurons (DIV 8–10) after pre-treating with either DMSO or TC-2153 (10 μM) for 1 h (Figure 4A-C). TC-2153 application hyperpolarized resting membrane potentials (RMPs) and decreased the input resistance (R_{in}), but did not affect membrane capacitance of recorded neurons (Figure 4D). Current clamp recording in the presence of synaptic transmission blockers revealed that TC-2153 application reduced instantaneous firing rates and the number of APs at 20 to 200 picoampere (pA) injections compared to DMSO or no treatment (Figure 4A-C). TC-2153 treatment also increased average rheobase current, interspike interval (ISI), AP rise time, AP decay time, and AP half width, while decreasing fast after-hyperpolarization (fAHP) amplitude at 100 pA injection (Table 1). These data indicate that TC-2153 decreases hippocampal neuronal excitability.

3.6 | TC-2153 treatment decreases sag voltage and I_h in cultured hippocampal neurons

To test if TC-2153 regulates intrinsic membrane properties of hippocampal pyramidal neurons upon membrane hyperpolarization, we measured the amplitude of voltage sag and rebound potential. We found that TC-2153 treatment significantly reduced voltage sag by 75.2% and rebound potentials by 51.6% at −200 to −40 pA current injections compared to DMSO or no treatment (Figure 5A-C).

Hyperpolarization-activated cyclic nucleotide-gated ion (HCN) channels produce a slowly depolarizing nonselective inward cationic current called I_h, which mediates voltage sag and rebound potentials and regulates RMP and excitability of the hippocampal pyramidal neurons.[31] Among HCN1-4 subunits, HCN1 and HCN2 subunits are predominantly expressed in the hippocampal and cortical excitatory neurons.[32] To test if TC-2153 modulates I_h, we first confirmed that cultured hippocampal neurons expressed HCN1 and HCN2 subunits (Figure S1C-D). Voltage-clamp recording upon membrane hyperpolarization revealed that TC-2153-treated neurons displayed smaller I_h density (pA/pF) at −70 mV to −120 mV compared to DMSO-treated and untreated neurons (Figure 5D-E). The normalized conductance (G/Gmax) and $V_{1/2}$ calculated from G/Gmax showed a depolarizing shift in voltage dependence (Figure 5F, Table S4). Consistent with decreased sag voltage and rebound potential (Figure 5A-C), these data indicate that TC-2153 downregulates I_h.

4 | DISCUSSION

The role of $STEP_{61}$ in weakening excitatory synaptic strength in the hippocampus and cortex has been well established,[7–10,13] but its effect on seizure susceptibility and regulation of hippocampal excitability remains elusive. In this study, we provide evidence that acute pharmacological inhibition of STEP with TC-2153 decreases KA-induced seizure severity and hippocampal excitability. Our study has also revealed a previously unknown action of TC-2153 in modulating intrinsic membrane properties.

4.1 | Genetic ablation of STEP exerts age- and sex-dependent effects on seizure severity

Because $STEP_{61}$ weakens excitatory synaptic strength in the hippocampus, seizure susceptibility should increase in STEP KO mice, which deletes all STEP isoforms.[8]

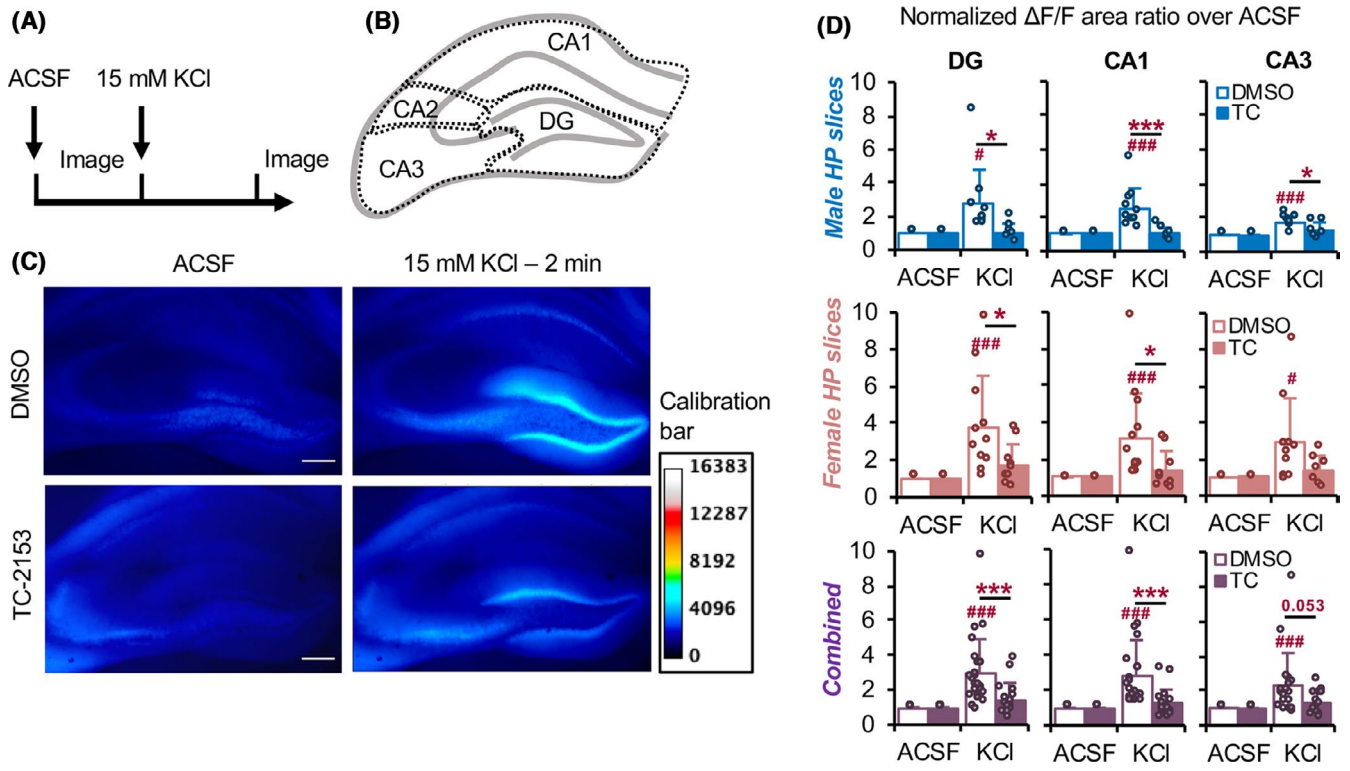

FIGURE 3 TC-2153 treatment reduces the excitability of acute hippocampal slices. (A) Experimental schematic of calcium imaging on coronal acute hippocampal slices prepared from Thy1-GCaMP6s mice at postnatal day (P) 28-90. The slices were incubated with dimethylsulfoxide (DMSO) (0.14%) or TC-2153 (10 μM) for 1 h, and subjected to GCaMP6s imaging first in artificial cerebrospinal fluid (ACSF) and then after 2 min of 15 mM KCl application. (B) Manual tracing of hippocampal CA1, CA3, and DG regions for analysis. (C) Representative GCaMP6s fluorescence images of slices. Raw pixel intensity is shown. (D) Quantification of GCaMP6s fluorescence (ΔF/F) in DG, CA1, and CA3 regions normalized to ACSF. The total number of slices imaged: DMSO-treated slices ($n = 23$ including 10 from four male and 13 from five female mice); TC-2153-treated slices ($n = 16$ including 7 from four male and 9 from five female mice). Compared to DMSO treatment, TC-2153 treatment reduces GCaMP6s signals in DG, CA1, and CA3 regions of hippocampal slices prepared from both male and female mice. The total number of analyzed slices from males: DG (10 DMSO, 7 TC-2153), CA1 (10 DMSO, 7 TC-2153), and CA3 (8 DMSO, 6 TC-2153). The total number of analyzed slices from females: DG (11 DMSO, 9 TC-2153), CA1 (11 DMSO, 9 TC-2153), CA3 (8 DMSO, 7 TC-2153). Data shown as mean ± SD. Post hoc Tukey test results are shown for ACSF vs KCl ($^{\#}p < .05$, $^{\#\#\#}p < .005$) and for DMSO + KCl vs TC + KCl ($^{*}p < .05$, $^{***}p < .005$). Table S2 shows two-way ANOVA test results with sex as one factor and treatment as the other

However, Briggs et al. combined both sexes for analyses and showed that STEP KO mice at 6–8 weeks of age are resistant to pilocarpine-induced seizures.[14] When we separated the sexes in our analysis for KA-induced seizure severity, there was a decrease in STEP KO males at 6–7 weeks of age but an increase at 8–12 weeks of age (Figure 1). STEP KO females in both age groups showed a decreasing trend in seizure severity (Figure 1). Our study thus demonstrates that genetic ablation of STEP, especially catalytic $STEP_{46}$ and $STEP_{61}$, most likely affects seizure severity in an age- and sex-dependent manner.

In rodents at 6 to 8 weeks of age, sexual maturation occurs in association with significant changes in sex hormone–dependent synapse formation and circuit maturation in the brain, particularly in the hippocampus and cortex.[28,29] The eighth week of age marks the end of the puberty and adolescence period.[28] These critical changes in hippocampal circuitry and synapse formation due to sex hormone shifts may influence sensitivity to pilocarpine and KA, which have different mechanisms for inducing limbic seizures[20,33] and may explain the age-dependent switch in seizure susceptibility of STEP KO mice.

4.2 | Antiseizure effect of the STEP inhibitor TC-2153

TC-2153 inhibits two catalytically active STEP isoforms: $STEP_{46}$ and $STEP_{61}$.[4] Both isoforms are expressed in the striatum and amygdala,[5] although only $STEP_{61}$ is expressed in the hippocampus and neocortex.[5] In both male and female mice, TC-2153 treatment decreased

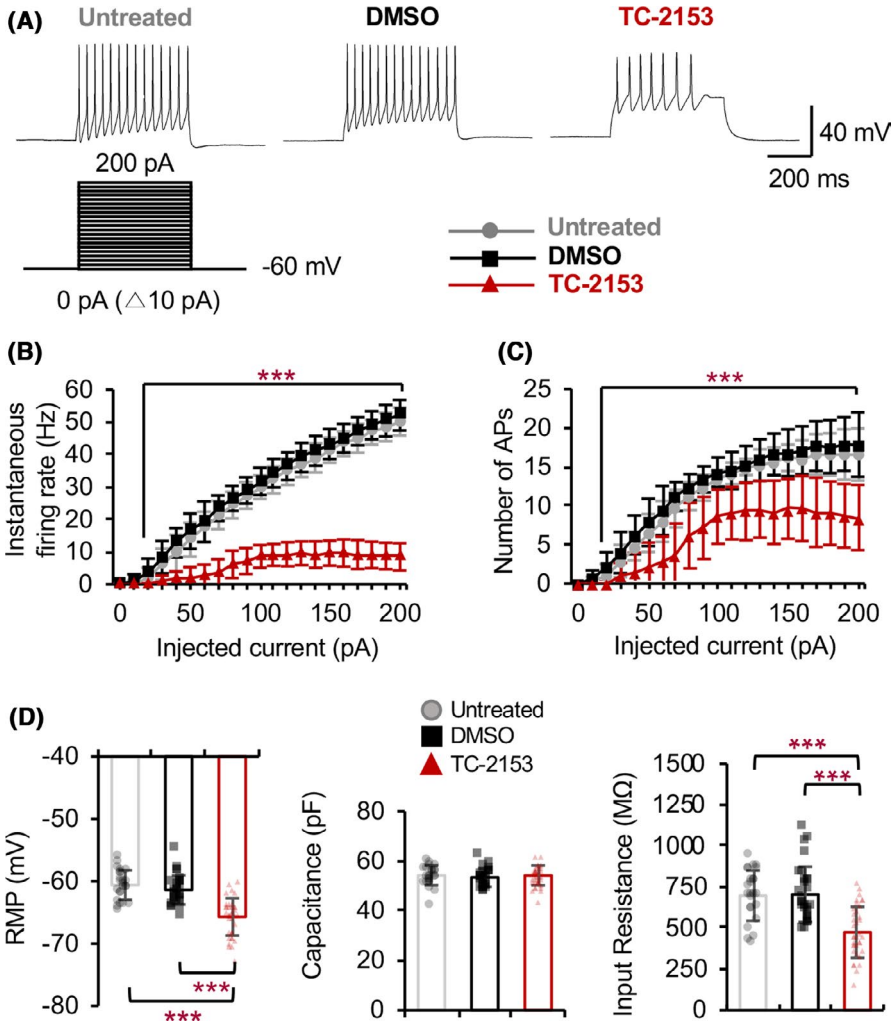

FIGURE 4 TC-2153 hyperpolarizes resting membrane potentials (RMPs) and reduces action potential (AP) firing and input resistance in cultured hippocampal neurons. Whole-cell current-clamp recording of hippocampal pyramidal neurons in dissociated culture (days in vitro 8–10) was performed in current clamp mode after 1 hour treatment with TC-2153 (10 μM) or DMSO (0.14%). (A-C) TC-2153 reduces AP firing in cultured hippocampal neurons. Spike trains were evoked in pyramidal neurons in the presence of synaptic transmission blockers by delivering constant somatic current pulses of 500 millisecond duration in the range 0–200 picoamperes (pA) with a step interval of 10 seconds at a holding potential of −60 millivolts (mV). (A) Representative traces of APs at 100 pA injection. (B) Average instantaneous AP firing rate. (C) Average number of APs. The number of recorded neurons: untreated (gray circle, $n = 12$), DMSO (black square, $n = 15$), or TC-2153 (red triangle, $n = 18$). (D) TC-2153 reduces hyperpolarizes RMP and decreases input resistance in cultured hippocampal neurons. Average resting membrane potential, capacitance, and input resistance in the recorded neurons: untreated ($n = 24$), DMSO ($n = 28$), or TC-2153 ($n = 36$). Data shown as mean ± SD. Post hoc Tukey test results are shown for TC-2153 vs untreated or DMSO (***$p < .005$)

hippocampal excitability (Figure 3) and the severity of KA-induced seizures (Figure 2) that arise mostly from the hippocampus (where KA subtype glutamate receptors are highly expressed), especially in the CA3 region compared to other brain regions including amygdala, striatum, and cortex.[20] Therefore, the majority of the antiseizure effect of TC-2153 is likely mediated by $STEP_{61}$ inhibition in the hippocampus, although we cannot exclude the possible contributions of inhibiting both $STEP_{46}$ and $STEP_{61}$ in other brain regions. Although our studies with TC-2153 pretreatment demonstrate its proof-of-concept anticonvulsant efficacy, investigating its pharmacokinetics and antiseizure effects during SE and TLE will be critical to assess its clinically relevant efficacy.

Compared to male mice, female mice displayed a greater trend in antiseizure effect of TC-2153, which was abolished by OVX (Figure 2). Greater antiseizure potency in female than male mice has also been reported for neurosteroids in various acute seizure models.[34] The possible involvement of ovarian-derived hormones[30] is interesting because prevalence, frequency, and semiology of focal

TABLE 1 AP properties of cultured hippocampal pyramidal neurons treated with DMSO or TC-2153 in Figure 5

	N	Rheobase (pA)	AP latency (ms)	V_T (mV)	ISI (ms)	AP height (mV)	AP rise time (ms)	AP decay time (ms)	AP HW (ms)	fAHP (mV)
Untreated	12	34.0 ± 13.46	16.9 ± 6.01	−36.5 ± 1.46	33.6 ± 4.11	53.5 ± 7.36	0.83 ± 0.15	2.73 ± 0.30	1.83 ± 0.33	21.5 ± 4.79
DMSO	15	33.2 ± 16.60	15.4 ± 3.08	−36.5 ± 2.35	32.1 ± 4.63	49.7 ± 7.12	0.83 ± 0.15	2.86 ± 0.35	1.91 ± 0.27	20.1 ± 2.29
TC-2153	18	63.2 ± 26.26*^	27.1 ± 24.50	−35.3 ± 4.22	48.1 ± 21.03*^	50.5 ± 12.35	1.18 ± 0.45*^	3.81 ± 1.46*^	2.58 ± 1.12*^	16.4 ± 4.90*^

Note: n, number; average rheobase current (the minimal current that elicited at least one spike). AP properties were measured from the first action potential evoked by a current step to 100 pA at a holding potential of −60 mV. AP latency was measured from the start time of current step to the peak of the first AP. V_T, voltage threshold for AP; Inter-spike interval (ISI); AP rise time, 10%–90% rise time of AP; AP decay time, 10%–90% decay time of AP; AP half-width (HW); fast afterhyperpolarization (fAHP). Each value represents the mean ± SD. Post hoc Tukey test results are shown for TC-2153 vs untreated (*$p < .05$) and TC-2153 vs DMSO (^$p < .05$). *$p < .05$ for untreated vs TC 2153. ^$p < .05$ for DMSO vs TC 2153.

seizures and TLE have been reported to differ by sex in both clinical patient populations and preclinical animal models due to their neurobiological actions.[35] Estrogen exacerbates seizures in women with epilepsy[36] and both estrogen and testosterone increase seizure susceptibility in rodent KA models.[37,38] In contrast, seizure frequency in mice and women with epilepsy is reduced by high progesterone level.[36,39]

It is interesting that the antiseizure effect of TC-2153 was lost in OVX female mice, whereas the effect was present in male mice (Figure 2). TC-2153 application also reduced the excitability of hippocampal slices from both male and female mice (Figure 3), even after circulating gonadal hormones had washed away while acclimating the slices in ACSF,[40] suggesting that gonadal hormone cannot fully explain the subtle sex difference in the response to TC-2153. Considering that women display higher drug concentrations in blood and longer duration for drug metabolism and clearance than men,[35] a greater trend in antiseizure effect in females may arise from higher brain concentration of TC-2153 in females than males. Alternatively, the enhanced seizure severity in OVX females compared to naive females (Figure S3) may also contribute to the diminished efficacy of TC-2153 in OVX females. Future studies shall evaluate the absorption, distribution, metabolism, and excretion properties of TC-2153 in both sexes and use gonadectomy in combination with hormone replacement to confirm whether TC-2153 decreases KA-induced seizure severity by altered pharmacokinetics of TC-2153 or suppressing proconvulsant actions of estrogen or testosterone, and/or potentiating anticonvulsant actions of progesterone.

4.3 | Decrease in hippocampal intrinsic excitability as a mechanism for the antiseizure effect of TC-2153

Our study demonstrates novel actions of STEP inhibitor TC-2153 (Figures 4 and 5). TC-2153 application markedly decreases the intrinsic excitability of cultured hippocampal pyramidal neurons (Figure 4). Considering that $STEP_{61}$ interacts with a variety of proteins including ion channels, ion transporters, and signaling proteins important for neuronal excitability and synaptic transmission,[12] our findings suggest a compelling possibility that $STEP_{61}$ may regulate ionic currents critical for intrinsic neuronal excitability and AP waveform, in contrast to its well-known role in weakening synaptic transmission.

Indeed, hyperpolarized RMP and decreased R_{in} in TC-2153-treated hippocampal neurons (Figure 4D) suggest the opening of potassium channels. Among major

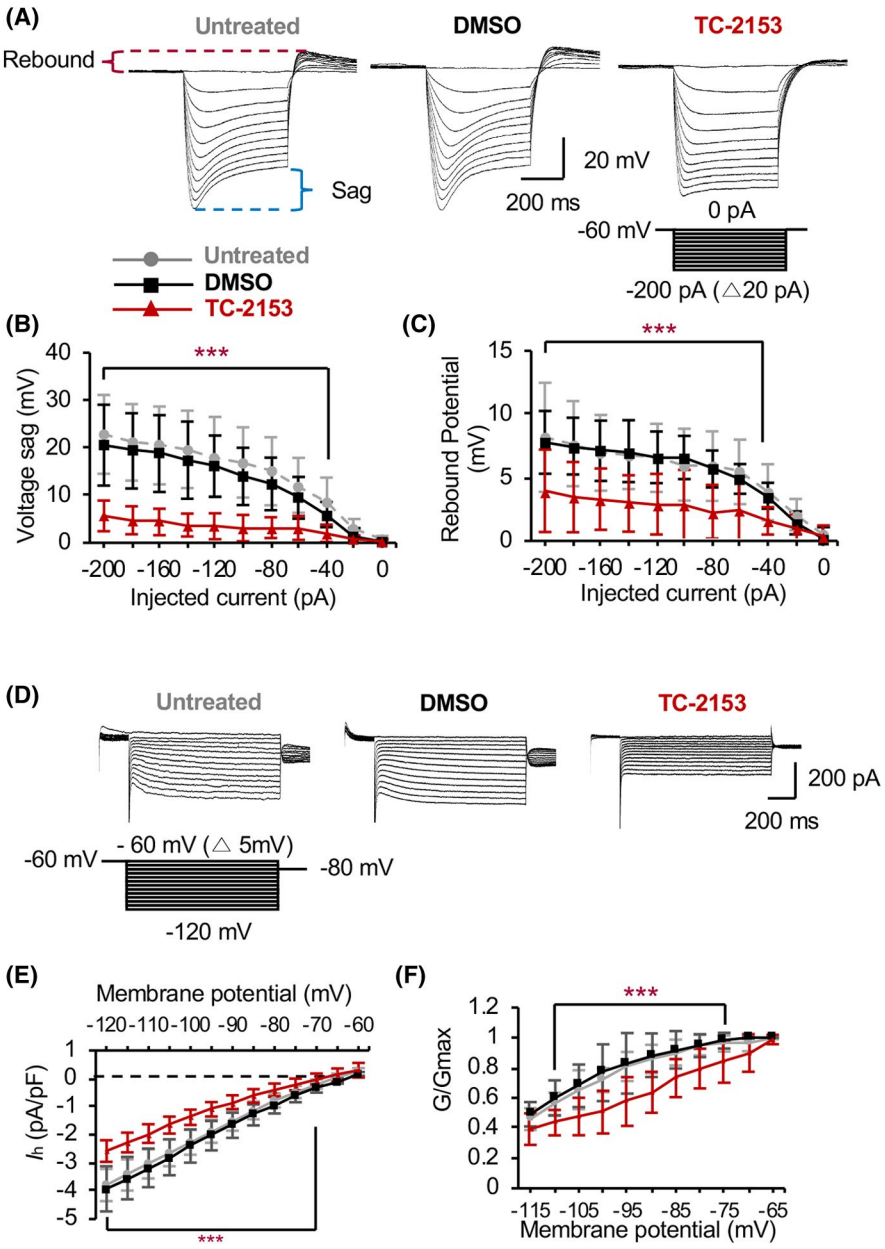

FIGURE 5 TC-2153 treatment reduces I_h in cultured hippocampal neurons. Whole-cell current clamp recording of cultured hippocampal neurons (days in vitro 8–10) was performed after 1 h treatment with TC 2153 (10 μM) or DMSO (0.14%). (A-C) TC-2153 reduces voltage sag and rebound voltage in cultured hippocampal neurons. (A) Representative responses to hyperpolarizing current steps from −200 to 0 picoamperes (pA) in 20 pA increments in current clamp mode. The amount of voltage sag (blue lines) and rebound potential (red lines) was determined as difference between the maximum and steady-state voltage during the hyperpolarizing current injection. (B) Average sag voltage. (C) Average rebound voltage. The number of recorded neurons: untreated (gray circle, $n = 12$), DMSO (black square, $n = 13$), or TC-2153 (red triangle, $n = 18$). (D-F) In voltage clamp mode, I_h was evoked by applying voltage steps from the holding potential of −60 millivolts (mV) to −120 mV in 5 mV decrements. (D) Representative traces of I_h. (E) I_h density at all voltage steps. (F) Normalized conductance (G/G_{max}) at all voltage steps. The number of recorded neurons: untreated ($n = 9$), DMSO ($n = 12$), TC-2153 ($n = 15$). Data shown as mean ± SD. Post hoc Tukey test results are shown for TC-2153 vs untreated or DMSO (***$p < .005$)

potassium currents in hippocampal pyramidal neurons,[41] fast-activating and inactivating I_A and fast-activating and slowly inactivating I_D delay the onset of firing and contribute to AP repolarization and firing rate.[41,42] Slowly activating and inactivating I_K mediates AP repolarization,[42] whereas slowly activating and non-inactivating I_M hyperpolarizes RMP and suppresses repetitive firing of APs without affecting their latency.[43] Calcium-activated I_C contributes to fAHP and regulates AP repolarization, firing rate, and half-width.[44] The effects of TC-2153 on ISI, AP rise and decay times, AP half width, and fAHP amplitude (Table 1) suggest that one or more of these potassium

currents may be regulated by TC-2153 to control intrinsic excitability and AP waveform.

4.4 | I_h as a novel target of TC-2153

We discover that TC-2153 treatment decreases I_h, which contributes to sag voltage and rebound potential evoked by membrane hyperpolarization (Figure 5). The role of I_h in hippocampal neuronal excitability is complex, as I_h exerts both excitatory and inhibitory effects on the ability of an excitatory postsynaptic potential (EPSP) to trigger an AP.[31] In hippocampal CA1 pyramidal neurons, HCN1 and HCN2 are preferentially enriched in the distal dendrites,[31] where I_h decreases EPSP summation,[45] but dendritic excitability can be enhanced or reduced by I_h.[46,47] I_h can also increase AP firing rate by depolarizing RMP and decreasing R_{in}.[48] Therefore, TC-2153-induced I_h reduction (Figure 5) may contribute to hyperpolarized RMP and decreased excitability seen in TC-2153-treated neurons (Figure 4).

How TC-2153 decreases I_h is unknown. Activation kinetics of the HCN2 channel is regulated by Src phosphorylation of its Tyr[476], whereas the receptor-like protein-tyrosine phosphatase-alpha (RPTPα) can dephosphorylate HCN2 and decrease its surface and current expression,[49] raising a possibility that $STEP_{61}$ may dephosphorylate HCN2, and its inhibition by TC-2153 may modify HCN2 channel function or expression. Alternatively, TC-2153 may directly bind to and decrease current expression of HCN1 and/or HCN2 channels. The mechanism underlying inhibitory actions of TC-2153 on I_h warrants future studies.

4.5 | Therapeutic potential of TC-2153

High levels of $STEP_{61}$ are associated with AD[7] and FXS.[15] Pharmacological inhibition of STEP with TC-2153 alleviates the excitatory synaptic defects and memory loss observed in the AD mouse model[16] and reverses the behavioral and synaptic deficits in the FXS mouse model.[15] In addition to these therapeutic potentials, our present study demonstrates that TC-2153 reduces seizure severity in both male and female mice and the activity of their hippocampi (Figures 1-3) and dampens the intrinsic excitability and I_h of hippocampal neurons (Figures 4 and 5). Clinical challenges exist in the use of antiseizure drugs that are ineffective or can differ by sex,[1,50] urging a need for new therapeutic targets. Our study presents TC-2153 as an attractive therapeutic candidate for epilepsy with novel mechanistic actions.

ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health under awards R01 NS083402, R01 NS097610, and R01 NS100019 (to H.J.C.); R01 NS105825 and R03 NS103029 (to C.A.C.-H.); R21 EB029076 (to G.R.H.); and R01 DC013073 and R01 DC016599 (to D.A.L.); It was also supported by the University of Illinois Campus Research Board RB21053 (to H.J.C.) and Carle Illinois Collaborative Research Seed Grant 083630 (to H.J.C. and G.R.H).

CONFLICT OF INTEREST

None of the authors have any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Jennifer M. Walters https://orcid.org/0000-0003-1995-1722
Eung Chang Kim https://orcid.org/0000-0002-8000-6271
Catherine A. Christian-Hinman https://orcid.org/0000-0003-3475-2166
Hee Jung Chung https://orcid.org/0000-0002-5555-9907

REFERENCES

1. Spencer SS. When should temporal-lobe epilepsy be treated surgically? Lancet Neurol. 2002;1:375–82. https://doi.org/10.1016/s1474-4422(02)00163-1
2. Goldberg EM, Coulter DA. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. Nat Rev Neurosci. 2013;14:337–49. https://doi.org/10.1038/nrn3482
3. Beck H, Yaari Y. Plasticity of intrinsic neuronal properties in CNS disorders. Nat Rev Neurosci. 2008;9:357–69. https://doi.org/10.1038/nrn2371
4. Goebel-Goody SM, Baum M, Paspalas CD, Fernandez SM, Carty Niki C, Kurup P, et al. Therapeutic implications for striatal-enriched protein tyrosine phosphatase (STEP) in neuropsychiatric disorders. Pharmacol Rev. 2012;64:65–87. https://doi.org/10.1124/pr.110.003053
5. Boulanger LM, Lombroso PJ, Raghunathan A, During MJ, Wahle P, Naegele JR. Cellular and molecular characterization of a brain-enriched protein tyrosine phosphatase. J Neurosci. 1995;15:1532–44.
6. Nakazawa T, Komai S, Tezuka T, Hisatsune C, Umemori H, Semba K, et al. Characterization of Fyn-mediated tyrosine phosphorylation sites on GluR epsilon 2 (NR2B) subunit of the N-methyl-D-aspartate receptor. J Biol Chem. 2001;276:693–9. https://doi.org/10.1074/jbc.M008085200
7. Kurup P, Zhang Y, Xu J, Venkitaramani DV, Haroutunian V, Greengard P, et al. Abeta-mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. J Neurosci. 2010;30:5948–57. https://doi.org/10.1523/JNEUROSCI.0157-10.2010
8. Zhang Y, Kurup P, Xu J, Carty N, Fernandez SM, Nygaard HB, et al. Genetic reduction of striatal-enriched tyrosine phosphatase (STEP) reverses cognitive and cellular deficits in an Alzheimer's disease mouse model. Proc Natl Acad Sci USA. 2010;107:19014–9. https://doi.org/10.1073/pnas.1013543107
9. Zhang Y, Venkitaramani DV, Gladding CM, Zhang Y, Kurup P, Molnar E, et al. The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate

receptor stimulation. J Neurosci. 2008;28:10561–6. https://doi.org/10.1523/JNEUROSCI.2666-08.2008
10. Jang SS, Royston SE, Xu J, Cavaretta JP, Vest MO, Lee KY, et al. Regulation of STEP61 and tyrosine-phosphorylation of NMDA and AMPA receptors during homeostatic synaptic plasticity. Mol Brain. 2015;8:55. https://doi.org/10.1186/s13041-015-0148-4
11. Won S, Incontro S, Nicoll RA, Roche KW. PSD-95 stabilizes NMDA receptors by inducing the degradation of STEP61. Proc Natl Acad Sci USA. 2016;113:E4736–44. https://doi.org/10.1073/pnas.1609702113
12. Won S, Incontro S, Li Y, Nicoll RA, Roche KW. The STEP61 interactome reveals subunit-specific AMPA receptor binding and synaptic regulation. Proc Natl Acad Sci USA. 2019;116:8028–37. https://doi.org/10.1073/pnas.1900878116
13. Pelkey KA, Askalan R, Paul S, Kalia LV, Nguyen TH, Pitcher GM, et al. Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. Neuron. 2002;34:127–38. https://doi.org/10.1016/s0896-6273(02)00633-5
14. Briggs SW, Walker J, Asik K, Lombroso P, Naegele J, Aaron G. STEP regulation of seizure thresholds in the hippocampus. Epilepsia. 2011;52:497–506. https://doi.org/10.1111/j.1528-1167.2010.02912.x
15. Chatterjee M, Kurup PK, Lundbye CJ, Hugger Toft AK, Kwon J, Benedict J, et al. STEP inhibition reverses behavioral, electrophysiologic, and synaptic abnormalities in Fmr1 KO mice. Neuropharmacology. 2018;128:43–53. https://doi.org/10.1016/j.neuropharm.2017.09.026
16. Xu J, Chatterjee M, Baguley TD, Brouillette J, Kurup P, Ghosh D, et al. Inhibitor of the tyrosine phosphatase STEP reverses cognitive deficits in a mouse model of Alzheimer's disease. PLoS Biol. 2014;12:e1001923. https://doi.org/10.1371/journal.pbio.1001923
17. Davis KE, Fox S, Gigg J. Increased hippocampal excitability in the 3xTgAD mouse model for Alzheimer's disease in vivo. PLoS One. 2014;9:e91203. https://doi.org/10.1371/journal.pone.0091203
18. Yan XX, Cai Y, Shelton J, Deng SH, Luo XG, Oddo S, et al. Chronic temporal lobe epilepsy is associated with enhanced Alzheimer-like neuropathology in 3xTg-AD mice. PLoS One. 2012;7:e48782. https://doi.org/10.1371/journal.pone.0048782
19. Khomenko TM, Tolstikova TG, Alexey B, Margarita D, Alla P, Dina K, et al. 8-(Trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine: novel aminobenzopentathiepine having in vivo anticonvulsant and anxiolytic activities. Lett Drug Des Discovery. 2009;6:464–7. https://doi.org/10.2174/157018009789057544
20. Levesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. Neurosci Biobehav Rev. 2013;37:2887–99. https://doi.org/10.1016/j.neubiorev.2013.10.011
21. Ujjainwala AL, Courtney CD, Rhoads SG, Rhodes JS, Christian CA. Genetic loss of diazepam binding inhibitor in mice impairs social interest. Genes Brain Behav. 2018;17:e12442. https://doi.org/10.1111/gbb.12442
22. Racine RJ, Gartner JG, Burnham WM. Epileptiform activity and neural plasticity in limbic structures. Brain Res. 1972;47:262–8. https://doi.org/10.1016/0006-8993(72)90268-5
23. Jang SS, Royston SE, Lee G, Wang S, Chung HJ. Seizure-induced regulations of amyloid-beta, STEP61, and STEP61 substrates involved in hippocampal synaptic plasticity. Neural Plast. 2016;2016:2123748. https://doi.org/10.1155/2016/2123748
24. Saavedra A, Ballesteros JJ, Tyebji S, Martínez-Torres S, Blázquez G, López-Hidalgo R, et al. Proteolytic degradation of hippocampal STEP61 in LTP and learning. Mol Neurobiol. 2019;56:1475–87. https://doi.org/10.1007/s12035-018-1170-1
25. Dana H, Chen TW, Hu A, Shields BC, Guo C, Looger LL, et al. Thy1-GCaMP6 transgenic mice for neuronal population imaging in vivo. PLoS One. 2014;9:e108697. https://doi.org/10.1371/journal.pone.0108697
26. Ratkai A, Tárnok K, Aouad HE, Micska B, Schlett K, Szücs A. Homeostatic plasticity and burst activity are mediated by hyperpolarization-activated cation currents and T-type calcium channels in neuronal cultures. Sci Rep. 2021;11:3236. https://doi.org/10.1038/s41598-021-82775-3
27. Bonin RP, Zurek AA, Yu J, Bayliss DA, Orser BA. Hyperpolarization-activated current (In) is reduced in hippocampal neurons from Gabra5-/- mice. PLoS One. 2013;8:e58679. https://doi.org/10.1371/journal.pone.0058679
28. Bell MR. Comparing postnatal development of gonadal hormones and associated social behaviors in rats, mice, and humans. Endocrinology. 2018;159:2596–613. https://doi.org/10.1210/en.2018-00220
29. Sisk CL, Zehr JL. Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol. 2005;26:163–74. https://doi.org/10.1016/j.yfrne.2005.10.003
30. Koebele SV, Bimonte-Nelson HA. Modeling menopause: the utility of rodents in translational behavioral endocrinology research. Maturitas. 2016;87:5–17. https://doi.org/10.1016/j.maturitas.2016.01.015
31. Biel M, Wahl-Schott C, Michalakis S, Zong X. Hyperpolarization-activated cation channels: from genes to function. Physiol Rev. 2009;89:847–85. https://doi.org/10.1152/physrev.00029.2008
32. Moosmang S, Biel M, Hofmann F, Ludwig A. Differential distribution of four hyperpolarization-activated cation channels in mouse brain. Biol Chem. 1999;380:975–80. https://doi.org/10.1515/BC.1999.121
33. Arshad MN, Naegele JR. Induction of temporal lobe epilepsy in mice with pilocarpine. Bio Protoc. 2020;10:e3533. https://doi.org/10.21769/BioProtoc.3533
34. Reddy DS, Carver CM, Clossen B, Wu X. Extrasynaptic gamma-aminobutyric acid type A receptor-mediated sex differences in the antiseizure activity of neurosteroids in status epilepticus and complex partial seizures. Epilepsia. 2019;60:730–43. https://doi.org/10.1111/epi.14693
35. Christian CA, Reddy DS, Maguire J, Forcelli PA. Sex differences in the epilepsies and associated comorbidities: implications for use and development of pharmacotherapies. Pharmacol Rev. 2020;72:767–800. https://doi.org/10.1124/pr.119.017392
36. Backstrom T. Epileptic seizures in women related to plasma estrogen and progesterone during the menstrual cycle. Acta Neurol Scand. 1976;54:321–47. https://doi.org/10.1111/j.1600-0404.1976.tb04363.x
37. Woolley CS. Estradiol facilitates kainic acid-induced, but not flurothyl-induced, behavioral seizure activity in adult female rats. Epilepsia. 2000;41:510–5. https://doi.org/10.1111/j.1528-1157.2000.tb00203.x
38. Mejias-Aponte CA, Jimenez-Rivera CA, Segarra AC. Sex differences in models of temporal lobe epilepsy: role of testosterone. Brain Res. 2002;944:210–8. https://doi.org/10.1016/s0006-8993(02)02691-4

39. Reddy DS, Castaneda DC, O'Malley BW, Rogawski MA. Anticonvulsant activity of progesterone and neurosteroids in progesterone receptor knockout mice. J Pharmacol Exp Ther. 2004;310:230–9. https://doi.org/10.1124/jpet.104.065268
40. Woolley CS. Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. Horm Behav. 1998;34:140–8. https://doi.org/10.1006/hbeh.1998.1466
41. Storm JF. Potassium currents in hippocampal pyramidal cells. Prog Brain Res. 1990;83:161–87. https://doi.org/10.1016/s0079-6123(08)61248-0
42. Bean BP. The action potential in mammalian central neurons. Nat Rev Neurosci. 2007;8:451–65. https://doi.org/10.1038/nrn2148
43. Brown DA, Passmore GM. Neural KCNQ (Kv7) channels. Br J Pharmacol. 2009;156:1185–95. https://doi.org/10.1111/j.1476-5381.2009.00111.x
44. Berkefeld H, Fakler B, Schulte U. Ca2+-activated K+ channels: from protein complexes to function. Physiol Rev. 2010;90:1437–59. https://doi.org/10.1152/physrev.00049.2009
45. Magee JC. Dendritic lh normalizes temporal summation in hippocampal CA1 neurons. Nat Neurosci. 1999;2:508–14. https://doi.org/10.1038/9158
46. Poolos NP, Migliore M, Johnston D. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. Nat Neurosci. 2002;5:767–74. https://doi.org/10.1038/nn891
47. George MS, Abbott LF, Siegelbaum SA. HCN hyperpolarization-activated cation channels inhibit EPSPs by interactions with M-type K(+) channels. Nat Neurosci. 2009;12:577–84. https://doi.org/10.1038/nn.2307
48. Gasparini S, DiFrancesco D. Action of the hyperpolarization-activated current (Ih) blocker ZD 7288 in hippocampal CA1 neurons. Pflugers Arch. 1997;435:99–106. https://doi.org/10.1007/s004240050488
49. Huang J, Huang A, Zhang Q, Lin YC, Yu HG. Novel mechanism for suppression of hyperpolarization-activated cyclic nucleotide-gated pacemaker channels by receptor-like tyrosine phosphatase-alpha. J Biol Chem. 2008;283:29912–9. https://doi.org/10.1074/jbc.M804205200
50. Perucca E, Battino D, Tomson T. Gender issues in antiepileptic drug treatment. Neurobiol Dis. 2014;72(Pt B):217–23. https://doi.org/10.1016/j.nbd.2014.05.011

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Walters JM, Kim EC, Zhang J, Jeong HG, Bajaj A, Baculis B, et al. Pharmacological inhibition of STriatal-Enriched protein tyrosine Phosphatase by TC-2153 reduces hippocampal excitability and seizure propensity. Epilepsia. 2022;00:1–14. https://doi.org/10.1111/epi.17192