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Supplementary Information for

Hippocampal deletion of $Na_V1.1$ channels in mice causes thermal seizures and cognitive deficit characteristic of Dravet Syndrome

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Supplementary Information Text

Methods.

Care and Maintenance of Mouse Lines. Mice were kept in standard mouse cages on a 12-hr light dark cycle with ad libitum water and food. *Scn1a* floxed mice were generated as described previously (1), and maintained on a C57BL/6J background (The Jackson Laboratory). *Scn1a* floxed mice maintained on a C57Bl6J are functionally wild-type and are behaviorally indistinguishable from their WT littermates. Mice were group-housed and maintained by backcrossing homozygous mice to C57BL/6J WT mice to yield *Scn1a* Flox heterozygous mice, which were bred to other, non-littermate, heterozygous mice to yield *Scn1a* homozygous Floxed mice. These homozygous *Scn1a* Flox mice were then bred with each other to yield experimental mice. Littermates of the same sex were randomly assigned to AAV-Cre or AAV-ΔCre experimental groups at P21, when the surgeries were performed. Epilepsy and electrophysiological studies contained both male and female mice,

as no difference was observed between the two groups, the data were pooled. Separate cohorts of mice were used for the behavioral experiments, which contained only male mice to maintain consistency with previously published behavioral *Scn1a*$^{+/-}$ data (1, 2). tdTomato reporter mice (stock # 007914) were purchased from The Jackson Laboratory and group housed. Flox *Scn1a* mice were genotyped using the primers FHY311 (5′-CTTGATGTGTTGAAATTCAC-3′) and FHY314 (5′-TATAGAGTGTTTAATCTCAAC-3′): WT allele, 846 bp; floxed allele, 1019 bp; and excised allele, 258 bp.

Immunohistochemistry. Immunohistochemical procedures were performed as described previously with slight modifications (1). Mice were anesthetized with isoflurane (Piramal Enterprises LTD, NDC 66794-017-25) and intracardially perfused with 4% paraformaldehyde (wt/vol) in 0.1 M Phosphate Buffer (PB) solution and allowed to post-fix for 1 h before being placed in a 15% sucrose solution in 0.1 M PB overnight. Subsequently, brains were allowed to sink for approximately 24 h in 30% sucrose in 0.1 M PB before being sectioned into 50 um slices and labeled as free-floating sections. Briefly, tissue was rinsed in 0.1 Tris buffer (TB) and 0.1 Tris buffered saline (TBS), blocked with an Avidin/Biotin blocking kit (Vector Biolabs, SP-2001). The primary antibody was rabbit anti-$Na_V1.1$ (1:175, Millipore AB 5204A), and the tissue was subsequently incubated in biotinylated goat anti-rabbit IgG (Vector Biolabs, BA-1000), and the secondary antibody was goat anti-rabbit IgG labeled with Streptavidin, Alexa Fluor™ 555 Conjugate (1:1000; Thermo Fisher, S32355). Tissue samples from AAV-Cre and AAV-ΔCre mice were processed simultaneously. Gain and offset matched images of GFP expressing cells were collected on a Leica SP8X confocal microscope at the Keck Imaging Facility of the University of Washington. Sections stained without primary antibody showed no detectable labeling. For all experiments, we verified that GFP reporter expression was present in the hippocampus; any mice with no expression or inappropriate expression were eliminated. To verify the activity of expressed AAV-Cre recombinase, we performed immunohistochemical staining and subsequent analysis using Imaris Image Analysis Software (Oxford Instruments) of AAV-Cre and AAV-ΔCre injected tissue labeled with the $Na_V1.1$ antibody (1:175, Millipore AB 5204A). Individual GFP positive cells (infected by virus) were analyzed for mean $Na_V1.1$ intensity through the entire volumetric cell using the Imaris Image Analysis Software (Oxford Instruments). Data files were analyzed by Imaris under the same parameters for both experimental conditions.

Thermal Induction and Analysis of Seizures. Thermal inductions were performed as described previously (3). Seizures were induced at P42 via a thermal induction protocol, three weeks after

injection of the virus or three weeks after injection of the virus and one week after EEG implantation. Briefly, mouse body temperature was measured continuously with a rectal thermometer probe connected to a feedback temperature recorder and a heat lamp (TCAT2DF; Physitemp). Mouse body temperature was held at 36.5°C for 10 min to habituate the mouse to the chamber. Following the habituation step, the internal temperature was elevated 0.5° every two min until either the mouse had a seizure or the internal body temperature reached 41.0 °. If the mouse experienced any behavioral seizure activity, the severity was assessed according to the Racine Scale system (4): 1, mouth and facial movements; 2, head nodding; 3, forelimb clonus, usually one limb; 4, forelimb clonus with rearing; and 5, generalized tonic-clonic seizure (GTC), rearing, clonus, and falling over. Due to the difficulty of assessing seizures with a severity below 3, we limited our assessment to Racine 3-5 seizures. If the mouse experienced a behavioral seizure, the trial was ended.

Behavioral Tests. For our behavioral tests, each mouse went through multiple, non-invasive procedures with a minimum of 48 h between each behavioral task to allow the mice to rest. Behavioral procedures were recorded with an HD ceiling mounted camera, and performed as described previously with slight modifications (2). Each data file was analyzed by Ethovision XT 14 (Noldus Technologies) according to the same parameters. For every mouse, the final behavioral test was the contextual fear conditioning, which requires an aversive stimulus. This test was performed last to avoid stressing the mice and confounding the other behavioral tests. Cohorts of male *Scn1a* floxed littermates were randomly assigned to experimental groups and injected at P21 with AAV-Cre or AAV-ΔCre. Behavioral experiments started at P80-90 and lasted to approximately P110. Mice were group-housed for most of this period and were separated to be singly housed and handled for 5 days before testing began. Only male mice were used for behavioral experiments to maintain consistency with previously published behavioral *Scn1a*$^{+/-}$ data (1, 2).

Open Field. Mice were placed in the lower left corner of a 40 cm X 40 cm arena and allowed to freely explore for 10 min. Their movement was recorded by a HD ceiling mounted camera. Total distanced traveled was measured, as well as the amount of time the mouse spent in the center of the arena, which comprised a circle having 25% of the area in the center of the chamber. Each data file was analyzed by Ethovision XT 14 (Noldus Technologies) according to the same parameters. The enclosure was wiped with paper towels and 70% ethanol between every subject.

Reciprocal Interaction. A stranger mouse (identified by a black mark on its tail) and experimental mouse were simultaneously placed into a 40 cm X 40 cm arena. The experimental mouse was allowed to freely interact with the sex matched stranger mouse for 10 min. Their movement was recorded by a ceiling mounted camera. The enclosure was wiped with paper towels and 70% ethanol in between every subject. The recorded video file was analyzed off-line by R.S, who was blinded to the identity of the experimental mouse until after analysis. Three distinct types of interactions were counted; nose to nose sniffing, nose to anogenital sniffing, and escape behavior, characterized by a darting movement away from the stranger mouse.

Three-Chamber Test of Social Interaction. The mice were tested for social interaction preference in a three-chambered apparatus (58 × 30 cm), which is a nontransparent plastic box with two partitions, which divide the box into three equivalently sized chambers (Left, Center, Right; each 30 × 19.3 cm). In the bottom center of each internal partition is a small square opening, which allows the mouse to freely pass into each of the three chambers. In the habituation phase, the experimental mouse was placed in the central chamber and allowed to explore the apparatus for 10 min. Following this 10-min habitation phase, the experimental mouse was removed and small cylindrical wire cages (10.5-cm diameter; Galaxy Pencil Cup; Spectrum Diversified Designs) were introduced into the top left and right corners of the apparatus. Cylindrical bottles of water were placed on top of these wire cups to prevent the mouse from climbing on top of them. One of the cages was empty and the other contained a sex- and aged-matched stranger C57BL/6J mouse. The experimental mouse was then re-introduced and allowed to explore the chambers again for 10 min. The side of the apparatus that contained the stranger mouse was counterbalanced in between trails. The wire cages and chamber were cleaned with 70% ethanol and wiped with paper towels between each test mouse. The experimental mouse's movement was recorded by a HD ceiling mounted camera. All data were analyzed under the same parameters between trials (EthoVision XT 14.0, Noldus Technology). Time spent in each chamber and time spent in a proximal circle 5 cm from each of the wire cages was recorded.

Barnes Maze. The Barnes maze apparatus is a white, opaque, circular piece of plexiglass (92 cm diameter) containing 20 equally spaced holes (7 cm diameter) located 5 cm from the perimeter. A black escape box (15 x 7 x 7 cm) is placed under one hole. The maze was set up 1 meter above the floor in a room containing a multitude of contextual cues, which did not change throughout the course of the experiment. Two bright lights (200W) were used to illuminate the surface of the maze. The experimental mouse's movement was recorded by a HD ceiling mounted camera. The

experimental mouse, after being allowed to habituate to the maze for 2 min on the first day, underwent 3 trials (with approximately 20 min between trials) per day for 4 days where the mouse was placed in the center of the maze and given 3 minutes to explore. If the mouse entered the escape hole, the trial finished and the lights were switched off for 1 min before placing the mouse back in its home cage. If the mouse didn't find the escape hole during the 3-minute trial, the trial was ended, the mouse was gently guided into the escape box and the lights were turned off. The escape box and maze were cleaned with 70% ethanol and wiped with paper towels between each test mouse. On the test day, the escape box was removed and mouse was placed on the maze for 3 min. Parameters measured (EthoVision XT 14.0, Noldus Technology) included time the mouse spent within a proximal circle of 5 cm around the correct hole, number of times the mouse placed its head into the target hole which used to contain the escape box, and latency to the correct hole. The wire cages and chamber were cleaned with 70% ethanol and wiped with paper towels between each test mouse, and all data was analyzed under the same parameters between trials.

Context-dependent Fear Conditioning. The contextual fear conditioning apparatus was a square (25 x 25) arena with clear plexiglass floor and a wire grid bottom which was connected to an amplifier which could be set to deliver shocks to the metal grid bottom and controlled by software (Freeze Frame 2.0, Actimetrics). The movement of mice was recorded by a USB webcam (LifeCam HD-6000, Microsoft) and PC-based video-capture software (WinAVI Video Capture, ZJMedia Digital Technology). On Day 1, a mouse was introduced into the chamber and allowed to habituate by exploring the chamber for 2 min. After two min, the mouse received a single mild foot shock (2 s, 0.6mA) and was removed from the chamber after an additional min. Thirty min after the habituation/training session, the mouse was returned to the chamber and recorded for 2 min. This same procedure was repeated 24 h after the training session and 7 days after the training session. The arena cleaned with 70% ethanol and wiped with paper towels between each test mouse. Percent freezing was calculated offline using the recorded video by the video-tracking software (EthoVision XT 9.0, Noldus Technology). Freezing behavior was defined as the amount of time in which the velocity of the experimental mouse was below 1.75 cm/s, and all data was analyzed under the same parameters between trials.

Novel Object Recognition. Novel object recognition was performed in a square, opaque, plexiglass chamber (40 x 40 cm). The experimental mouse's movement was recorded by an HD ceiling mounted camera. During the habituation session, a test mouse was allowed to freely explore for 10 min. Following this habituation stage, two objects of similar size but different shape and

color (Duplo Bricks) were placed in opposite corners of the arena 14 cm from the corner. The mouse was allowed to explore these objects for another 10 min. After a 2 min delay, the test mouse was placed back into the chamber, in which one of the “familiar” objects had been replaced by a novel object. The mouse was allowed to explore these objects for another 10 min. To avoid odor cues, a duplicate of the familiar object was used. The arena and objects were cleaned with 70% ethanol and wiped with paper towels between each test mouse. Time spent within a 5 cm proximal circle centered on each object was measured using Ethovision XT 14 (Noldus Technology), and all data was analyzed under the same parameters between trials. The arena cleaned with 70% ethanol and wiped with paper towels between each test mouse.

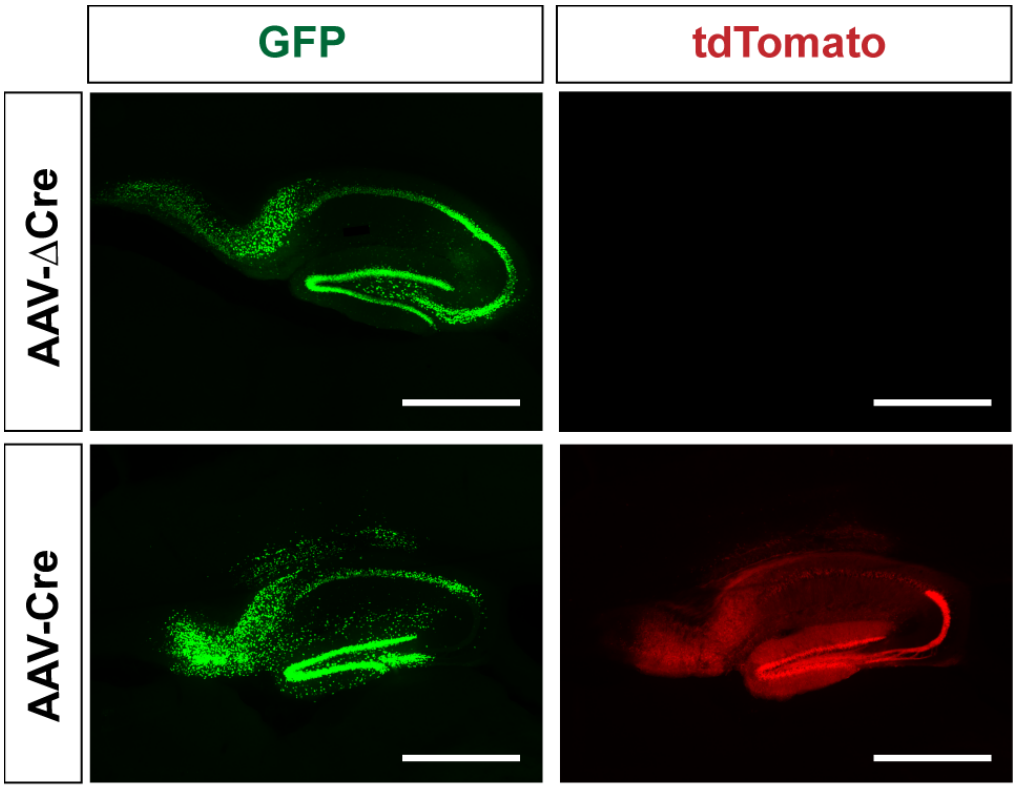

Fig. S1. AAV-Cre expressing virus activates reporter expression. AAV-ΔCre and AAV-Cre injection in the hippocampus (GFP, green) and tdTomato reporter expression (tdTomato, Red). Scale bar = 500 μm.

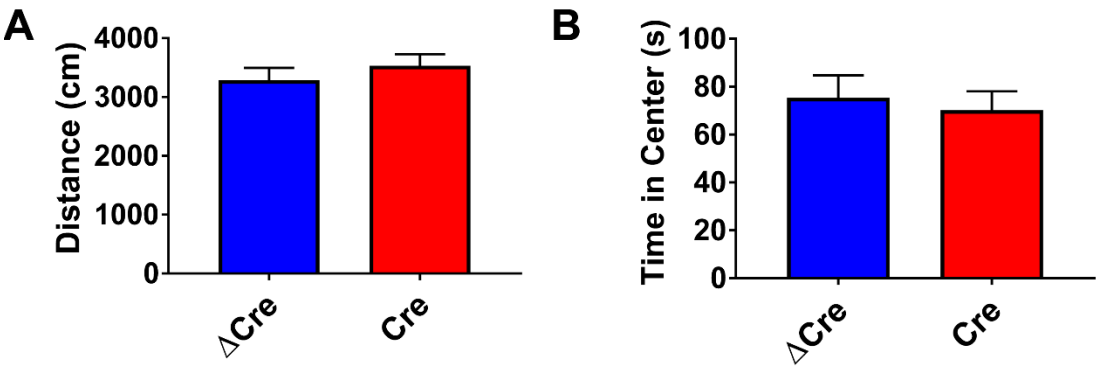

Fig. S2. Deletion of *Scn1a* in the hippocampus does not affect behavior in the open field. (*A*) Distance travelled during time min spent in open field chamber. Unpaired two-tailed Student's t test: Left, AAV-ΔCre: 3285 ± 210, n=16; Right, AAV-Cre: 3531 ± 198, n=17; P = 0.40. (*B*) Amount of time spent in center of open field. Unpaired two-tailed Student's t test: Left, AAV-ΔCre: 75.4 ± 9.39, n=16; Right, AAV-Cre: 70.2 ± 7.80, n=17; P = 0.67. Data are represented as mean ± SEM.

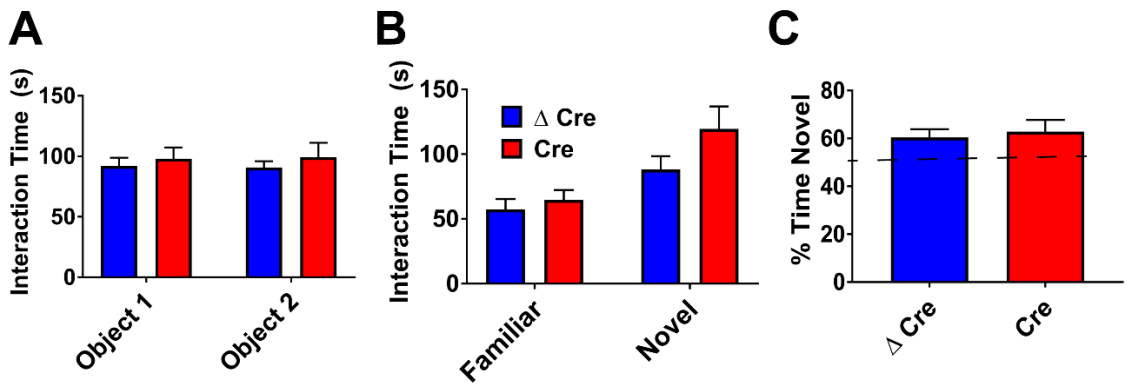

Fig. S3. *Scn1a* deletion in hippocampus does not cause impairments in novel object recognition. (*A*) Amount of time (s) AAV-ΔCre (Blue) and AAV-Cre (Red) spent with the familiar object. (*B*) Amount of time (s) spent with a familiar object vs. a novel object. (*C*) Ratio of time spent with novel object to total time spent interacting with both objects. Unpaired two-tailed Student's t test: AAV-Cre: 60.4 ± 3.50, n=10; AAV-ΔCre: 62.8 ± 4.85, n=9; P = 0.69. Data are represented as mean ± SEM.

References

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