PNAS

Supplementary Information for

Loss of Cav1.3 RNA editing enhances mouse hippocampal plasticity, learning and memory

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Supplemental Experimental Procedures

Generation of genetically modified $Ca_V1.3^{\Delta ECS/\Delta ECS}$ mice

The strategy to generate ECS targeting construct is summarized in Figure S1A. Briefly, a 9.2 kb mouse genomic DNA fragment spanning 5.1 kb upstream and 4.1 kb downstream of ECS was digested with *XhoI* and *BamHI* from mouse bacterial artificial chromosome (BAC) DNA (Invitrogen, Carlsbad, CA) and subcloned into pBluescript SK+ vector. The ECS sequence was then replaced with the selection marker cassette containing the NeoR gene flanked by loxP sites obtained from the NEB4 vector. The sequence of the 3.5 kb targeting backbone containing 400bp upstream of ECS, Neo cassette and 900bp downstream of ECS was confirmed by automated DNA sequencing before digesting with *BsaBI* and *BstBI* and re-ligated back into the $Ca_V1.3$-pBluescript SK+ vector to generate the targeting construct.

The ES cells were first transfected with targeting construct by electroporation (240 V 500 µF, 7.48 sec). Then after 250 µg/mL G418 (geneticin) neomycin drug selection, surviving colonies were picked into 96-well plates for further selection. About 300 G418-resistant ES colonies were screened to identify those that had undergone homologous recombination via genotyping by PCR and then confirmed by Southern blot analysis. The SV129 mouse blastocysts were injected with positive ES cells (Service from Biological Resource Centre Department I). Among the pups, 15 of them were chimeric mice. Male and high agouti-colour coated F0 mice were selected to mate with C57BL/6 female mice to generate F1 pups.

To generate $Ca_V1.3^{\Delta ECS}$ mice, genotyping by PCR and Southern blot analysis were performed to select male and female F1 with the desired genotype, Neo/WT, for breeding to generate ECS null mice with Neo/Neo genotype (F2). F2 mice were then crossed with actin-Cre (FVB/N-Tg(ACTB-cre)2Mrt/J) mice for the deletion of selection maker Neo cassette (F3). To confirm the deletion, F3 mice were crossed with C57BL/6J to generate F4 with heterozygous genotype. Then male and female F4 mice with the desired genotype were crossed to generate $Ca_V1.3^{\Delta ECS}$ mice. The $Ca_V1.3^{\Delta ECS}$ mice were backcrossed with C57BL/6J mice for 6 generations to obtain >99% of C57BL/6J genetic background.

For genotyping of $Ca_V1.3^{\Delta ECS}$ mice and $Ca_V1.3^{ECS}$ littermates following generations of backcrossing, two pairs of primers were used in two separate PCR reactions. An amplicon of 191 bp would indicate the KO allele with LoxP Fw and GT Rs Primers; an amplicon of 145 bp would indicate the WT allele with ECS Fw and GT Rs primers. For Southern blot analysis, the genomic DNA was digested by *SspI* restriction enzyme O/N before running on 0.7% agarose gel. A 500 bp Probe was used at 41°C for hybridization. A 11kb band indicated KO allele while a 5.3kb band indicated WT allele.

Evaluation of IQ-domain RNA editing in $Ca_V1.3$ channels

Total RNA was isolated from brain tissues using the TRIzol method (Invitrogen) and first strand cDNA was synthesized with Superscript II and oligo(dT)18 primers (Invitrogen). RNA was treated with DNase I (Invitrogen) before subjected to reverse transcription. Negative control reactions without reverse transcriptase were performed in all reverse transcription RT-PCR experiments to exclude contamination by genomic DNA. Reverse transcription to generate the first strand cDNA was performed by standard methods. For tissues collected from mouse, rat and macaca fascicularis, all experimental procedures were approved by IACUC at the National University of Singapore. For the post-mortem brain samples from various animals from Mandai Wildlife Group, all procedures were approved by IACUC at the MWG Research Panel. For human, Whole Brain Marathon-Ready™ cDNA library (Clontech, Takara) was used. PCR was performed with primers designed for different species (listed in Table S1). PCR products were then separated by electrophoeresis on a 1% agarose gel, extracted and then purified using the Qiagen gel extraction kit. The PCR products were sent for direct automated DNA sequencing (1st Base, Singapore).
The level of RNA editing was calculated by measuring the heights of the peaks of A/G on the electropherograms obtained from DNA sequencing:

The editing level (%)=height (G) / [height (A)+height (G)] *100%

Evaluation of transcription level of mCacna1d by real-time PCR

The total RNA obtained from whole brain tissues of $Ca_V1.3^{\Delta ECS}$ mice and $Ca_V1.3^{ECS}$ mice was normalized to a concentration of 1mg/μl after DNase treatment and then used in reverse transcription to synthesize first strain cDNA. The transcription level was detected using Taqman gene expression assay (Applied Biosystems, CA, USA). The transcription level of mCacna1d gene (Mm01209919_m1, NM_028981.2) was detected and normalized to the level of internal control gene Actb (Mm00607939_s1, NM_007393.3). The real-time PCR was run by the 7500 real-time PCR system according to the manufacturer's instructions. ΔCT value was used for statistical analysis by two-tails, unpaired Student's T-test.

Investigation of C-terminal alternative splicing pattern of $Ca_V1.3$ channels

The first strand cDNAs obtained from both $Ca_V1.3^{\Delta ECS}$ mice and $Ca_V1.3^{ECS}$ mice as described previously were used as templates to amplify a fragment of the C-terminus of $Ca_V1.3$ channels spanning exons 40 to 48, using the MusCTail Fw and Rs Primers. The PCR products were then used as templates for examination of splicing patterns in the C-terminal by DNA sequencing (1). Primers used are listed in Table S1.

Immunoblotting

To validate the expression level of targeted proteins, brain tissues or hippocampal slices were dissected on ice and were snap-frozen immediately. The CA1 region was then isolated carefully under a dissection microscope at 4 °C. Total protein from the CA1 regions was extracted by using the T-PER Tissue Protein Extraction Kit (no. 78510; Thermo Fisher Scientific), cOmplete Protease Inhibitor Cocktail (#11836145001; Roche). Quick Start Bradford Protein Assay Kit was used to quantify the protein level in the samples (#5000201; Bio-Rad). Thirty milligrams of protein extracts were separated on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride transfer membranes. The membranes were blocked with 5% BSA or nonfat dry milk and incubated with primary antibodies overnight at 4 °C. The primary antibodies used were as follows: anti-$Ca_V1.3$ (rabbit; AC-005; Alomone), anti-CaMKII (rabbit; 3362; Cell Signalling), anti-CaMKII (phospho Thr286; rabbit; 12716; Cell Signalling), anti-CREB (rabbit; 48H2; Cell Signalling), anti-CREB (phosphor Ser133; rabbit; 9198; Cell Signalling), anti-RED1(rabbit; ab64830; Abcam), anti-SRSF9 (rabbit; RN081PW; MBL) and anti-tubulin monoclonal antibody (mouse; T8328; Sigma-Aldrich). Anti- phospho-Synapsin and anti-Synapin antibodies (rabbit) were obtained from Prof. Thomas C. Südhof's lab (Stanford University)(2).

Membranes were incubated with the HRP-conjugated secondary antibody on the following day (anti-rabbit; anti-mouse; Sigma-Aldrich) for 1 h. The immune-precipitated proteins were detected by using a chemiluminescence detection system according to the manufacturer's instructions (Supersignal West Pico HRP detection kit; Pierce Biotechnology) and developed on an imager (Azure Biosystems). ImageJ software was used to quantify the optical density of each protein band. Each lane of protein band density was normalized with α-tubulin protein density and then the average level of corresponding protein from untrained $Ca_V1.3^{ECS}$ Mice.

Whole-cell patch-clamp electrophysiological recordings

Soma of CA1 and CA3 pyramidal cells were visually identified using a Nikon Eclipse FN1 equipped a 40× water immersion objective and a CoolSnap EZ camera. Brain slices were constantly perfused with the above mentioned oxygenated ACSF at a flow rate of 1.5 to 2 ml/min at room temperature. Whole-cell patch clamp electrophysiological recordings were conducted using EPC-9 or EPC-10 USB amplifier controlled by Patchmaster (Heka Elektronik, Lambrecht, Germany). Patch pipettes of 3–5 MΩ were pulled from borosilicate glass capillaries using a vertical Narishige PC-10 puller (Japan).

For current clamp recordings, patch pipettes were filled with (in mM): 140 K-$MeSO_4$, 5 K-Cl, 5 EGTA, 10 N-(2-Hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES); 0.5 Na_3GTP, 4 MgATP and 10 Na-Phosphocreatine. After formation of giga seal (>1 GΩ), input resistance was

monitored regularly by measuring voltage response by a 20 pA current injection. The reported membrane potential was corrected for the liquid junction potential 14.5 mV.
For voltage-clamp recording of Ca^{2+} currents in CA1 neurons, the internal solution contained (in mM): 130 Cs-$MeSO_3$, 4 CsCl, 5 EGTA, 10 HEPES, 0.5 Na_3GTP, 4 Mg-ATP, 10 Na-Phosphocreatine, 5 QX-314 (pH 7.3 adjusted with CsOH, 280 and 290 mosmol/L). For recording of K^+ currents, the internal solution for current clamp recording was used, and 5 mM BAPTA was used instead of EGTA when stated. Recordings with series resistance over 20 MΩ or changed by more than 20% were rejected. 1μM TTX (Alomone) was always included in the bath solution when recording Ca^{2+} and K^+ currents, a cocktail of 10mM TEA-Cl, 5mM 4-AP and 5 mM CsCl was added in the external solution when recording Ca^{2+} current, 1μM and 10μM nifedipine (L-type channel blocker; Sigma-Aldrich) was applied to bath sequentially for isolation of $Ca_V1.3$ channel currents. For isolation of IbTX sensitive BK currents, 100 nM IbTX (BK channel blocker; Alomone) was applied to the bath solution.
To record miniature excitatory postsynaptic currents (mEPSCs), cells were held at -70 mV holding potential. The mEPSCs were recorded in the presence of 0.5 μM TTX and 20 μM bicuculine (Tocris) in the bath solution. And 0.5 mM EGTA was used in the internal solution with the rest of the components remaining the same as mentioned above.
To measure paired-pulse ratios, the triple patch-clamp EPC10 amplifier and software Patch-master (HEKA Electronics) were used for data acquisition. In addition, a CED 1401 analog-to-digital converter (Cambridge Electronic Design) and a custom-made software program were used to regulate stimulation in the record paired-pulse ratio experiments. EPSC evoked by stimulation of Schaffer-collateral fibres in the stratum radiatum of CA1 by using a tungsten stimulating electrode (AM Systems). Schaffer collateral projections were stimulated at intervals from 20 ms to 200 ms by 0.1 ms voltage pulses generated by an isolated pulse stimulator (Model 2100; AM Systems). The stimulation strength ranged from 4-8 V. The ratio between the peak amplitude of the second and the first response was computed.
To measure AMPAR/NMDAR ratio, the patch-clamp EPC10 USB amplifier and software Patch-master (HEKA Electronics) were used for data acquisition. The stimulus isolator A365 (WPI) was used to regulate stimulation. Evoked EPSCs were obtained at -70 mV and +40 mV respectively by the same intensity stimulation of Schaffer-collateral fibres in the stratum radiatum of CA1 using a bipolar stimulating electrode (WPI) placed 200 μm away from the recorded neuron. AMPAR and NMDAR-mediated EPSCs were discriminated by their different kinetics as described in a previous study. Briefly, AMPAR current at +40 mV was determined by the eEPSC trace recorded at -70 mV, and the NMDAR current at +40 mV was measured at the time AMPAR current decayed (usually around 60 ms after peak) at -70 mV (see Fig. 3D Inset).

Field Recording

Transverse hippocampal slices of 400 μm thickness were prepared from the right and left hippocampus using a manual tissue chopper (Stoelting, Wood Dale, Illinois), and transferred onto a nylon net placed in an interface chamber (Scientific Systems Design, Ontario, Canada) and incubated at 32 °C at an ACSF flow rate of 1 ml/min and carbogen consumption of 16 l/h. The entire process of animal dissection, hippocampal slice preparation and placement of slices on the chamber was done within approximately five minutes to ensure that hippocampal slices were in good condition for electrophysiology studies. The slices were incubated for at least 3 h before starting the experiments.
In all the field electrophysiology recordings, single-pathway experiments were performed. A single monopolar lacquer-coated stainless-steel electrode (5MΩ; AM Systems, Sequim) was positioned in the stratum radiatum of the CA1 region for stimulating a neuronal population, thus evoking field excitatory postsynaptic potentials (fEPSP) from Schaffer collateral/commissural-CA1 synapses (Fig.1A). One electrode (5MΩ; AM Systems) represented as 'rec' was placed in the CA1 apical dendritic layer for recording fEPSP. After the pre-incubation period, a synaptic input-output curve (afferent stimulation vs. fEPSP slope) was generated. Test stimulation intensity was adjusted to elicit fEPSP slope of 40% of the maximal slope response for both synaptic inputs S1 and S2. The signals were amplified by a differential amplifier, digitized using a CED 1401 analog-to-digital converter (Cambridge Electronic Design, Cambridge, UK) and monitored online with custom-made

software. To induce late LTP, a “strong” tetanization (STET) protocol consisting of three trains of 100 pulses at 100 Hz (single burst, stimulus duration of 0.2 ms per polarity), with an inter-train interval of 10 min, was used. A weak tetanization (WTET) protocol consisting of one 100 Hz train (21 biphasic constant-current pulses; pulse duration per half-wave, 0.2 ms) was used to induce E-LTP. For inducing depotentiation, a weak low-frequency stimulation protocol consisting of 900 pulses (1 Hz; duration, 0.2 ms/phase) was applied 5 min after the induction of E-LTP in the same synaptic input. Paired pulse ratio (PPR) was evoked using an interstimulus interval of 50 ms at 40 % of maximum stimulus intensities. PPR was expressed as the ratio of the fEPSP slope of second stimulus to the first stimulus.10 μm AM251 (Tocris) was applied in the bath solution for 30min for blocking of CB1 receptor. In all experiments, a stable baseline was recorded for at least 30 min using four 0.2-Hz biphasic constant-current pulses (0.1 ms per polarity) at each time point. Four 0.2-Hz biphasic, constant current pulses (spaced at 5 s) given every five minutes were used for post-induction recordings also and the average slope values from the four sweeps was considered as one repeat while used for plotting. Initial slopes of fEPSPs were expressed as percentages of baseline averages.

Optokinetic drum vision test

Animals were placed on the platform in the center of drum for 3 min. Two different width black and white strips (0.5 cm and 1 cm) were used with rotating in clock wise and counter-clock wise. The optokinetic reflex of the tested animals is observed as its gaze following the strips. Trials were scored as 1 for successful observation of gaze behaviour 3 times during the test and 0 for animals who failed to do so.

Acoustic startle response test

Acoustic startle responses of animals were carried out using SR-LAB startle chambers (San Diego Instruments, San Diego, CA). The animals were placed inside the plexiglass cylinder to acclimate for 5 min before playing the sound stimulus. Sound stimulus with intensity of 0, 70, 75, 80, 85, 90, 100, 110 and 120 dB and duration of 40 ms were played in random orders for 5 times. The startle responses of animals were measured by the sensors that convert small movements to voltage. The magnitude of the change in voltage represented the startle response of the tested animals. Average change in voltage in response to the sound stimulus with same intensity was calculated from the 5 repeated trials.

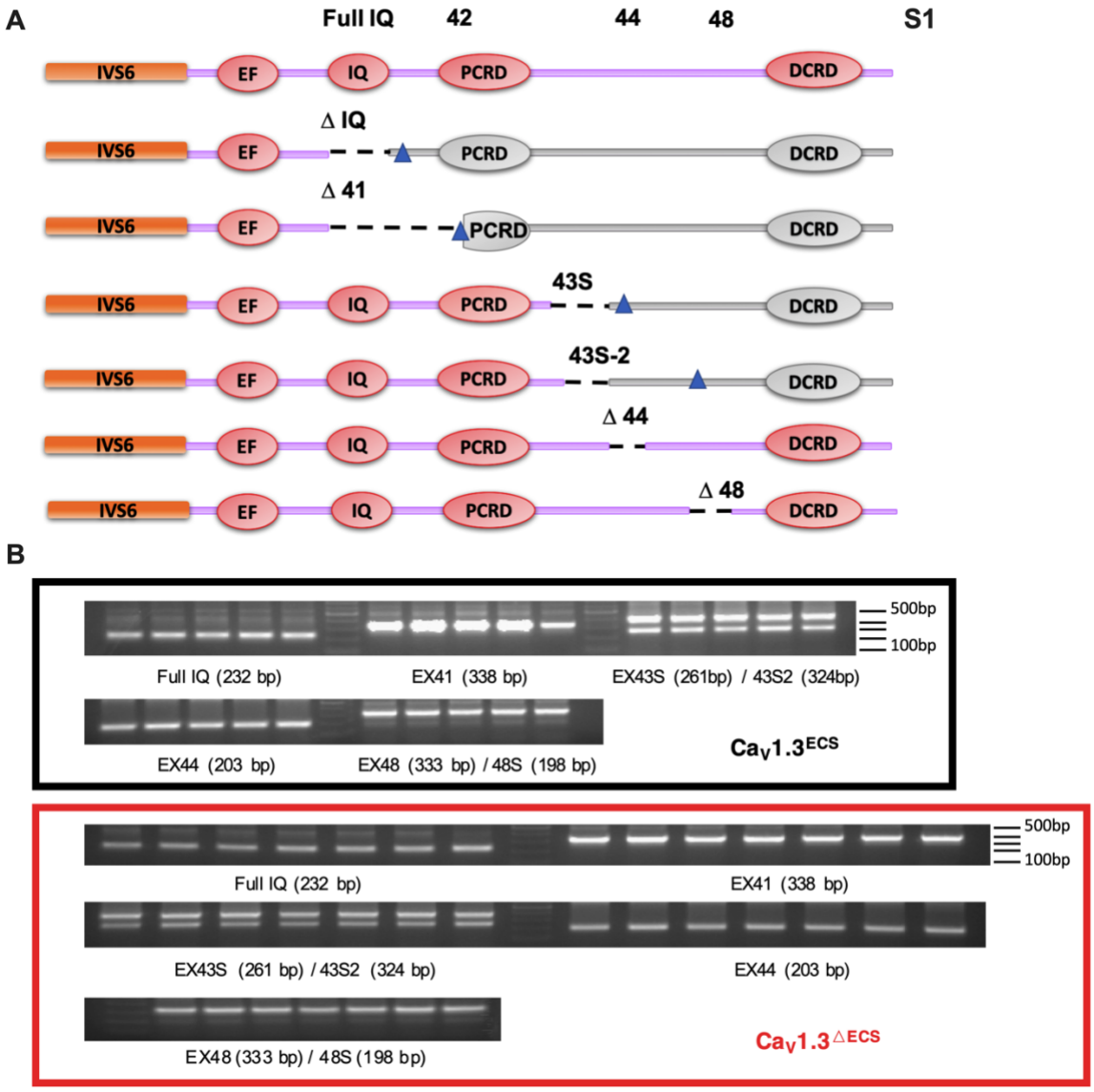

Fig. S1. C-terminal splicing $Ca_V1.3$ channel of $Ca_V1.3^{\Delta ECS}$ mice.

(A) Schematic illustrations of C-terminal splice variants of $Ca_V1.3$ channel. The stop codons for ΔIQ, Δ41, 43S, and 43S-2 are indicated by blue triangles. (B) Patterns of C-terminal splice variants detected from whole brain cDNA of $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice are similar. Inclusion of full IQ domain is indicated by 232 bp band; inclusion of exon 41 is indicated by 338 bp band; inclusion of exon 43S and 43S2 are indicated by band of 261 bp and 324 bp size respectively; inclusion of exon 44 is indicated by 203 bp bans; inclusion of exon 48 and exon 48S are indicated by band of 333 bp and 198 bp size respectively.

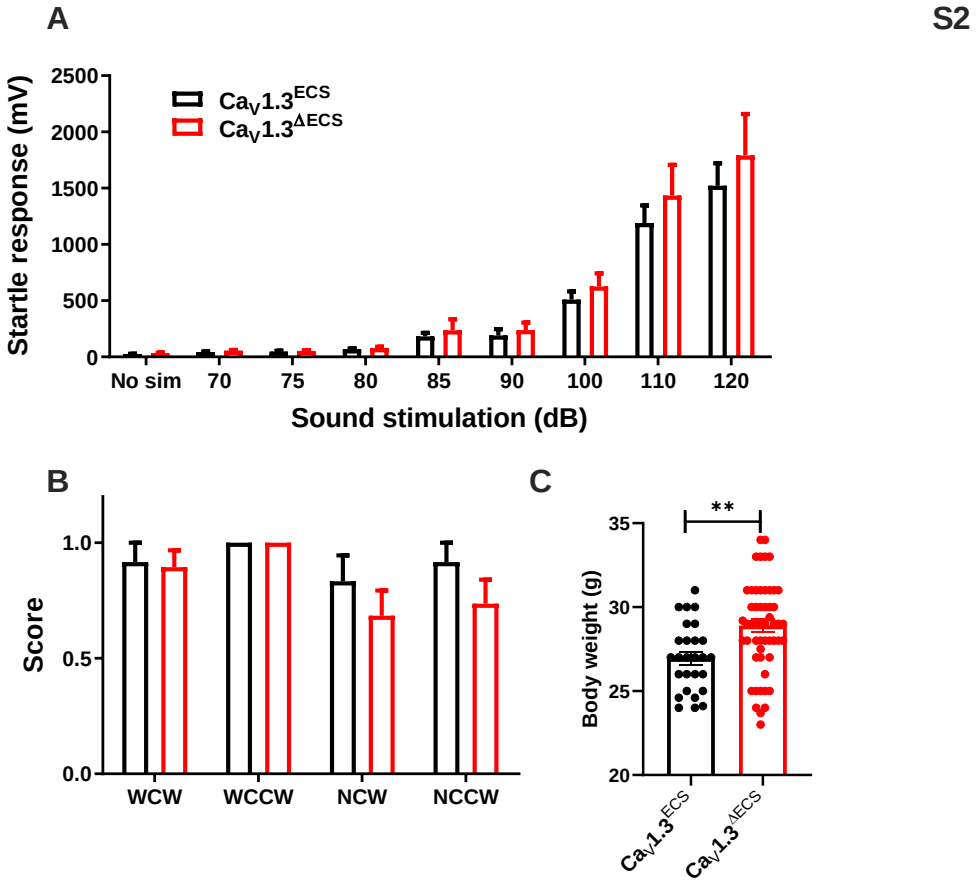

Fig. S2. Gross phenotypes of $Ca_V1.3^{\Delta ECS}$ mice.

(A) Acoustic startle response test. Startle responses of animals were recorded with various sound stimulations (70-120 dB). Both $Ca_V1.3^{ECS}$ (N = 13) and $Ca_V1.3^{\Delta ECS}$ (N = 19) mice showed increasing startle response with rising intensity of sound stimulus. (B) Optokinetic drum vision test. Black and white strips with two width 0.5 cm and 1 cm were rotated with the drum in clockwise and counter-clockwise (N: narrow, W: wide; CW: clockwise; CCW: counter-clockwise). The optokinetic reflex of the tested animals was observed as its gaze following the strips. Trials were scored as 1 for success and 0 for failure. Both $Ca_V1.3^{ECS}$ (N = 12) and $Ca_V1.3^{\Delta ECS}$ (N = 19) mice showed normal optokinetic reflex. (C) Body weight of $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ male mice at 3-month of age. $Ca_V1.3^{\Delta ECS}$ mice were about 2g heavier than $Ca_V1.3^{ECS}$ mice. Data represent means ± SEM. Scatters in bars indicate the number of analysed animals. The two-tailed unpaired Student's t-test was used, ** $p < 0.01$.

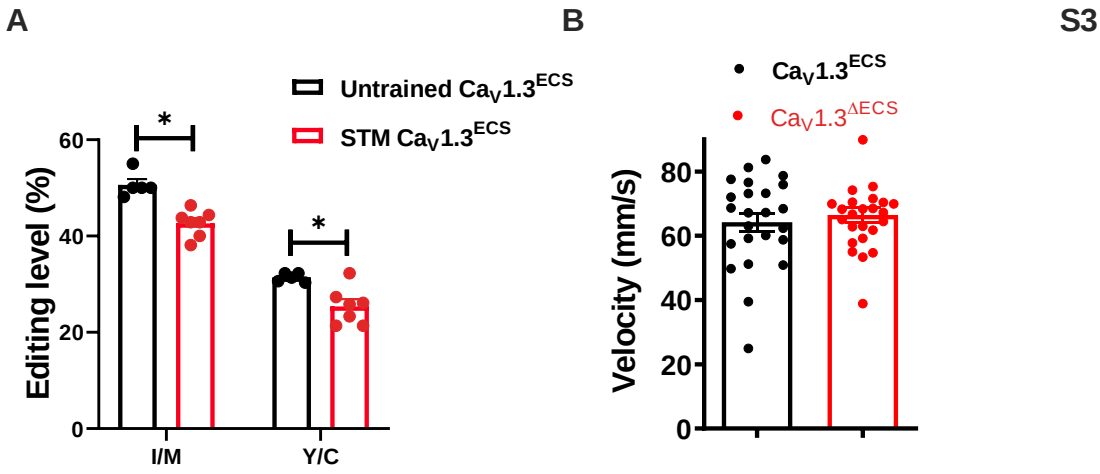

Fig. S3. Down-regulation of $Ca_V1.3$ RNA editing in hippocampus after STM probe test and velocity of animals in Morris water maze.
(A) Quantification of IQ-domain RNA editing levels at I-to-M and Y-to-C sites of $Ca_V1.3$ channels in whole hippocampal from $Ca_V1.3^{ECS}$ mice with (N = 6) and without (N = 5) water maze training. (B) Velocity of $Ca_V1.3^{\Delta ECS}$ mice (N = 25) measured during LTM probe test was not different from $Ca_V1.3^{ECS}$ mice (N = 24). Data represent means ± SEM. Scatters in bars indicate the number of analysed animals. Two-tailed unpaired Student's t-test was used for data in (A and B), * $p < 0.05$.

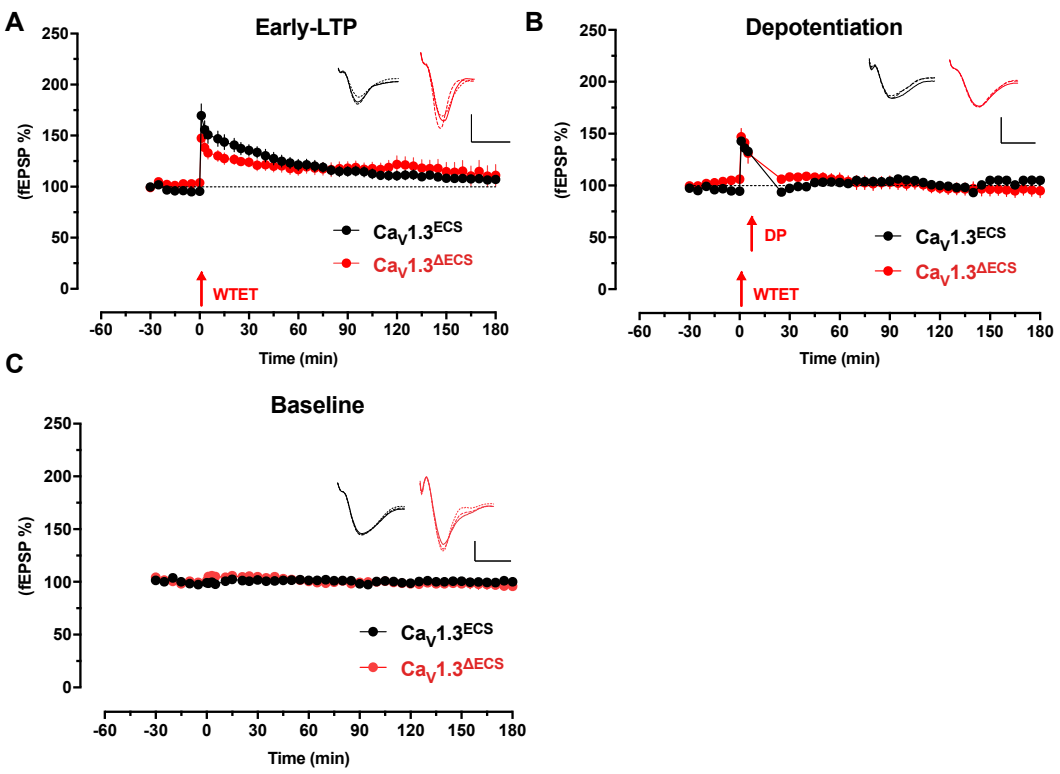

Fig. S4. Early-LTP and depotentiation from field recordings were unaltered in Ca$_V$1.3$^{\Delta ECS}$ mice.

(A) Early-LTP recorded from hippocampal slices with WTET at Schaffer collateral of Ca$_V$1.3^{ECS} and Ca$_V$1.3$^{\Delta ECS}$ mice. Scale bar 5 ms, 2 mV. (B) Depotentiaton (DP) induced by a weak low frequency stimulation, 5 min after WTET, were not different between Ca$_V$1.3^{ECS} and Ca$_V$1.3$^{\Delta ECS}$ mice. Scale bar 5 ms, 2 mV. (C) The fEPSP slope values did not show any statistically significant difference from its baseline values recorded from the hippocampal slices of Ca$_V$1.3^{ECS} and Ca$_V$1.3$^{\Delta ECS}$ mice with test stimulus given every 5 min at any time point. Scale bar 5 ms, 0.5 mV. Analog traces represent typical fEPSPs of input S1 recorded 15 min before (dotted line), 30 min after (dashed line), and 180 min (solid line) after tetanisation. Wilcox test was used, $p>0.05$.

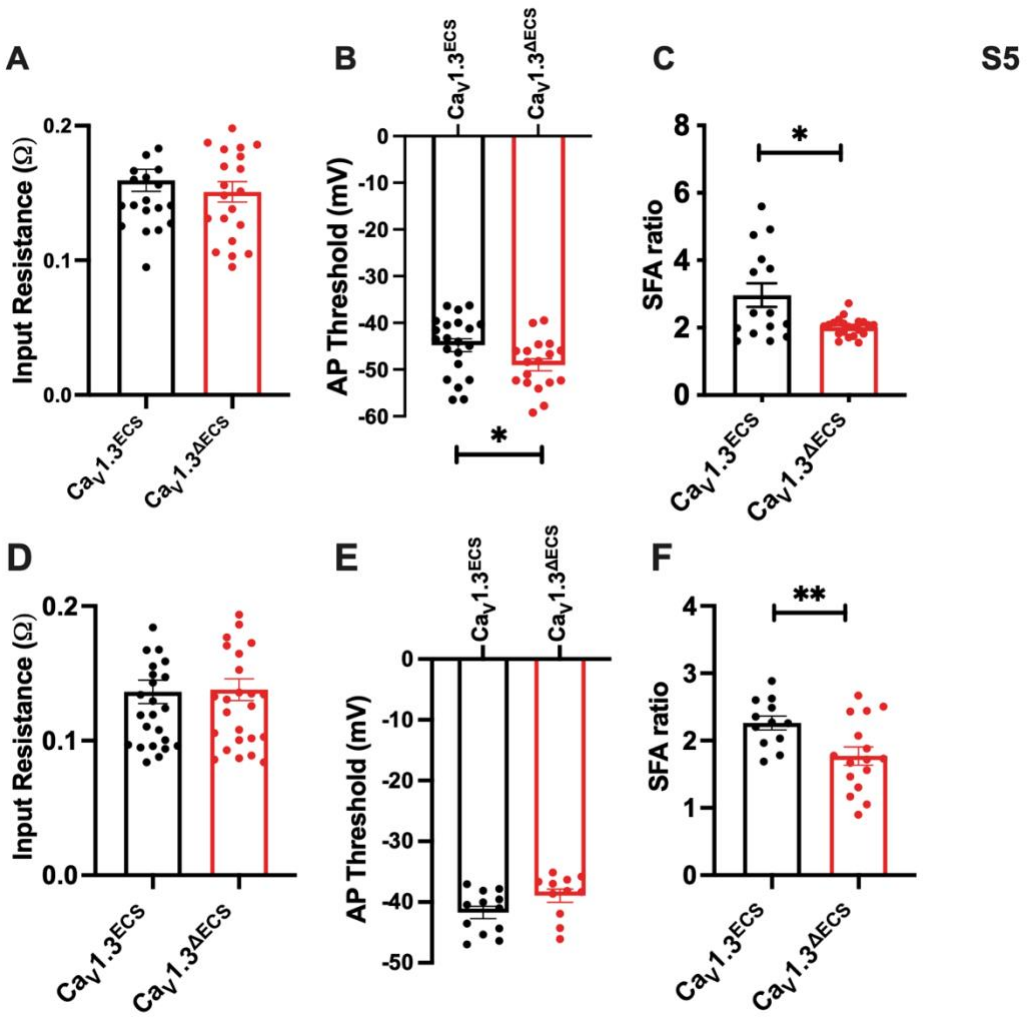

Fig. S5. Intrinsic properties of CA1 and CA3 pyramidal neurons.

(A) Input resistance of CA1 pyramidal neurons in $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. (B) AP threshold measured from 1st AP induced by 200 pA current injection in CA1 pyramidal neurons from between $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. (C) SFA ratio of CA1 pyramidal neurons in $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. (D) Input resistance of CA3 pyramidal neurons in $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. (E) AP threshold measured from 1st AP induced by 250 pA current injection in CA3 pyramidal neurons from between $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. (F) SFA ratio of CA3 pyramidal neurons in $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. Data represent means ± SEM. Scatters in bars indicate the number of analysed neurons. The two-tailed unpaired Student's t-test was used for data in all panels. *$p < 0.05$, **$p < 0.01$.

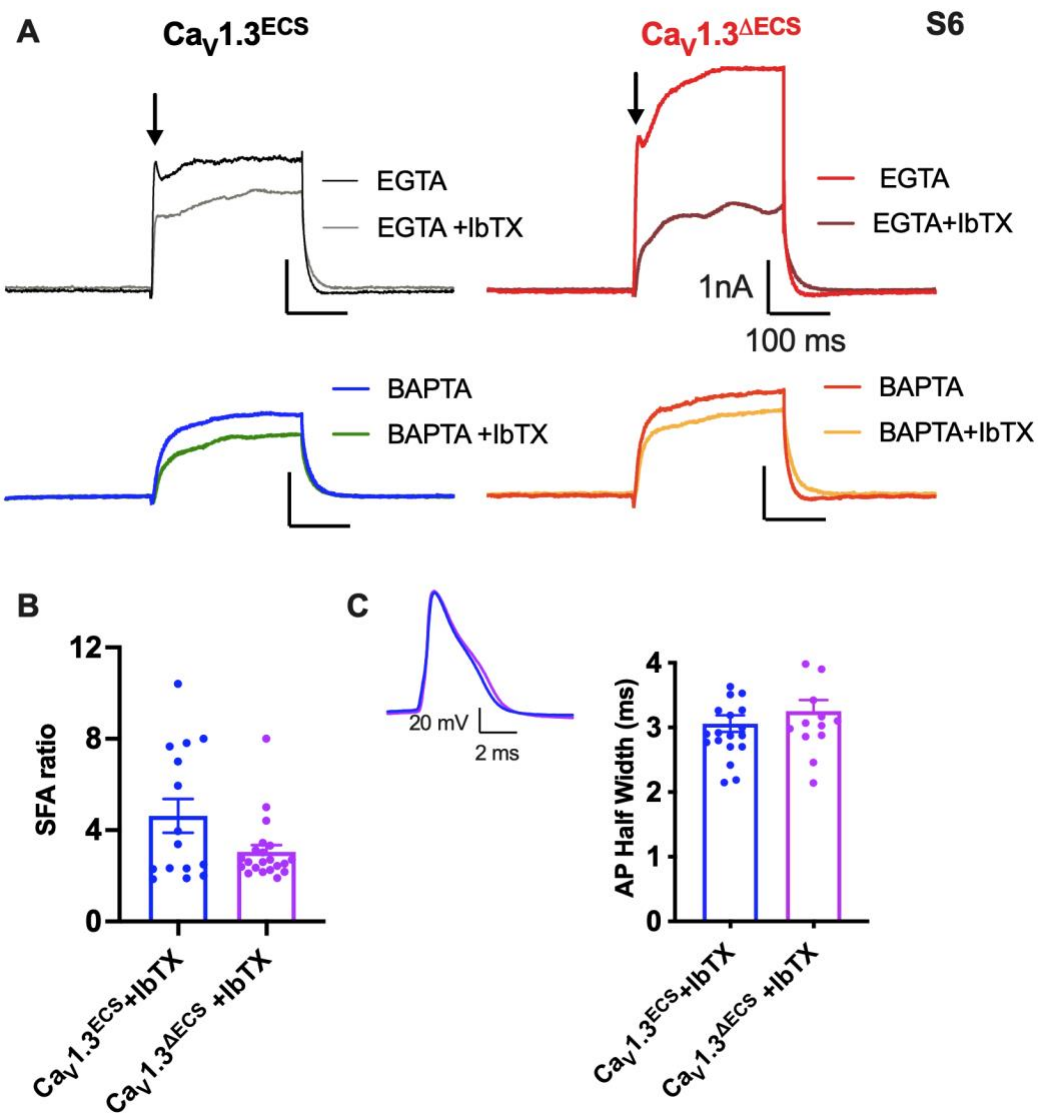

Fig. S6. IbTX-sensitive current before and after IbTX treatment.
(A) Representative traces of I_K currents recorded with EGTA and BAPTA in the pipette solution from CA1 pyramidal neurons before and after IbTX treatment in hippocampal slices from $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. Arrow indicating the fast-activated I_K current measured for quantification. (B) SFA ratio of CA1 pyramidal neurons in $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice after IbTX treatment. (C) Representative traces and quantification of AP half-width of of CA1 pyramidal neurons in $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice after IbTX. Data represent means ± SEM. Scatters in bars indicate the number of analysed neurons. Two-tailed unpaired Student's t test was used for analysis of data in all panels, $p>0.05$.

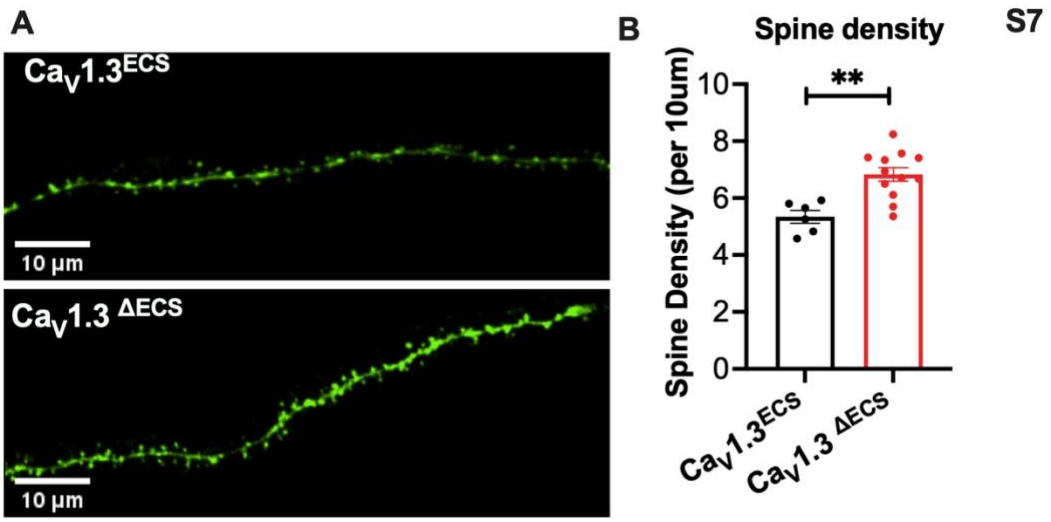

Fig. S7. Increased spine density of CA1 pyramidal neurons in $Ca_V1.3^{\Delta ECS}$ mice. (A) Representative images of dendritic branch of CA1 pyramidal neurons from $Ca_V1.3^{ECS}$ and $Ca_V1.3^{\Delta ECS}$ mice. (B) Quantitation of spine density measured from basal dendritic branch 100-200 um from soma of CA1 pyramidal neurons. Data represent means ± SEM. Scatters in bars indicate the number of analysed dendrites. The two-tailed unpaired Student's t-test was used, **p < 0.01.

LoxP Fw	5’- ATAACTTCGTATAATGTATGC-3’
ECS Fw	5’- TGTTTTAGGGTGGTGGCATGGA-3’
GT Rs	5’- GGAGTGTTGCAGGCTCGTGA-3’
MusCTail Fw	5’- AACCTCTTTGTGGCTGTCATC-3’
MusCTail Rs	5’- GGGTCTGGCTCCTCGTCACT-3’
E40_Fw	5’- GAACCTGGAGCAAGCTAATGAAG -3’
E41_Rs	5’-CTGTAGGGCAATCGTGGTGTT -3’
E42_Rs	5’- CATCTTCTTCTTCTGGTTTGGAGT -3’
E42_Fw	5’- AATCCGACGGGCTATATCCTGTGA -3’
E43_Rs	5’- TCGATCATGCTTGCAGGAGTAA -3’
E43_Fw	5’- CAGCATTGGGAACCTTGAG -3’
E45_Rs	5’- CCAAGCAGCGGGGGTCTC -3’
E47_Fw	5’- GCAAAGCAGCCAAGACGA -3’
E48_Rs	5’- AGACTAGCTGGCGTGAAAG -3’
Mus_1D Fs	5’- CTCCGAGCTGTGATCAAGAAAATCTGG-3’
Mus_1D Rs	5’- GGTTTGGAGTCTTCTGGCTCGTCA-3’
Zoo_1D Fw	5’- GCCATGAACATGCCTCTCAACAGTGATGG-3’
Zoo_1D Rs	5’- CTGTAGGGCAATTGTGGTGTTCTTCGC-3’
Mac_1D Fw	5’- GAACCTGGAGCAAGCTAATGAAG-3’
Mac_1D Rs	5’- TGTAGGGCAATTGTGGTGTTCT-3’
Hum_1D Fw	5’- CTTTGGTTCGAACGGCTCTTA-3’
Hum_1D Rs	5’- TGTAGGGCAATTGTGGTGTTCT-3’

Table S1. Primer Sequences used in the study.

1. Tan BZ, *et al.* (2011) Functional characterization of alternative splicing in the C terminus of L-type CaV1.3 channels. *The Journal of biological chemistry* 286(49):42725-42735.
2. Patzke C, *et al.* (2019) Neuromodulator Signaling Bidirectionally Controls Vesicle Numbers in Human Synapses. *Cell* 179(2):498-513 e422.