Supporting Information

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SI Materials and Methods

Generation of Calcium-Dependent Protein for Secretion 2–KO Mice Carrying the GAD67-EGFP Transgene and Analysis of GABAergic Interneurons. The generation of calcium-dependent protein for secretion 2 (CAPS2)-KO mice was described previously (1). CAPS2-KO ($CAPS2^{-/-}$) and WT littermates containing the glutamate decarboxylase 67 (GAD67) promoter-driven GFP transgene were generated by breeding CAPS2 heterozygous ($CAPS2^{+/-}$) mice with GAD67-GFP knock-in heterozygous ($GAD67^{GFP/+}$) mice (2). Genotyping was performed by PCR. $CAPS2^{+/+}GAD67^{GFP/+}$ (WT/GAD67-GFP) and $CAPS2^{-/-}GAD67^{GFP/+}$ (KO/GAD67-GFP) mice were generated by crossing $CAPS2^{+/-}GAD67^{GFP/+}$ mice with $CAPS2^{+/-}$ mice. The number of GFP-fluorescent interneurons in the CA1, CA3, and dentate gyrus regions of interest (200 mm × 200 mm) was quantified.

DNA-Expression Constructs. The BDNF cDNA (3) was fused to four tandem repeated sequences of superecliptic pHluorin (generously provided by J. E. Rothman, Yale University School of Medicine, New Haven, CT) and was subcloned into pEF-BOS to create pEF-BOS-BDNF-4xpHluorin. The CAPS2 cDNA (3) was fused to tdTomato (generously provided by R. Y. Tsien, University of California at San Diego, La Jolla, CA) and subcloned into pEF-BOS to create pEF-BOS-CAPS2-tdTomato.

Hippocampal Primary Cell Cultures and Transfection. Primary cultured hippocampal neurons from CAPS2-KO mice were prepared using previously described methods (4), with slight modifications. Briefly, CAPS2-KO mouse hippocampi were dissected on postnatal day 0, dissociated, and plated onto poly-D-lysine-coated glass-bottomed dishes (MatTek). On days 6–8 in vitro, neurons were transfected with BDNF-4× pHluorin, with or without CAPS2-tdTomato, using Lipofectamine 2000 (Invitrogen). Time-lapse live-cell imaging was performed 24–48 h after transfection. Transfected cells, which emitted green (BDNF-4× pHluorin) fluorescence and/or red (CAPS2-tdTomato) fluorescence, were used for subsequent imaging experiments.

Hippocampal Slice Preparation and Electrophysiology. Acute hippocampal slice preparations were prepared according to previously described methods (5), with slight modifications. Briefly, under deep anesthesia, CAPS2 WT and KO mice (2- to 3-wk old and 6- to 8-wk old) were decapitated, and the brains were removed rapidly. Transverse hippocampal slices (400–500 μm in thickness) were cut using a vibratome (PRO 7; Dosaka) and were maintained in artificial cerebrospinal fluid (ACSF) at room temperature. A bipolar platinum iridium-stimulating electrode was placed in the CA1 stratum radiatum region. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the CA1 stratum radiatum following a 0.05-Hz test pulse. Long-term potentiation (LTP) was induced electrically using one of the following protocols. High-frequency stimulation (HFS)-induced LTP was elicited by 100-Hz, 100-pulse stimulation. Theta-burst stimulation (TBS)-induced LTP was elicited by four trains, with 10-s intervals between trains. Each train had five bursts separated by 200 ms and included four pulses delivered at 100 Hz, which were at 20% of maximal stimulus intensity. For LTP recording during the inhibition of GABAergic transmission, 100 μM picrotoxin (Sigma) was dissolved in ACSF. For LTP recording in the presence of BDNF, recombinant BDNF (100 ng/mL) and BSA (1 μg/mL) were applied during the LTP experiment at least 20 min before TBS. Whole-cell patch-clamp recordings were performed from CA1 pyramidal neurons under visual guidance using IR differential interference contrast optics (Hamamatsu Photonics). Patch pipettes (2–5 MΩ) were pulled from borosilicate glass capillaries (GC150F-10; Harvard Apparatus). Data were amplified using a MultiClamp 700A (Molecular Devices) and were digitized at 10 kHz and filtered at 2 kHz using a Digidata 1440 system with pCLAMP9 software (Molecular Devices). Cells with a high-seal resistance >1 GΩ and a series resistance <25 MΩ were included in the analysis. Series resistance and input resistance were controlled before and after each recording, and cells were discarded if either or both parameters were altered by >20%. Miniature inhibitory postsynaptic currents (mIPSCs) were isolated pharmacologically using bath application of 1 mM kynurenic acid (TCI) and 1 μM tetrodotoxin (Tocris). Miniature excitatory postsynaptic currents (mEPSCs) were isolated pharmacologically using bath application of 100 μM picrotoxin (Sigma) and 1 μM tetrodotoxin (Tocris). Cells were voltage clamped to −80 mV, and mIPSCs and mEPSCs were recorded for 3 min. The high-sucrose cutting solution contained (in mM): 234 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 10 $MgSO_4$, 26 $NaHCO_3$, and 11 D-glucose, gassed with 95% O_2/5% CO_2. ACSF contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, and 11 D-glucose, gassed with 95% O_2/5% CO_2. The intracellular solution contained (in mM): 124 CsCl, 8 KCl, 8 NaCl, 10 Hepes, 0.1 EGTA, 4 MgATP, 0.3 NaGTP, and 5 QX-314 (Sigma) for mIPSC recording. CsCl was replaced with cesium methanesulfonate for mEPSC recording.

Immunohistochemistry and Immunocytochemistry. Immunochemical staining of hippocampal cultures and slices was performed as described previously (1). For immunohistochemistry, postnatal day 28 (P28) and 8-wk-old C57BL/6J mice were anesthetized with diethyl ether and were transcardially perfused with saline, followed by 4% paraformaldehyde/PBS. Brains were dissected, postfixed in 4% paraformaldehyde at 4 °C for 5 h, and cryoprotected by immersion in 15% sucrose/PBS overnight at 4 °C. After embedding in Tissue-Tek OCT compound (Sakura Finetechnical), brains were frozen in dry ice powder, and 14-μm sagittal sections were cut using a cryostat (Leica CM1850; Leica Microsystems) at −18 °C. Sections then were air-dried for 1 h and rinsed three times in PBS. For immunocytochemistry, cultured neurons were fixed with 4% paraformaldehyde/PBS and then were washed with PBS. After blocking with 5% normal donkey serum/PBS (Vector Laboratories), sections were incubated with primary antibody at 4 °C overnight, rinsed in PBS, incubated with secondary antibody at room temperature for 1 h, and then rinsed in PBS. Sections then were mounted with Vectashield Mounting Medium (Vector Laboratories) and examined using an epifluorescent microscope (Eclipse E800; Nikon) equipped with a cooled CCD camera (SPOT model 1.3.0; Diagnostic Instruments) or with a confocal laser microscope (FV1000; Olympus). Antibodies used were as follows: guinea pig polyclonal anti-CAPS2 antibody (1:10000) (3), rabbit polyclonal anti-BDNF antibody (1:100) (6), mouse monoclonal anti-Tau antibody (1:1,000; BD Biosciences), mouse monoclonal anti-MAP2 antibody (1:500; Sigma), guinea pig polyclonal anti-vGluT1 antibody (1:2,000; Chemicon), and mouse monoclonal anti-vGAT antibody (Synaptic Systems) (1:500). The number of excitatory and inhibitory terminals in the CA1 stratum radiatum region of interest (10 μm × 10 μm) was quantified.

Electron Microscopy. Under deep Nembutal anesthesia (250 mg/kg, i.p.), 3-mo-old WT and CAPS2-KO mice were transcardially perfused with 2 mL saline followed by 20 mL 4% paraformaldehyde/2% glutaraldehyde/0.1 M phosphate buffer (pH 7.4). The brains were removed and immersed in the same fixative at 4 °C overnight. Transverse hippocampal slices (300 μm thick) were cut using a microtome (VT1000S; Leica) and were post-fixed in cold 1% osmium tetraoxide solution for 1 h at 4 °C. After dehydration in a series of graded alcohols, the sections were embedded in epoxy resin (EPON812; Taab). Serial ultrathin sections (70 nm thick) were cut using an ultramicrotome, were mounted on uncoated 200-μm mesh copper grids, and then were stained with uranyl acetate/lead citrate. Dendritic profiles were traced serially and examined using an electron microscope (JEM-1200EX; Jeol), and digital image data from imaging plates were scanned and digitized using the FDL imaging system (Fujifilm). Digitized images were processed using Image Gauge (Fujifilm) and Photoshop (Adobe).

Behavioral Experiments. ***Open field test.*** Open field tests, with or without a novelty object, were performed as previously described (1) using 3-mo-old male mice. Locomotor activity was measured in an open field apparatus (60 cm × 60 cm), with a light level of 50 lx at the surface level. Each mouse was placed in the center of the open field, and horizontal movements were monitored for 15 min using a CCD camera. The images were processed using Image J OF software (O'Hara). Total activity for 15 min was used in statistical analysis.

Elevated plus maze test. Three-month-old male mice were used. An elevated plus maze (closed arms: 25 cm × 5 cm × 15 cm; open arms 25 cm × 5 cm × 0.3 cm) was used in a soundproof room (2 m × 2 m × 2 m). The floor of each arm was made of white plastic, and the walls and ridges were made of clear plastic. Closed arms and open arms were orthogonally arranged, 60 cm above the floor. The light level was 70 lx at the center of the maze platform (5 cm × 5 cm). Individual mice were put onto the center platform facing an open arm and were allowed to move freely in the maze for 5 min. The total distance traveled, percent of time in the open arms, and percent of entries to the open arms were measured as indices. Data were collected and analyzed using Image J EPM software (O'Hara).

Novelty suppressed feeding test. Four-month-old male mice were weighed, and food was removed from the cage. Water was available ad libitum. Twenty-four hours after food restriction, mice were transferred to a testing room and placed in a novel arena with a Petri dish containing a food pellet in the center. Each subject was placed in the corner of the testing area, and the latency to eat the pellet was recorded during a 10-min period. All experiments were performed using normal, ambient, overhead lighting and were performed during the light phase of the cycle, between 1300 hours and 1800 hours.

Eight-arm radial maze test. All training and testing were performed with 4-mo-old male mice in an open, white, one-unit, eight-arm radial maze with transparent walls 11.5 cm high and corridors 9.0 cm wide. The eight arms radiated equidistantly from a circular central area with a diameter of 29.0 cm. Each arm was 40.5 cm long, and at the end of the arm a circular food well (diameter: 1.5 cm, depth: 0.5 cm) contained 25-mg food pellets (Precision Food Pellets; O'Hara). The maze was placed in the middle of a well-lit room, and no other animals were present during training and testing. A multitude of 2D and 3D distal cues were available. During the training trials, four arms were baited. The same arms remained baited for all training and retention trials. Four patterns of baited arms were used, and the patterns were chosen to reflect equal difficulty and to minimize nonspatial search strategies (i.e., more than two consecutive visits to unbaited arms). Four massed trials were conducted per day for each subject and were preceded by a 1-min confinement in the center platform. The trial was terminated when the mouse entered and ate from all baited arms or after a maximum latency of 10 min. At the end of 10 trials, the mouse was returned to the home cage. This procedure was repeated for 9 training d (36 trials in total). Retention testing was conducted 24 h after the final training trials, with four trials as described under the training procedure. Horizontal movements and arm entries were monitored for 10 min using a CCD camera. Images were processed using Image J OF software (O'Hara), and animals were scored based on number of entries into baited and unbaited arms, time to retrieve bait rewards, and sequence of arms visited. The number of arm entries/min also was recorded. Data were analyzed for the following measures: percent correct, number of reference memory errors, and number of working memory errors. Reference memory errors were scored when the mouse visited an arm that was never baited with food. Working memory errors were scored when a mouse revisited an arm during a trial from which it had already obtained the food reward.

Y-maze test. Three-month-old male mice were used. The Y-maze apparatus (O'Hara) was made of gray plastic and consisted of three compartments (3 cm wide on the bottom and 10 cm wide on the top, 40 cm long, and 12 cm high) radiating out from a central 3 cm × 3 cm × 3 cm triangular platform. The maze was set 80 cm above the floor, and desks and test apparatus surrounding the maze provided spatial cues. Each mouse was placed in the center of the maze facing one of the arms and was allowed to explore freely for 5 min. Experiments were performed at a light intensity of 70 lx at the center of platform. An arm entry was defined as four legs entering one of the arms, and the sequence of entries was quantified using a television monitor, which was placed behind a partition. An alternation was defined as consecutive entry into all three arms (the maximum number of alternations was the total number of entries minus 2). Percent alternation was calculated as (actual alternations/maximum alternations)/100. Percent alternation was designated as spontaneous alternation behavior and represented working memory.

Zero maze test. A zero maze (O'Hara) 40 cm in diameter and 5 cm wide, which consisted of two enclosed areas and two open areas, was used in a soundproof room (2 m × 2 m × 2 m and 50 cm above the floor); 5-mo-old male mice were used. Mice were placed in the closed section of the maze and were allowed free access to all maze areas for 5 min at a light intensity of 70 lx. Total distance traveled, percent time spent in open arms, and percent entries into open arms were measured as indices. Data were collected and analyzed using Image J OF software (O'Hara).

Morris water maze. A circular maze made of white plastic (1 m in diameter, 30 cm in depth) was filled with water (temperature about 25 °C) to ≈20 cm in depth; 4-mo-old male mice were used for testing. The water was colored with white paint so that the mice could not see the platform (20 cm high, 10 cm in diameter; 1 cm below the water surface) or other cues under the water. Extramaze landmark cues (i.e., calendar, figure, plastic box) were visible to the mice. Mouse movements were recorded in the maze and analyzed using Image J WM (O'Hara). The mice underwent six trials in one session per day for 4 consecutive d. Each acquisition trial was initiated by placing the mouse into the water facing the outer maze edge randomly at one of four designated starting points. However, the position of the submerged platform remained constant for each mouse throughout testing. A trial was terminated when the mouse reached the platform, and latency and distance swam were measured. Cutoff time of the trial was 60 s, and mice that did not reach the platform within 60 s were removed from the water and placed on the platform for 30 s before being dried and placed back in the home cage. The intertrial interval was ≈6 min. After 4 d of training, a probe test was conducted. During the probe test, the platform was removed. Each mouse was placed into the water at the point opposite the target platform and was allowed to swim in the

maze for 60 s. Distance swum, number of target quadrant crossings and remaining three quadrants crossings, and time spent in each quadrant were measured.

Barnes maze. On the first day, habituation training was conducted with 3-mo-old male mice. Individual mice were placed on the center of the maze, which consists of a circular table with 12 circular holes around the circumference of the table, and were allowed to explore freely (5 min per mouse, without escape cage). Following habituation training, escape trainings were conducted. For escape training, mice were placed into a small plastic container in the maze center for 10 s The container then was opened, and mice were led to the escape hole and allowed to escape into the hole (the tail was gently pinched if necessary). Escape training was repeated five times by altering the position of the escape hole (e.g., 1, 3, 5, 8, or 11). Escape training was conducted three times per day for 4 d. Each trial was terminated when the mouse escaped into the correct hole (cage) within a maximum of 120 s after the start. Mice were left in the cage for 30 s and then returned to the home cage. The intertrial interval was ≈15 min. After the final escape training on the fifth day, probe tests were performed for a duration of 120 s using the same paradigm as for maze training, except for the absence of a target cage.

Contextual fear conditioning. Fear conditioning was performed with 3-mo-old male mice and consisted of three parts: a conditioning trial (day 1), a context test trial (day 2), and a cued test trial (day 3). Mice were placed in a clear plastic chamber (34 × 26 × 30 cm) equipped with a stainless steel grid floor wired to a shock generator. Mouse behavior was monitored and recorded using a CCD camera attached to the chamber ceiling. White noise (65 dB) was supplied from a loudspeaker as an auditory cue (conditioned stimulus, CS). An unconditioned stimulus (US) (footshock: 0.5 mA, 2 s) was administered at the end of the 30-s CS period. The conditioning trial consisted of a 2-min exploration period, followed by two CS–US pairings separated by 1 min. Context tests were performed 24 h after conditioning trials in the same conditioning chamber for 3 min in the absence of white noise. Furthermore, a cued test was performed in an alternative context with distinct cues; the test chamber varied from the conditioning chamber in brightness (by 0–1 lx), color (white), floor structure (no grid), and shape (triangular). The cued test was conducted 24 h after the contextual test was finished; it consisted of a 2-min exploration period (no CS) to evaluate nonspecific contextual fear, followed by a 2-min CS period (no foot shock) to evaluate acquired cued fear. Rate of freezing response (immobility, excluding respiration and heartbeat) was measured as an index of fear memory. Data were collected and analyzed using Image J FZ2 software (O'Hara).

Forced swim test. Four-month-old male mice were placed into plastic buckets (19 cm in diameter and 23 cm deep) containing 25 °C water. Mobility was recorded for 6 min. The last 5 min were scored for immobility. Immobility duration was scored and analyzed using FST software (O'Hara).

Tail suspension test. Three-month-old male mice were held by the tail using adhesive tape placed ≈1.5 cm from the tip of the tail; the adhesive tape was attached to a wire 30 cm above the floor. Movement was monitored for 5 min using a CCD camera, and immobility duration was scored and analyzed using Image J TS software (O'Hara).

Sucrose preference test. Five-month-old male mice were used to determine preference for sucrose solution, as previously described (7). Mice were placed in their home cages and provided with access to two water bottles, one with water and the other with a 2% sucrose solution. The amount of each liquid consumed over a 48-h period was measured.

Theta Oscillation Recording. A commercial miniature electrode-interface printed circuit board (PCB) with an Omnetics connector (EIB-27 micro; Neuralynx) was used to construct an implantable electrode array. Two square metallic rods (~1 mm across) were soldered to the PCB, which held a cannula (~0.2 mm diameter). Four insulated tungsten microwires (California Fine Wire), which served as recording electrodes, were inserted through the cannula and were connected to the PCB using gold connecting pins (EIB Pins Small; Neuralynx). Electrode tips were cut with fine scissors, and electrode lengths were adjusted so that the tips were ~200–300 μm apart.

Male CAPS2-KO and WT littermates, age 8 and 12 wk, were used in the chronic recording study. The mice were anesthetized with a mixture solution containing 2% ketamine and 0.1% xylazine (10 mL/kg). Two small holes (1-mm diameter) were drilled above the cerebellar cortex, and a screw electrode was placed in each hole to serve as ground and reference for differential recording. A small craniotomy was made on the right side of the skull at 1.7 mm posterior to bregma and 1.0 mm lateral from the midline. The electrode array was inserted through the dura mater; the longest electrode reached a depth of 2.0 mm from the surface. The craniotomy then was covered with a mixture of paraffin and paraffin oil, and the headstage was rigidly cemented with dental acrylic (Super-Bond C&B; Sun Medical). After the dental acrylic had solidified, a protective wall of one-sided vinyl-laminated paper was attached around the headstage. Chronic electrophysiological recordings were performed 5 d after surgery. Once the preamplifier cable was plugged into the headstage, at least 30 min of settling time was allowed before recording. Sleep recordings were performed in home cages. While the animal was sleeping, rapid eye movement (REM) sleep was recognized by the appearance of theta (6–12 Hz) activity, which was preceded by a slow-wave sleep EEG pattern. At the end of REM sleep, theta activity disappeared. Occasionally, the mouse exhibited a brief twitch-like motion, followed by resumed sleep. During some recordings, mouse behavior was videotaped simultaneously. Electrophysiological data were obtained from four electrodes and were digitized simultaneously at 32.556 kHz (bandwidth 0.1 Hz to 9 kHz) using a 16-bit multichannel data acquisition system (Digital Lynx; Neuralynx). For analysis of EEG, acquired data were digitally resampled to 1.25 kHz and analyzed using custom software written with MATLAB (8).

1. Sadakata T, et al. (2007) Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients. *J Clin Invest* 117:931–943.
2. Tamamaki N, et al. (2003) Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J Comp Neurol* 467:60–79.
3. Sadakata T, et al. (2004) The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. *J Neurosci* 24:43–52.
4. Shiraishi Y, Mizutani A, Yuasa S, Mikoshiba K, Furuichi T (2004) Differential expression of Homer family proteins in the developing mouse brain. *J Comp Neurol* 473:582–599.
5. Liu L, et al. (2004) Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* 304:1021–1024.
6. Katoh-Semba R, Takeuchi IK, Semba R, Kato K (1997) Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain. *J Neurochem* 69:34–42.
7. Maguire J, Mody I (2008) GABA(A)R plasticity during pregnancy: Relevance to postpartum depression. *Neuron* 59:207–213.
8. Sakatani S, Seto-Ohshima A, Itohara S, Hirase H (2007) Impact of S100B on local field potential patterns in anesthetized and kainic acid-induced seizure conditions in vivo. *Eur J Neurosci* 25:1144–1154.

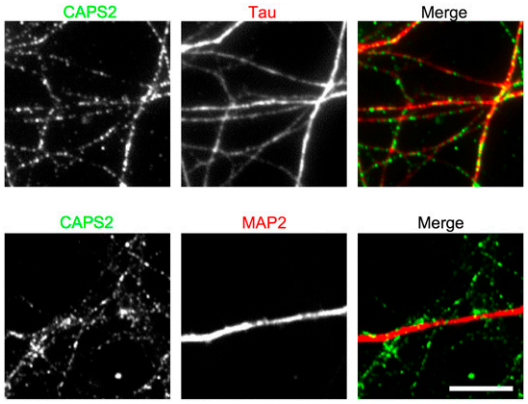


Fig. S1. CAPS2-immunopositive puncta are localized in axons but not in dendrites in cultured hippocampal neurons. CAPS2-immunopositive puncta are localized in Tau-immunopositive axons (*Upper*) but not in MAP2-immunopositive dendrites (*Lower*). (Scale bar, 10 μm.)

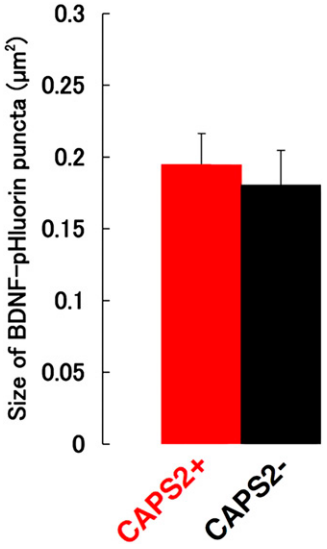


Fig. S2. CAPS2 overexpression does not increase the size of BDNF-pHluorin fluorescent puncta. Quantitation of the average size of BDNF-pHluorin fluorescent puncta in CAPS2-tdTomato overexpressing ($CAPS2^+$) and CAPS2-KO ($CAPS2^-$) neurons (n = 57 in WT mice and 63 in KO mice). Error bars represent SEM.

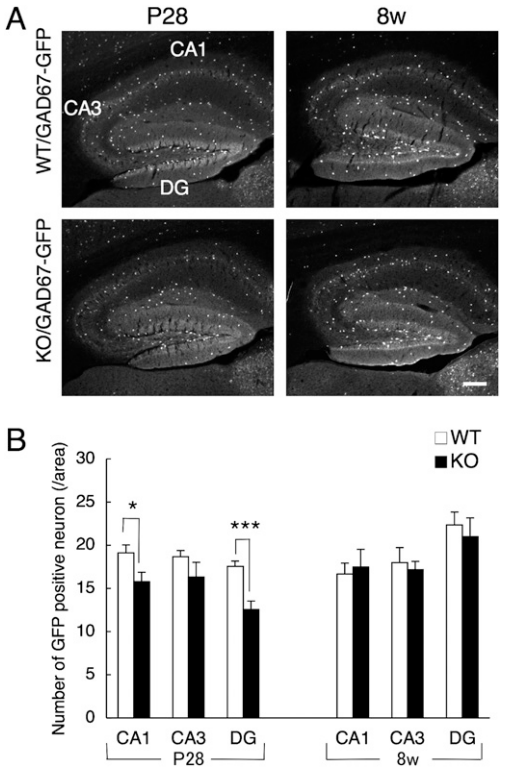


Fig. S3. GABAergic interneuron numbers are reduced at P28 but recover by 8 wk in the CAPS2-KO mouse hippocampus. (*A*) Representative pictures of P28 and 8-wk-old WT and CAPS2-KO mouse hippocampi. GABAergic interneurons were visualized by crossing CAPS2-KO mice with GAD67-GFP–knock-in mice. The dentate gyrus (DG), CA3, and CA1 regions were analyzed. (Scale bar, 100 μm.) (*B*) Number of GFP^+ interneurons in WT and CAPS2-KO mice. The number of GFP neurons is reduced in the CA1 and DG of CAPS2-KO mice at P28, but there is no difference between WT and KO mice at 8 wk of age ($n = 9$ each for WT and KO for P28, and $n = 6$ each for WT and KO for 8 wk, respectively). Error bars represent SEM. $^*P < 0.05$; $^{***}P < 0.001$ using Student's t test.

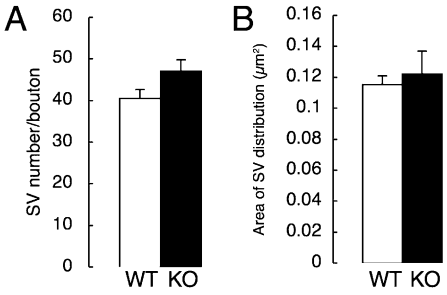


Fig. S4. Number and distribution of synaptic vesicle (SVs) per excitatory terminal is indistinguishable between WT and CAPS2-KO mice. (*A*) Number of SVs per excitatory terminal. (*B*) Area of SV distribution. Error bars represent SEM (n = 146 in 8-wk-old WT mice and 175 in CAPS2-KO mice).

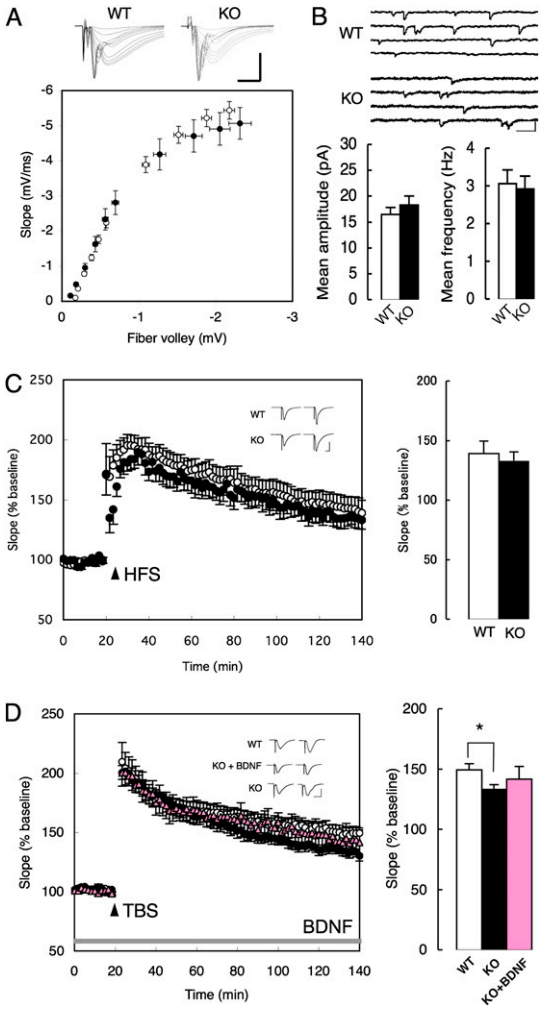


Fig. S5. Basal transmission and HFS-induced CA3-CA1 LTP are unchanged in the CAPS2-KO mouse hippocampus but TBS-induced LTP and the effect of BDNF are impaired. (*A*) Input/output curve in the hippocampus remains unchanged between WT and KO mice. (*Upper*) Representative fEPSP traces of WT and CAPS2-KO mice. (Scale bars: horizontal bar, 10 ms; vertical bar, 2 mV.) (*Lower*) Average fEPSP slope vs. amplitude of fiber volley (n = 13 slices from four WT mice and 14 slices from five KO mice). (*B*) (*Upper*) Representative traces of mEPSCs recorded from neurons in the hippocampal CA1 region in WT and CAPS2-KO slices. (Scale bars: horizontal bar, 100 ms; vertical bar, 10 pA.) (*Lower*) Statistical data of mEPSC amplitude (*Left*) and frequency (*Right*) are shown. mEPSC amplitude and frequency are unchanged in CAPS2-KO mice (n = 9 cells/slices from three WT and three KO mice). Error bars represent SEM. (*C*) (*Left*) Time course of CA3-CA1 LTP induced by HFS. (*Inset*) Representative traces immediately before (*Left*) and 120 min after (*Right*) HFS in each genotype. (Scale bars: horizontal bar, 10 ms; vertical bar, 1 mV.) (*Right*) Results 120 min after HFS (n = 13 slices from five WT mice and 14 slices from six KO mice). Error bars represent SEM. (*D*) (*Left*) Time course of TBS-LTP with 100 ng/mL BDNF application (gray bar) as indicated in Fig. 4*D Left*. (*Inset*) Representative traces immediately before (*Left*) and 120 min after (*Right*) TBS in each genotype. (Scale bars: horizontal bar, 10 ms; vertical bar, 1 mV.) (*Right*) Results 120 min after TBS (for the BDNF experiment, n = 10 CAPS2-KO slices from five mice; WT and CAPS2-KO data are replicated from Fig. 4*D*). Error bars represent SEM. *P < 0.05 using one-way ANOVA and post hoc Dunnett's *t* test (n = 10 slices in each group).

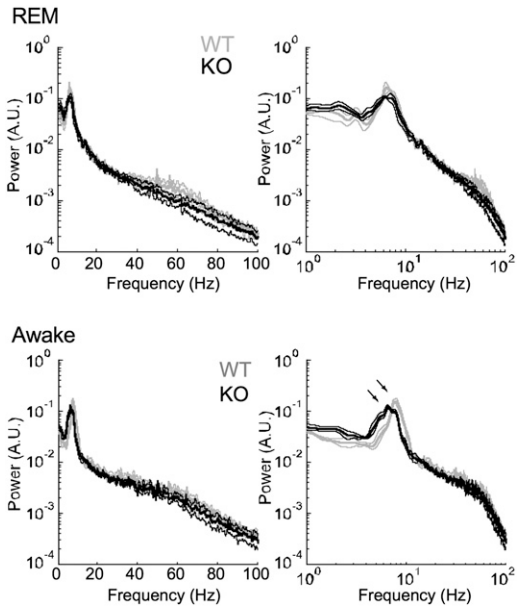


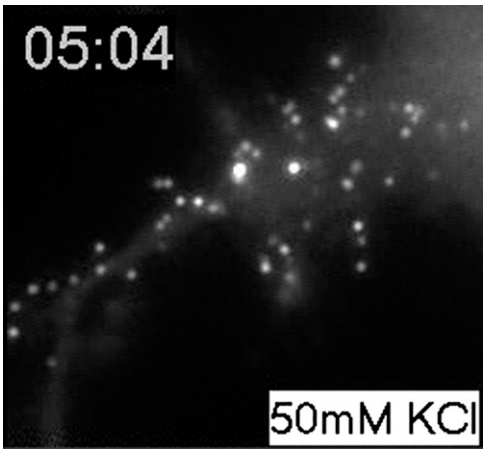
Fig. S6. Reduction of the frequency of hippocampal CA1 theta oscillations in awake CAPS2-KO mice. Theta oscillations were recorded from the hippocampal CA1 region from unanesthetized mice during REM sleep (*Upper Left*) and from awake mobile mice (*Lower Left*). Note that the peak frequency of theta oscillations is shifted to the left in KO mice (black) compared with WT mice (gray), as indicated by arrows in the logarithmic frequency scale plots (*Upper* and *Lower Right*). A.U. arbitrary unit.

Table S1. CAPS2-KO mice exhibit increased anxiety-like behavior

Behavioral tests	WT	KO	*P*
Anxiety			
Open field (% time in center)	17.7 ± 0.9	16.1 ± 2.1	Ns
Open field (total distance: AU)	5200 ± 325	4892 ± 504	Ns
Open field —novel object (% time in center)	17.2 ± 1.4	3.8 ± 2.9	**
Open field— novel object (total distance: AU)	6840 ± 482	4518 ± 1024	*
Elevated plus maze (% time in open arm)	20.9 ± 4.9	3.2 ± 1.7	*
Novelty suppressed feeding (latency: s)	114 ± 29	217 ± 36	*
Eight-arm radial maze (number of total arm choices)	10.8 ± 0.3	7.8 ± 0.2	***
Y-maze (number of total arm choices)	20.2 ± 1.3	15.7 ± 1.1	*
Zero maze (% time in open arm)	45.4 ± 1.1	45.5 ± 1.2	Ns
Learning and memory			
Eight-arm radial maze (reference memory error: %)	42.6 ± 1.2	47.6 ± 1.8	*
Eight-arm radial maze (working memory error: %)	36.0 ± 1.5	34.1 ± 0.7	Ns
Morris water maze (% in quadrant)	22.3 ± 4.4	22.1 ± 7.1	Ns
Y-maze (% alteration)	63.9 ± 3.4	62.8 ± 3.9	Ns
Barnes maze (% target)	45.1 ± 4.2	55.8 ± 4.9	Ns
Contextual fear (% freezing)	16.5 ± 2.9	20.1 ± 3.5	Ns
Depression			
Forced swim (time immobile: s)	118.3 ± 9.3	145.7 ± 8.4	*
Tail suspension (time immobile: s)	75.0 ± 6.5	70.4 ± 6.0	Ns
Sucrose preference (% sucrose)	85.0 ± 0.8	83.5 ± 1.8	Ns

AU, arbitrary unit; Ns, not significant.

*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ using the Student's *t* test.



Movie S1. Time-lapse live-cell imaging of BDNF-pHluorin secretion from CAPS2-KO mouse hippocampal neurons transfected with CAPS2-tdTomato, related to Figs. 1 and 2. This movie is an example of pHluorin fluorescence dynamics on an axon from 2 min before to 8 min after 50 mM KCl stimulation. KCl stimulation was applied at 2 min, as shown in the movie.

Movie S1