Supporting Information

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SI Methods

RNA Extraction and Real-Time RT-PCR. Total RNA was isolated and extracted from the frontal cortex by using TRIzol (Invitrogen) according to the manufacturer's instructions. RNA was subsequently purified by using an RNeasy minicolumn (Qiagen) with on-column DNase removal. RNA was quantified by using the NanoDrop spectrophotometer (Agilent Technologies), and 1 μg of total RNA was reverse transcribed into single-stranded cDNA by using Superscript III (Invitrogen) following the manufacturer's protocol. Quantitative RT-PCR was performed by using the IQ5 iCycler (Bio-Rad) using iQ SYBR GreenER supermix (Invitrogen) with 1 μL of synthesized cDNA. PCR efficiencies of primers were examined by standard curve of serial-diluted cDNA and melting-curve functionality. Individual mRNA levels were normalized for each well to the actin mRNA levels. I: (F) AGTCTCCAGGACAGCAAAGC, (R) ACACCTGGGTAGGCCAAGTT, II-c: (F) ATCACCAGGATCTAGCCACC, (R) GCCTTCATGCAACCGAAGTA, III: (F) CTGCTTCTCAAGGGAAGGG, (R) GCCTTCATGCAACCGAAGTA, IV: (F) AGAGCAGCTGCCTTGATGTT, (R) GGTAGGCCAAGTTGCCTTGT, IXA: (F) CTGTCCCCGAGAAAGAAAGTT, (R) GCCTTCATGCAACCGAAGTA, VI: (F) GACCAGAAGCGTGACAACAA, (R) GCCTTCATGCAACCGAAGTA, coding: (F) TGGCTG ACACTTTTGAGCAC, (R) AAGTGTACAAGTCCGCGTCC.

Histology and Immunohistochemistry. WT and BDNF-KIV mice with or without KA treatment were anesthetized (50 mg/kg pentobarbital) and transcardially perfused (4% paraformaldehyde, 2% picric acid, and 0.1 M phosphate buffer, pH 7.2). The brains were postfixed for 2 h, cryoprotected in 30% sucrose solution, and cut with a frozen microtome in coronal 40-μm sections containing the mPFC (1.9 through 2.1 mm from bregma). Nissl staining was performed according to the manufacturer's instruction (FD Neuro Technologies). For immunohistochemistry, the free-floating sections were washed with TBS-T wash buffer [10 mM Tris·HCl, pH 7.4; 150 mM NaCl; and 0.025% (vol/vol) Tween 20] and incubated with 0.3% H_2O_2 for 5 min in methanol and then TBS blocking buffer (10% normal goat serum, 1% BSA, and 0.3% Triton X-100) for 2 h. BDNF signal was detected by using a Vectastain ABC kit (Vector Laboratories) as follows: anti-BDNF antibody (1:200, kindly provided by Xinfu Zhou (Flinders University, Australia), 4 °C overnight); biotinylated anti-rabbit IgG (1:200, for 2 h at room temperature); ABC complex (1 h, room temperature); and diaminobenzidine. Immunofluorescence staining was performed for other proteins by using following primary antibodies: MAP2 (1:500; m9942; Sigma), GFAP (1:500; Chemicon), GluR1 (1:500; AB1504; Chemicon), synaptophysin I (1:500; ab14692; Abcam), parvalbumin (1:500; AB9312; Chemicon), calbindin (1:1,000; AB9481; Abcam), calretinin (1:1,000; AB5404; Chemicon), and GAD67 (1:500; MAB5406; Chemicon). Immunoreactivities were detected by Cy3-conjugated anti-mouse or anti-rabbit antibody, or FITC-conjugated anti-mouse or anti-rabbit antibody (1:200; Jackson ImmunoResearch). The density per area of staining was measured by using the National Institutes of Health Image-J program. Immunofluorescent images were analyzed by using a Nikon ECLIPSE 800 Fluorescent Microscope and StereoInvestigator Stereology 8.0 Software (Microbright Fields). In brief, the observed brain regions were traced in 3–7 (3 sections PFC, 4 sections amygdale (AM), 7 sections hippocampus (HP) systematically sampled sections (480 μm apart), and positive cell bodies were manually counted. Cell densities were calculated as cells per cubic millimeter.

Electrophysiology. Coronal prefrontal cortical slices (300 μM) were prepared from 6- to 10-week-old BDNF-KIV and age-matched WT littermates in accordance with National Institutes of Health guidelines. Brain slices were cut by using a VF-200 tissue slicer (Precisionary Instruments) in a buffer that consisted of the following (in mM): sucrose 200; $NaHCO_3$ 26; D-glucose 10; H_2PO_4 1.25; KCl 3; $MgCl_2$ 3; and $CaCl_2$ 1. Slices were then transferred to a holding chamber for approximately 1 h to recover in modified artificial cerebrospinal fluid (ACSF) that contained the following (in mM): NaCl 125; $NaHCO_3$ 26; D-glucose 10; H_2PO_4 1.25; KCl 3; $MgCl_2$ 1.2; and $CaCl_2$ 2.4, bubbled with carbogen gas (95% O_2/5% CO_2). This composition of ACSF was used for both storage during recovery and perfusion during all experiments.

Whole-cell recordings were performed at 31 °C to 34 °C and were made by using an Axopatch 200B amplifier (Axon Instruments), sampled at 10 kHz, digitized by a DigiData 1322A, and later analyzed offline by either ClampFit (Axon software) or MiniAnalysis (Synaptosoft software). The magnitude and timing of extracellular stimulation and whole-cell current injection were controlled with a Master-8 stimulator (AMPI). Monopolar stimulation was administered via a silver wire housed in an ACSF-filled glass electrode (1–2 MÙ) placed in layer II/III of the PFC slice. Whole-cell patch pipettes with resistances ranging between 3 and 7 MÙ were pulled by using standard borosilicate capillaries by an upright PB-7 electrode puller (Narishige). Three different intracellular solutions were used for the whole-cell experiments. For the measurement of sEPSCs and evoked *N*-methyl-D-aspartic acid receptor-mediated synaptic currents, the solution contains (in mM): cesium gluconate 125; CsCl 20; NaCl 10; MgATP 2; Na_2GTP 0.3; EGTA 0.2; Hepes 10; and QX-314 2.5. For sIPSC recordings, an elevated chloride intracellular solution was used (in mM): $CsCl_2$ 134; NaCl 4; MgATP 2; Na_2GTP 0.5; Na_2phosphocreatine 5; EGTA 1; and Hepes 10. To record EPSPs during spike-timing-dependent potentiation experiments, the following solution was used (in mM): potassium gluconate 130; KCl 10; MgATP 2; Na_2GTP 0.2; Na_2phosphocreatine 5; EGTA 0.5; Hepes 10. For all intracellular solutions, the pH and osmolarity were adjusted to 7.2–7.3 and 290–300 mOsm, respectively.

For spike-timing-dependent experiments, monosynaptic EPSPs were evoked every 15 s by using an extracellular stimulation electrode positioned in layer II/III. The initial slope of the evoked EPSP was used as an index of synaptic strength. All evoked EPSP responses were normalized to the mean baseline control (at least 20 consecutive sweeps). During the conditioning period, presynaptic–postsynaptic stimulation (Δ5 ms) was repeated 50 times with a 7-s interpairing interval. Throughout all experiments, cell input resistance was monitored by applying a hyperpolarizing pulse (10 pA, 100 ms). Any experiments with changes greater than 20% were excluded. For all electrophysiology experiments, data are displayed as mean ± SEM, with $P < 0.05$ indicating statistical significance.

All pharmacological drugs were bath applied through gravity perfusion. Stock solutions were stored at −20 °C and were diluted to the proper concentration before each experiment. The NMDA receptor antagonist D-(−)-2-amino-5-phosphonopentanoic acid (D-APV, 50 μM), and AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM) were ob-

tained from Sigma–RBI, whereas the $GABA_A$ receptor antagonist bicuculline methiodide (10 μM) was obtained from Tocris Cookson.

KA Treatment. KA (Sigma) was administered i.p. at 30 mg/kg in 0.9% NaCl. The behavior of mice was monitored continuously up to 3–6 h. Within the first hour after injection, all animals developed seizures evolving into recurrent generalized convulsions. These mice were killed at 3-h or 6-h time points, and their brains were used for RT-PCR, Western blotting, or immunohistochemistry, as described below.

Cell Culture and Stimulation. Cell culture experiments were performed by using cortical neurons from embryonic day 16/17 (E16/E17) mice. Cortex was dissociated in Ca^2-free and Mg^2-free HBSS containing 0.125% trypsin for 15 min, triturated in DMEM/10% FBS, and plated at 1×10^6 cells per well in 12-well plates. Cells were grown at 37 °C, 5% CO_2 and 95% humidity, first in 10% FBS/DMEM, and 1 day later were switched to serum-free medium Neurobasal plus B27 (Life Technologies). Cultures were grown in serum-free medium for 6 days before being used for experiments, and the medium was changed every 3 days. Fresh medium was applied 24 h before each experiment. Tetrodotoxin (1 μM) was added the night before stimulation to reduce endogenous synaptic activity. Thirty minutes before stimulation, 100 μM APV was added to block NMDA receptor activity. KCl (25 mM) was used to induce membrane depolarization.

BDNF ELISA. Age-matched WT and BDNF-KIV mice injected with or without KA were killed by deep anesthesia. FC from each mouse was homogenized by sonication in homogenization buffer (20 mM Tris, pH 7.5; 150 mM NaCl; 1% Nonidet P-40; 1 mM EDTA; 10 μg/mL aprotinin; 1 μg/mL leupeptin; 1 μg/mL pepstatin; and 1 mM PMSF), followed by centrifugation for 5 min at 12,000 $\times$ *g* at 4 °C. The supernatants were collected, and their protein concentrations were measured by Bio-Rad protein assay (Bio-Rad Laboratories). BDNF protein levels were determined by ELISA (BDNF immunoassay system; Promega) as described by the manufacturer. The BDNF signal was normalized to the total soluble protein in each sample, and the data were expressed as the mean ± SE.

Western Blotting. The samples, prepared in the same way as that in ELISA, were mixed with 4× NuPAGE LDS sample buffer (Novex) and 5% 2-mercaptoethanol, heated (80 °C, 10 min), and electrophoresed on a 10% (wt/vol) NuPAGE Bis-Tris gel in Mes buffer. Immediately after electrophoresis, proteins in the gel were electrotransferred to a 0.45-μm poly(vinylidene difluoride) membrane (Millipore). The membrane was blocked with 5% (wt/vol) skim milk in TBS-T (TBS with 0.05% Tween-20) for 1 h at room temperature, and was incubated at 4 °C overnight with anti-BDNF rabbit polyclonal antibody (1:1,000; Santa Cruz Biotechnology) or anti-GFP rabbit polyclonal antibody (1:1,000; ab290; Abcam). The membrane was then incubated with anti-rabbit IgG antibody conjugated with horseradish peroxidase (1:1,000; Sigma; 1 h, room temperature) and developed with the chemiluminescence ECL PLUS Western Blotting system (Amersham Biosciences).

Statistical Analysis. Comparison between 2 genotypes was made using unpaired Student's *t* test, as indicated in the text. Repeated measures of variance analysis (ANOVA) were used for multiple variables and, when warranted, post hoc multiple comparisons were carried out. Data were presented as means ± SEM. Statistical significance was set at $P \leq 0.05$ as * and $P \leq 0.01$ as **.

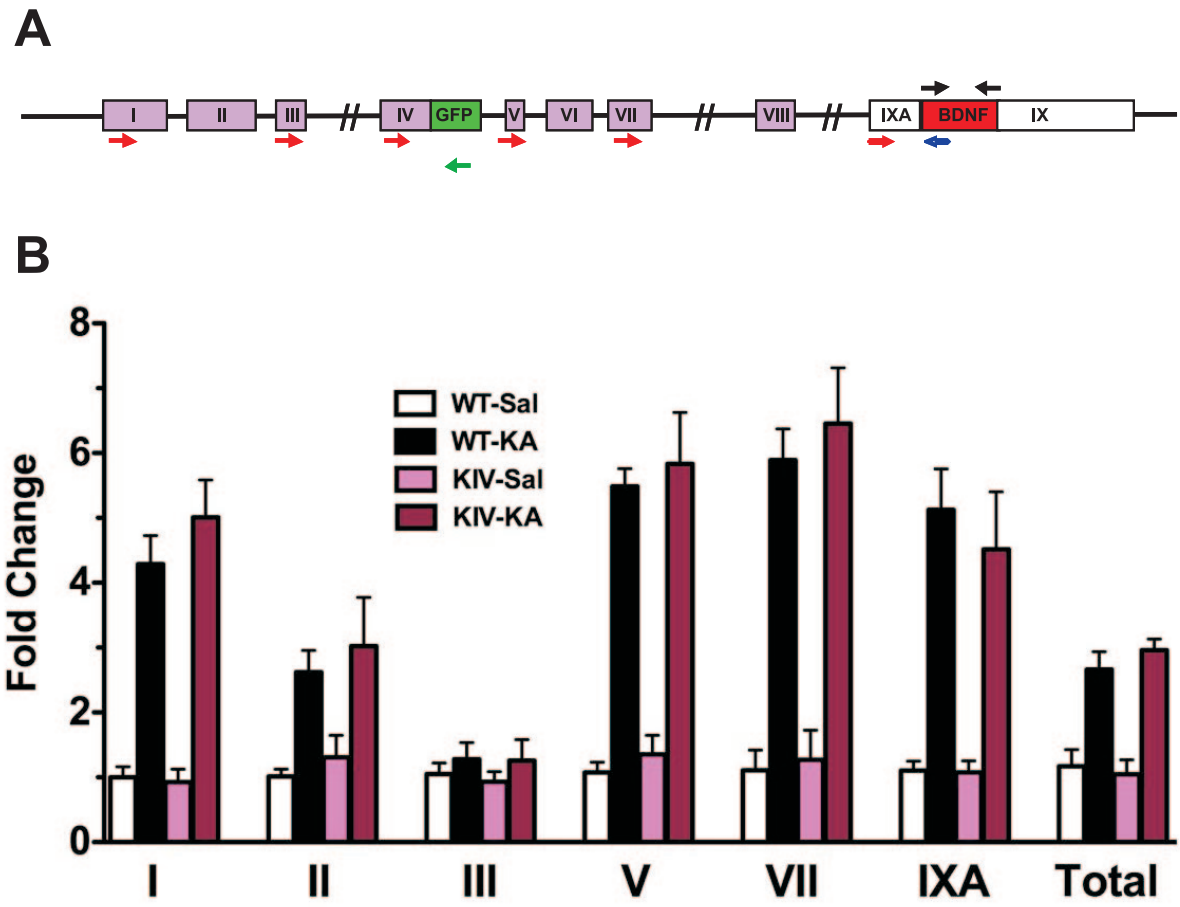

Fig. S1. KA-induced changes in different BDNF transcripts. Cortices were dissected from mice 3 h after administration of Saline (Sal) or KA. Quantitative PCR analysis was performed by using primer pairs specific for exons I, II, III, IV, V, VII, and IXA (red: forward; blue: reverse). For GFP, a reverse primer (green) in the GFP coding region was used. For total BDNF transcripts, a set of primers (black) corresponding to the BDNF coding region was used. Data on exon IV and GFP are shown in Fig. 2*A*. (*A*) Schematic diagram of the genomic structure of BDNF gene, showing all BDNF exons and locations of RT-PCR primers. (*B*) Effect of KA BDNF transcription through various promoters in WT and KIV cortex.

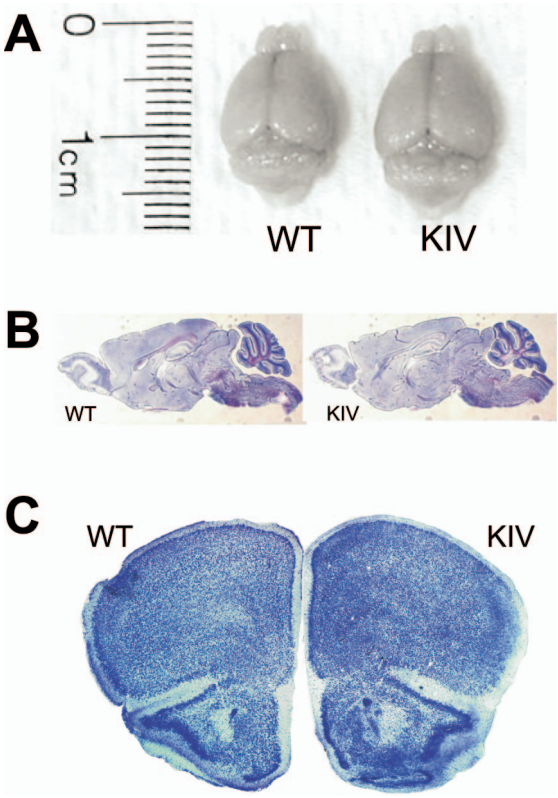

Fig. S2. Normal brain anatomy, dendritic morphology, and synaptic architecture. (*A*) Normal size and gross morphology of adult brain (P60) from WT and mutant (KIV). (*B*) Sagittal view of Nissl-stained sections. No significant difference was found between genotypes. (*C*) Coronal view of Nissl-stained sections showing normal structure and cell density of mPFC at bregma 2.1 mm in WT and KIV.

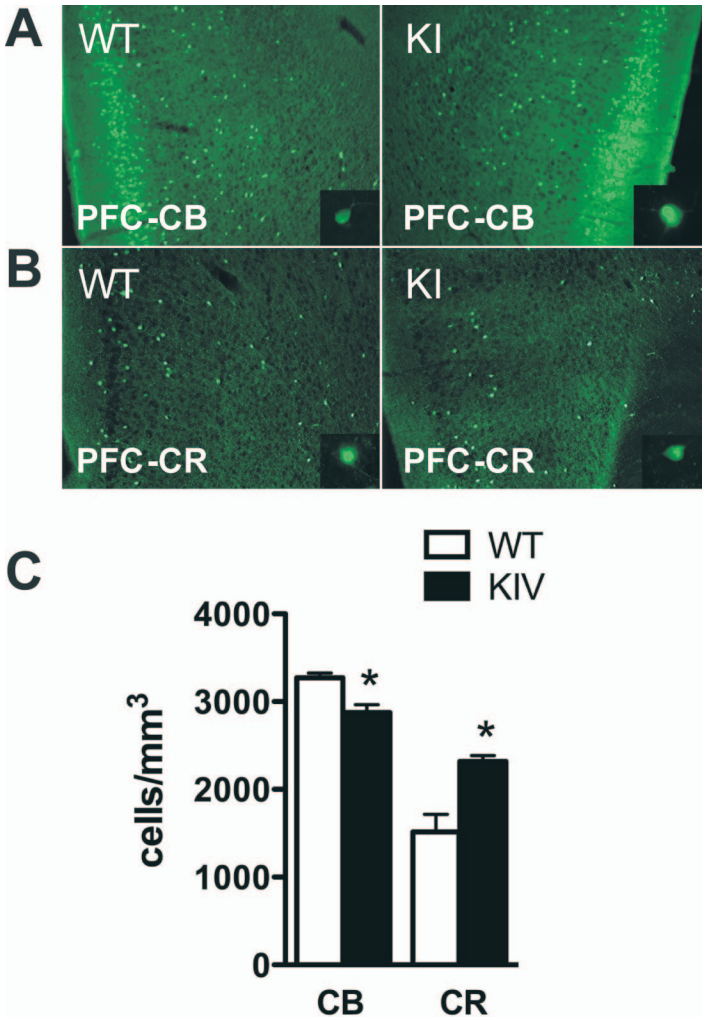

Fig. S3. Immunohistochemical characterization of CB- and CR-GABAergic interneurons. (*A*) Representative photomicrograph of calbindin (CB)-positive interneurons in the mPFC. In both *A* and *B*, examples of immunostained neurons are shown in the lower right corner of each image (Magnification: 20×). (*B*) Representative photomicrograph of CR-positive interneurons in the mPFC (Magnification: 20×). (*C*) Quantification of cell densities of CB and CR interneurons in the mPFC of WT and KIV mice. $n = 3$ pairs of mice.

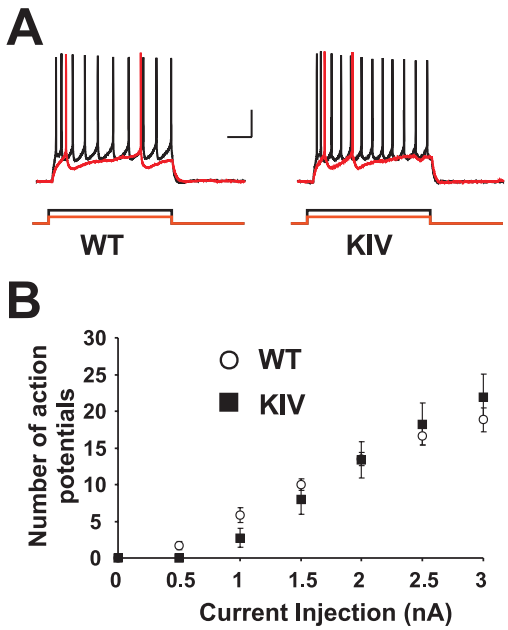

Fig. S4. Normal excitability of layer V pyramidal neurons in mPFC slices from KIV. (*A*) Representative current clamp traces evoked by depolarizing current injection (bottom) at either 0.5 nA (red) or 1.5 nA (black) in mPFC slices. (Scale bars: 20 mV, 200 ms.) (*B*) Normal excitability of layer V pyramidal neurons in mPFC slices. The number of action potentials increases with the increasing current injections.

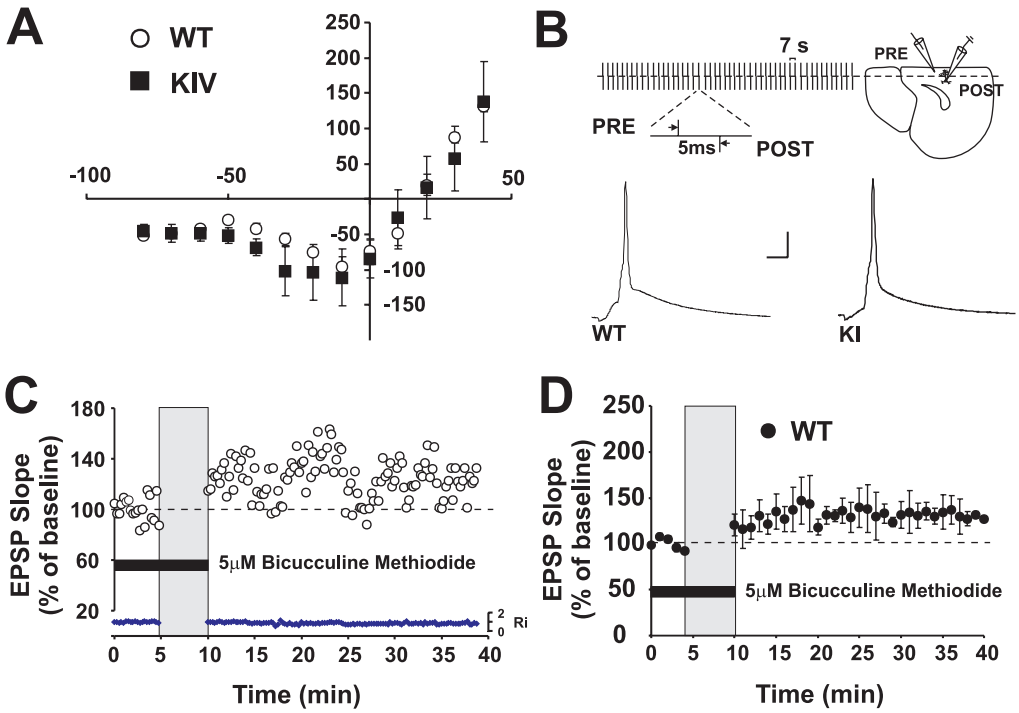

Fig. S5. Regulation of STDP by GABAergic inhibition. (*A*) Normal NMDAR-mediated synaptic currents in KIV neurons. Pharmacologically isolated NMDA currents in pyramidal neurons in layer V of mPFC were evoked by stimulating layer II/III at different holding potentials. The *I–V* curve in BDNF-KIV neurons is similar to that in WT neurons. (*B*) Normal action potential profile in BDNF-KIV during spike-timing pairing protocol. Representative current-clamp traces of action potentials during pairing of presynaptic stimulation with postsynaptic current injection. (*Inset*) Schematic and timing of the STDP induction protocol. (Scale bars: 20 mV, 5 ms.) (*C*) An example of robust STDP when GABAergic transmission is blocked. The $GABA_A$ antagonist bicuculline (5 μM) was perfused to the WT mPFC slices before and during the pairing stimulation. (*D*) Summary plot showing the persistent increase in the EPSP slopes after repetitive correlated presynaptic and postsynaptic stimulation in the presence of the GABA antagonist.

Table S1. Similar electrophysiological properties of WT and BDNF-KIV

	Resting membrane properties		Action potential				sIPSC		sEPSC	
Mice	V_m, mV	R_i, MΩ	$V_{threshold}$, mV	Amplitude, mV	Width, ms	Latency to first spike, ms	Rise, ms	Decay, ms	Rise, ms	Decay, ms
WT	−65.3 ± 0.5	256 ± 25	−37.0 ± 2	74 ± 3	0.73 ± 0.05	80 ± 12	2.2 ± 0.1	8.5 ± 1.0	2.3 ± 0.1	11.1 ± 0.9
BDNF-KIV	−66.0 ± 0.5	267 ± 27	−38 ± 1	73 ± 2	0.68 ± 0.05	61 ± 11	2.1 ± 0.1	8.2 ± 0.6	2.4 ± 0.1	11.3 ± 1.0