Supporting Information

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SI Methods

Generation of mice lacking protein interacting with C kinase 1 (PICK1) protein is described in fig. 5 of ref. 1.

All experiments were conducted in accordance with the policies of the Animal Care and Use Committee of The Johns Hopkins University.

External Solutions. The external solutions used were (in mM):

Artificial cerebrospinal fluid 1 (ACSF1): 124 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 $NaH2PO_4{\cdot}H_2O$, 11 glucose, $xCaCl_2$, $xMgCl_2$.
Artificial cerebrospinal fluid 2 (ACSF2): 125 NaCl, 3.25 KCl, 25 $NaHCO_3$, 1.25 $NaH2PO_4{\cdot}H_2O$, 11 glucose, $xCaCl_2$, $xMgCl_2$.
Artificial cerebrospinal fluid 3 (ACSF3): 119 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1 $NaH2PO_4{\cdot}H_2O$, 11 glucose, $xCaCl_2$, $xMgCl_2$.
Dissection buffer 1 (DB1): 2.6 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 211 sucrose, 10 glucose, 0.5 $CaCl_2$, 5 $MaCl_2$.
Dissection buffer 2 (DB2): 119 choline Cl, 2.5 KCl, 26 $NaHCO_3$, 1 $NaH_2PO_4{\cdot}H_2O$, 30 glucose, 1 $CaCl_2$, 7 $MgCl_2$ 1 kynurenic acid, 1.3 Na ascorbate, 3 Na pyruvate.

Slice Preparation. Hippocampal slices were prepared from PICK1-KO and WT littermate mice ranging in age from 2 wk to 3 mo. Mice were anesthetized with an i.p. injection of a mixture of tribromoethanol at 0.25 mg/g body weight and 2-methyl-2-butanol at 0.16 μL/g body weight in water before decapitation or cardiac perfusion. Transverse hippocampal slices were prepared with a vibratome following dissection of the hippocampus in ice-cold oxygenated (95% O_2/5% CO_2) ACSF or DB (see below).

In Figs. 1*B*, 2*C*, 3 *A* and *C*, and 4 and Figs. S1–S3, 300-μm slices were prepared. Dissection/slicing occurred in ACSF1 (0.5 mM $CaCl_2$ and 5 mM $MgCl_2$). Slices were recovered at RT for at least 1 h on the surface of cell-culture inserts in contact with ACSF1 (2 mM $CaCl_2$ and 2 mM $MgCl_2$) and were continuously supplied with humidified oxygen.

In Figs. 1 *A* and *C*, 2*B*, and 3*B*, 400-μm slices were prepared. Dissection/slicing occurred in ACSF2 (Figs. 2*B*, 3*B*, and 5*B*) with 2.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, or in DB1 (Fig. 1 *A* and *C* and 4× theta in adult) with 0.5 mM $CaCl_2$, and 5 mM $MaCl_2$. Slices were recovered in ACSF2 with 2.5 mM $CaCl_2$, and 1.5 mM $MgCl_2$ at RT (Figs. 2*B* and 3*B*) or 30 °C (Fig. 1 *A* and *C* and 4× theta in adult mice) for at least 1.5 h before recording.

In Fig. 2*A*, intracardiac perfusion with ice-cold DB2 preceded decapitation and preparation of 300-μm slices in DB2. Slices were recovered in ACSF3 with 2.5 mM $CaCl_2$, and 1.3 mM $MgCl_2$ supplemented with 1 mM kynurenic acid at 35 °C for 45 min. Slices then were transferred to ACSF3 without kynurenic acid and maintained at RT for at least 45 min.

Before recording, a cut was made between cornu ammonis 3 (CA3) and cornu ammonis 1 (CA1) to minimize recurrent activity.

Intracellular Recordings. Hippocampal CA1 pyramidal neurons were targeted for whole-cell patch-clamp recording using differential interference contrast imaging on a fixed-stage upright microscope under 40× magnification (Axioscope; Carl Zeiss, or E600FN; Nikon). Electrical signals were recorded using an Axopatch 1D or Multiclamp 700B amplifier (Axon Instruments). Patch pipettes (6–10 MΩ) were pulled from borosilicate glass. Access resistance was monitored every sweep and measured as the peak current of a 2 mV/30 ms or 5 mV/10 ms hyperpolarizing voltage step. Cells in which the access resistance varied by more than 25% were discarded.

Internal Solutions. In Figs. 1*B*, 3*A*, and 4*B* and Figs. S1–S3, the internal solution contained 130 mM Cs-methanesulfonate, 20 mM $CsCl_2$, 10 mM Hepes, 2 mM MgATP, 0.3 mM Na GTP, 0.2 mM EGTA, pH 7.3, at 300 mOsm. In Figs. S1 *D* and *E* and S3*D* [current–voltage (I–V) curves], 100 μM spermine and 5 μM QX-314 were added.

In Fig. 2*A*, the internal solution contained 115 mM Cs-methanesulfonate, 2.8 mM NaCl, 20 mM Hepes, 4 mM MgATP, 0.5 mM Na GTP, 0.4 mM EGTA, 10 mM Na phosphocreatine, 5 mM tetraethylammonium chloride, pH 7.2, at 290 mOsm.

Current–voltage analysis. Whole-cell evoked current–voltage relationship was measured by averaging 10–20 traces at each holding potential. Recordings were made in ACSF1 with 100 μM D,L-2-amino-5-phosphonocaleric acid (AP5) and 100 μM picrotoxin at 35 °C.

Extrasynaptic AMPAR analysis. Outside-out soma patches were made after whole-cell patch configuration was established by slowly pulling the patch electrode. The AMPA-containing bathing solution included cyclothiazide (100 μM) to block desensitization of AMPAR currents. The voltage ramp was −70 mV to +60 mV at a rate of 50 mV/s, resulting in total ramp duration of 2,600 ms. Recording temperature was 35 °C.

Miniature excitatory postsynaptic currents measurement. Recordings were made in ACSF1 with 2.5 mM $CaCl_2$ and 1.3 mM $MgCl_2$ supplemented with 1 μM tetrodotoxin, 100 μM AP5, 100 μM picrotoxin, and 10 μM bicuculline. Cells were held at −68 mV, and miniature excitatory postsynaptic current (mEPSC) measurements were collected for ∼20 min. The Mini Analysis Program (v 6.0 Synaptosoft, Inc.) was used to analyze mEPSCs with the following parameters: amplitude threshold, 3.5 pA; period to average a baseline, 2 ms; period to search a local maximum, 10–20 ms; time before a peak for baseline, ∼4–6 ms; period to search decay time, 30 ms; number of points to average peak, five; area threshold, 6 coulombs.

Long-term potentiation. In Fig. 3*A* and Fig. S2, long-term potentiation (LTP) was induced by applying 200 pulses (2 Hz) at 0 mV depolarization. Recordings were made in ACSF1 with 2.5 mM $CaCl_2$ and 1.3 mM $MgCl_2$ supplemented with 100 μM picrotoxin at 35 °C. Responses were evoked at 0.1 Hz. Data are presented as responses averaged at 2-min intervals and then normalized to the average of baseline recording. In Fig. 2*A*, LTP was induced by applying 120 pulses (0.66 Hz) at 0 mV depolarization. Recordings were made in ACSF3 with 4 mM $CaCl_2$ and 4 mM $MgCl_2$ supplemented with 20 μM picrotoxin at RT. Responses were evoked at 0.1 Hz. Data are presented as responses averaged at 1-min intervals and then normalized to the average of baseline recording.

Long-term depression. Long-term depression (LTD) was induced by applying 200–300 pulses (0.5–1 Hz) at −40 mV depolarization. Recordings were made in ACSF1 with 2.5 mM $CaCl_2$ and 1.3 mM $MgCl_2$ supplemented with 100 μM picrotoxin at 35 °C. To monitor slice stability, the field excitatory postsynaptic potential (fEPSP) amplitude was recorded simultaneously with a Dagan EX1 differential amplifier. Only slices that had no reduction of the fEPSP were chosen for analysis. Responses were evoked at 0.1 Hz. Data are presented as responses averaged at 2-min intervals and then normalized to the average of baseline recording.

Extracellular Recordings. fEPSPs were evoked with a 125-μm metal concentric bipolar electrode (FHC) placed in the middle of the stratum radiatum of CA1, and a 1–2 MΩ glass recording electrode filled with ACSF was positioned ∼200–400 μm away. In-

put–output curves were obtained for each slice, and responses were set to ~40% of maximum for LTP experiments and ~55% of maximum for LTD experiments. Recording conditions for Figs. 2*C*, 3*C*, and 4*A* were ACSF1 with 2.5 mM $CaCl_2$ and 1.3 mM $MgCl_2$ at 35 °C. Recording conditions for Figs. 2*B*, 3*B*, and 1 × 100 Hz LTP in adult mice were ACSF2 with 2.5 mM $CaCl_2$, and 1.5 mM $MgCl_2$ at RT. Recording conditions for Fig. 1 *A* and *C* and 4× theta in adult mice were ACSF2 with 2.5 mM $CaCl_2$, and 1.5 mM $MgCl_2$ at 30 °C.

The following stimulations were used:

High-frequency stimulation (HFS) LTP: 2 × 100 Hz, 20-s intertrain interval.

Theta-burst LTP: One or four trains of 10 bursts at 5 Hz (~theta frequency), each burst consisting of four responses at 100 Hz, 10-s intertrain interval.

Low-frequency stimulation (LFS) LTD: 900 single pulses at 1 Hz.

Paired-pulse LFS LTD: 900 paired pulses (50-ms interpulse interval) at 1 Hz in the presence of 100 μM AP5.

Behavior. Mice were handled for 3 min on each of 5 consecutive days before beginning experiments. The step-through inhibitory avoidance (IA) apparatus consisted of a rectangular chamber 35.56 cm wide × 17.78 cm deep × 30.40 cm high (Coulbourn Instruments) divided into two separate compartments. The "light" compartment was made of clear Plexiglas and illuminated by a small, overhead light, and the "dark" compartment was made of black Plexiglas and was not illuminated. The compartments were connected by a guillotine-style door, and both compartments contained a metal grid floor connected to an electric generator source that delivered a scrambled 0.3-mA, 2-s shock.

For habituation (day 1), a mouse was placed in the light side of the chamber facing the wall opposite the guillotine door. After 30 s the door was opened. The mouse was allowed to explore until it crossed to the dark side. The door closed immediately after the mouse crossed to the dark side and the mouse was returned promptly to the home cage after entering the dark side of the chamber.

For training (day 2), the mouse again was placed in the light side of the chamber facing the wall opposite the guillotine door. The door was opened after 30 s, and latency to cross to the dark side following door opening was recorded. The guillotine door closed immediately after the mouse entered the dark side, and 3 s later the mouse received a 2-s, scrambled, 0.3-mA foot shock. The mouse remained in the dark chamber for 30 s following foot shock after which it was returned gently to the home cage.

For testing (day 3), 24 h after training the mouse was reintroduced to the light side facing the wall opposite the door. The door opened 30 s after the mouse was placed in the chamber, and the latency to step through to the dark side was recorded as a measure of memory retention (compared with step through latency on training day). The maximum latency was set at 7 min.

Data Analysis and Statistics. All plasticity experiments are presented as responses normalized to the average of baseline values. Approximate 5-min averages from the end of each experiment were used to calculate the magnitude of plasticity for statistical tests. All error bars represent SEM. In Fig. 5 and Figs. S1*B* and S3*B*, statistical significance was determined with a one-way ANOVA and Tukey post hoc analysis. For all other figures, a two-tailed unpaired Student's *t* test was used at $P < 0.05$ to determine statistical significance.

Data acquisition and analysis except mEPSCs were done using custom software written in AXOBASIC 3.1 or pCLAMP/Clampfit (Axon Instruments). Data were compiled in Microsoft Excel and plotted in Excel or Microcal Origin.

1. Gardner SM, et al. (2005) Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. *Neuron* 45:903–915.

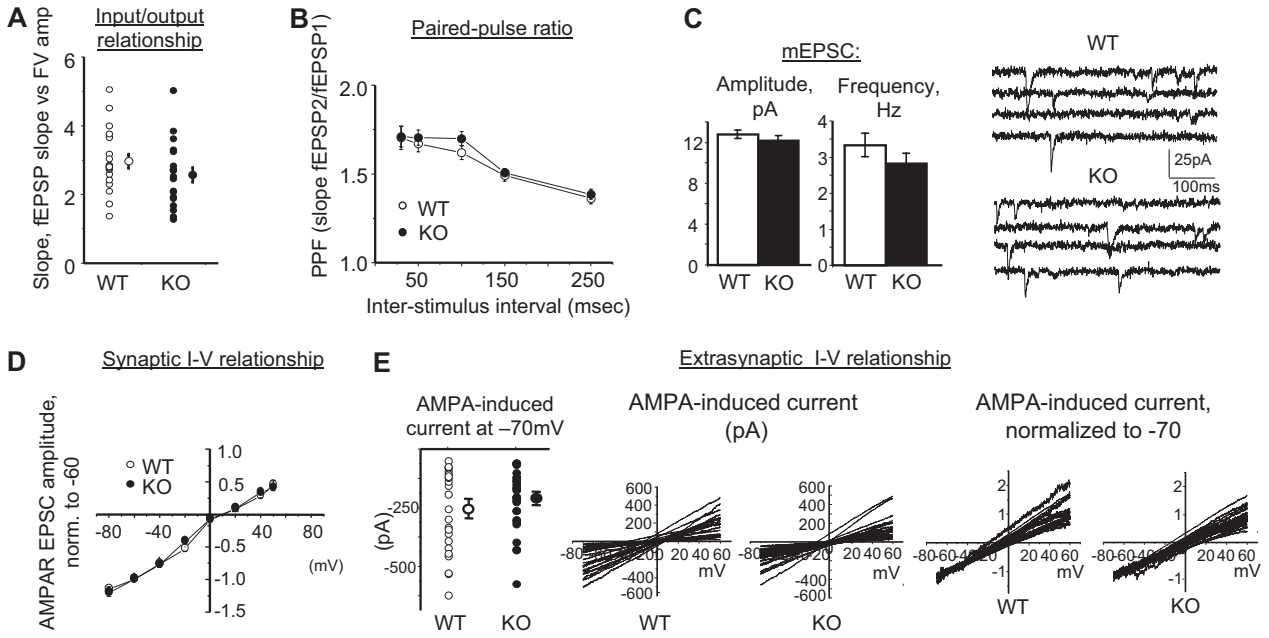

Fig. S1. Basal transmission in hippocampal pyramidal neurons is unaffected in adult (2- to 3-mo-old) PICK1-KO mice. (*A*) The slope of the input–output curve is not altered in adult Pick1-KO mice. WT (n = 19): 2.97 ± 0.21 ms^{-1}; KO (n = 19): 2.57 ± 0.22 ms^{-1}; $P > 0.05$. FV, fiber volley. (*B*) Paired-pulse facilitation (PPF) is not altered in adult PICK-KO mice. WT, n = 19, KO, n = 20; $P > 0.05$ at all interstimulus intervals (ISI). (*C*) mEPSC amplitude and frequency are unaffected in adult PICK1-KO mice. WT (n = 25 cells): amplitude = 12.8 ± 0.40 pA, frequency = 3.34 ± 0.33 Hz; KO (n = 22 cells): amplitude = 12.2 ± 0.44 pA, $P > 0.3$; frequency = 2.83 ± 0.27 Hz; $P > 0.2$. (*D*) Synaptic I–V relationship in CA1 pyramidal neurons. WT, n = 12; KO, n = 10. (*E*) AMPA (20 μM)-induced currents during voltage ramps (50 mV/s, 2,600-ms duration) from −70 mV to +60 mV from the somatic outside-out patch membrane was measured in WT and PICK-KO mice. Average distribution of AMPA-induced current at −70 mV was not different in WT (213 ± 28 pA) and PICK1-KO (172 ± 20 pA) mice (P = 0.12). In all figures, error bars indicate SEM.

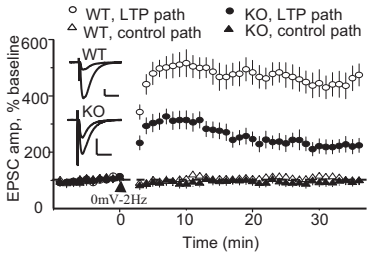

Fig. S2. LTP induced by pairing 200 pulses at 2 Hz with 0 mV depolarization (35 °C) is reduced in PICK1-KO mice. WT (n = 7): 437 ± 44%; KO (n = 10): 221 ± 24%; $P < 0.001$. (*Inset* scale bars: 100 pA, 20 ms.)

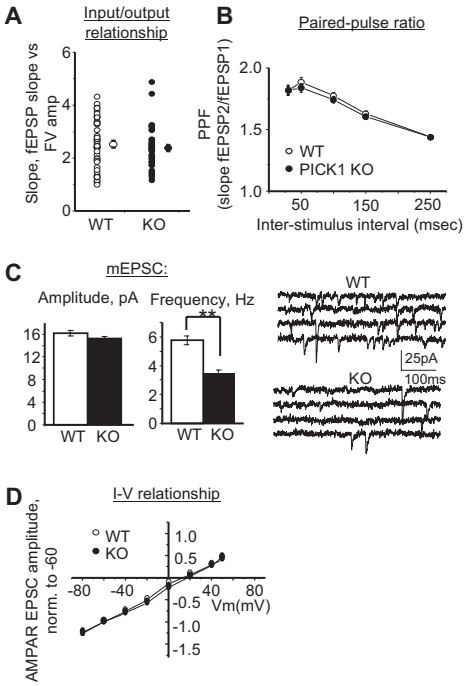

Fig. S3. Basal transmission in hippocampal pyramidal neurons is modestly affected in juvenile (2- to 3-wk-old) PICK1-KO mice. (*A*) The slope of the input–output curve is not altered in juvenile PICK1-KO mice. WT ($n = 47$): 2.53 ± 0.13 ms^{-1}; KO ($n = 41$): 2.39 ± 0.12 ms^{-1}; $P > 0.05$. (*B*) PPF is not altered in juvenile PICK1-KO mice. WT, $n = 47$; KO, $n = 41$; $P > 0.05$ at all ISI. (*C*) mEPSC amplitude is unaffected in juvenile PICK1-KO mice [WT ($n = 35$ cells): 16.6 ± 0.47 pA; KO ($n = 37$ cells): 15.35 ± 0.35 pA; $P > 0.1$], whereas mini frequency is significantly lower in KO mice (WT: 5.76 ± 0.32 Hz; KO: 3.46 ± 0.23 Hz; $P < 0.001$). (*D*) I–V relationship in CA1 pyramidal neurons. WT, $n = 12$; KO, $n = 18$.