Supplementary materials and methods

Physical health

Measures of general health and neurological reflexes were assessed in the mice as described previously (31, 22). Briefly, general health measures included, body weight (g), coat and whisker condition, and body and limb tone. Evaluations of posture, physical condition of the fur, and observations for unusual home cage behaviors, and irregular spontaneous behaviors such as excessive grooming, digging, rearing, or stereotypy were performed. Neurological reflexes examined included trunk curl, forepaw reaching, corneal reflex, ear twitch, whisker twitch, and the righting reflex. The wire hang test to evaluate limb strength was performed by placing the mouse on a set of wire bars and then inverting the lid for up to 60 s (as previously described (31)).

Contextual fear memory protocol: Day 1 (conditioning phase; 510 s): 120 s no stimulus to measure baseline freezing, then a tone (28 s) paired with a co-terminating foot shock (0.7 mA for 0.2 s) for 3 consecutive times 90 s apart (% freezing was measured for each shock during allotted time), followed by 120 s recovery time. Day 2 (Context phase; 300 s), was performed 24 h after the initial training in the same conditioning chamber without either tone or foot shock (% freezing was measured over the total allotted time).

Fear extinction learning protocol: Day 1 (conditioning phase; 1870 s): 5 tones of 30 s each (intervals 200 s, 220 s, 80 s, 150 s), 80 s of no stimulus and a 30 s tone paired with 0.5 s shock (0.7 mA) for 5 times (intervals 200 s, 220 s, 80 s, 150 s). In day 1 the % freezing was graphed for each single tone or tone plus shock during allotted time. Days 2 and 3 (extinction phase; 2300 s) took place after 24 and 48 h respectively from day 1: 30 tones (30 s each) at variable intervals (60 s, 60 s, 30 s, 30 s, 30 s, 30 s, 60 s, 30 s, 30 s, 30 s, 30 s, 60 s, 60 s, 60 s, 60 s, 30 s, 30 s, 60 s, 30 s, 60 s, 30 s,

30 s, 60 s, 60 s, 60 s, 60 s, 30 s, 60 s, 30 s, 60 s) without foot shock. In day 2 and 3 the % freezing was graphed every 5 tones during the allotted time.

Temporal order recognition test: The temporal order object recognition test, in which discriminations are based on the relative recency of presented objects, was assessed as described previously (31, 39, 40). Briefly, the sample phase comprised of two sample periods of 5 min each with objects positioned in the two corners at the same side of the arena; during sample 1 the mouse is allowed to explore two identical copies of the same object (novel object 1). Sample 2 took place one hour later, with two identical copies of a different object (novel object 2). The test phase occurred with a 3 hour delay from sample 2; wherein a copy of novel object 1 and a copy of novel object 2 were presented for 5 min in the same arena positioned in the two corners at the same side of the arena. The discrimination ratio was calculated as the difference in time spent sniffing novel object 1 and novel object 2 divided by total sniff time. If temporal order discrimination memory is intact, subjects exhibit a discrimination ratio >0.

Novel object preference task: The novel object recognition test was used as described previously (31). A separate group of naive mice was used. In the sample phase two identical copies of the same object (novel object 1) were positioned in the two corners at the same side of the arena. The subject is placed in the arena to explore for 10 min. During the test trial (two hours after sample phase), the subject is returned to the arena for 5 min and allowed to explore a copy of the novel object 1 and a new object with different shape and color (novel object 2) placed in the two corners on the same side of the arena. The preference index is calculated as ratio of the time spent sniffing the novel object 2 over the total sniffing time during test trial. If novel object memory is intact, subjects exhibit a preference index >0.5.

Object location recognition task: The object location test was used as described previously (31, 39). A separate group of naive mice was used. In the sample phase, two identical copies of the same object (novel object 1 and novel object 2) were positioned in the two corners at the same side of the arena. The experimental mouse was placed in the arena and allowed to explore for 10 min. During the test trial that took place 1 h after the sample phase, the experimental mouse returned the arena for 5 min to explore the novel object 1 in an unchanged location and the novel object 2 positioned in a new location. The preference index was calculated as the ratio of the time spent sniffing the novel object 2 over the total sniffing time during the test phase. If object location memory is intact, subjects exhibit a preference index >0.5.

Hippocampal Electrophysiology: Coronal hippocampal slices 400 μm thick were cut. Slices were then transferred to a holding chamber containing oxygenated artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$ and 10 D-Glucose) for 30 min at 34°C and for another 30 min at 22°C for recovery, and then transferred to a submersion recording chamber continually perfused with 32°C oxygenated ACSF (rate: 2 ml/min). Slices were equilibrated for at least 15 min before each recording.

Field recording: ACSF-filled glass electrodes (resistance <1 MΩ) were positioned in the stratum radiatum of area CA1 for extracellular recording. Synaptic responses were evoked by stimulating Schaffer collaterals with 0.1 ms pulses with a bipolar tungsten electrode (WPI Inc., Sarasota, FL, USA) once every 20 s. To measure PPF, synaptic responses were elicited by paired pulses at different inter-pulse intervals (IPI, 12.5, 25, 50, 100, 200, 400, 800 and 1600 ms, respectively). To measure LTP, the stimulation intensity was systematically increased to determine the maximal field excitatory post-synaptic potential (fEPSP) slope and then adjusted to yield 40-60% of the maximal (fEPSP) slope. Experiments with maximal fEPSPs of less than 0.15 mV or with

substantial changes in the fiber volley were rejected. After recording of a stable baseline for at least 15 min, LTP was induced by 4TBS (4 theta-burst trains, each of 4 pulses at 100 Hz). Field EPSPs were recorded (Axopatch 200B amplifier, Molecular Devices, Sunnyvale, CA, USA), filtered at 2 kHz, digitized at 10 kHz (Axon Digidata 1321A), and stored for off-line analysis (Clampfit 10). Initial slopes of fEPSPs were expressed as percentages of baseline averages. In summary graphs, each point represents the average of 3 consecutive responses. The time-matched, normalized data were averaged across experiments.

Electrophysiology mPFC: Coronal slices containing mPFC 300 μm thick were cut. The slices were initially incubated at 34°C in ACSF containing 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 26 mM $NaHCO_3$ and 10 mM glucose, bubbled with carbogen gas (95% O_2 - 5% CO_2, pH 7.4), and then kept at room temperature. Slices were allowed to recover for at least 60 min before experiments were started. Following recovery, the mPFC slices were transferred into a recording chamber at approximately 32-34°C. For current clamp, the recording pipettes were filled with intracellular solution containing the following (in mM): 130 K-gluconate, 1 MgCl2, 5 EGTA, 5 MgATP, 10 HEPES, and 0.4 Na2GTP, pH 7.2 with KOH. Patch pipette resistance was 4–7 MΩ. Whole-cell recordings were obtained with Axopatch 200B amplifiers (Molecular Devices), filtered at 2 kHz, and sampling was acquired at intervals of 20–100μs through a DigiData 1321A interface with program CLAMP10 (Molecular Devices), as described previously (31, 72). Repetitive firings were evoked by various depolarizing steps, and action potential numbers were plotted against the depolarizing currents injected into neurons in the layer II/III and layer V of mPFC. For voltage clamp, the recording pipettes were filled with intracellular solution containing (in mM) 130 K-gluconate, 1 $MgCl_2$, 5 EGTA, 5 MgATP, 10 HEPES and 0.4 Na_2GTP (pH 7.2 with KOH). The resistances of patch pipettes were 3-6 MΩ.

mEPSC and mIPSC to pyramidal neurons in the layers II-III and V were detected by whole-cell recordings. mEPSCs were recorded with a holding potential at -70 mV in the presence of 50 μM bicuculline and 1.5 μM tetrodotoxin (Sigma, St. Louis, MO, USA) to block $GABA_A$ receptor currents and voltage-gated sodium currents, respectively. To record mIPSCs, a different Cs^+-based intracellular solution (140 mM $CsCl_2$, 2 mM $MgCl_2$, 2 mM Na_2-ATP, 0.5 mM Na_2GTP, 5 mM Na_2-phosphocreatine, 1 mM EGTA, 10 mM HEPES, and 5 mM QX-314, pH 7.25 with CsOH) was used. The mIPSCs were recorded at -70 mV in the presence of 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Sigma) as competitive AMPA/kainate receptor antagonist, 50 μM (2*R*)-amino-5-phosphonovaleric acid; (2*R*)-amino-5-phosphonopentanoate (APV, Sigma) as selective NMDA receptor antagonist, and 1.5 μM tetrodotoxin (Sigma) as blockers of sodium channels. The access resistance was monitored during recordings, and the data were excluded from analysis if the series resistance changed more than 20% from control levels. All recordings were made with an Axopatch 200B amplifier through a DigiData 1321A interface, recorded and analyzed by Clampfit 10 (Molecular Devices, Sunnyvale, CA, USA). Pyramidal neurons of the mPFC were identified by their shape, location and adaptive firing characters with wider action potentials.

Western blotting: The following antibodies were used in this study: anti-AKT-1 (1:500, #07-416; Millipore, Billerica, MA, USA), anti AKT-2 (1:5000; kind gift of Dr Morris Birnbaum, University of Pennsylvania School of Medicine (73), anti-AKT-3 (62A8 1:1000, #3788; Cell Signaling, Beverly, MA, USA), anti-pAKTser473 (1:50, #05-669-K; Millipore), anti- pAKTthr308 (1:1000, #2965; Cell Signaling), anti-pAKT2ser474 (1:1000, #8599, Cell Signaling) anti-mTOR (1:1000, #2972; Cell Signaling), anti- pmTOR-Ser2448 (1:1000, #2971; Cell Signaling), anti-S6 (1:1000, #2317; Cell Signaling), anti-pS6 (1:2000, #4858; Cell Signaling), anti-p-70S6K (1:1000, #9202;

Cell Signaling), anti-pp-70S6K (1:1000, #9205; Cell Signaling), anti-GSK3β (1:2500, #610201; BD transduction Laboratorie, San Jose, CA, USA), anti-pGSK3βser9 (1:1000, #9323; Cell Signaling), anti-PI3Kinase p110δ (A-8 1:500, #sc-55589; Santa Cruz, CA, USA), anti-PDK1 (1:1000, #3062; Cell Signaling), anti-pPDK1 (Ser241, 1:1000, #3061; Cell Signaling), NMDAζ1 (H-300 1:200, #sc-9058; Santa Cruz), anti-AMPA (GluA 2/3/4 1:1000, #2460; Cell Signaling), anti-GAT-1 (1:500, #ab426; Abcam, Cambridge, MA, USA), anti-PSD95 (1:1000, #2507; Cell Signaling).