Supplementary Material

Materials and Methods

Generation of CA3-NR1 knockout mice. We isolated an approximately 120-kb-long bacterial artificial chromosome (BAC) clone BAC1, that contained a part of the genomic sequence of mouse KA-1, one of the kainate receptor subunits, by screening a C57BL/6 (B6) BAC library (Genome Systems) with a probe composed of the 5' untranslated region of a mouse KA-1 cDNA clone (S1). The BAC1 clone contained the genomic DNA sequence of the putative promoter, exon 1 (~338 bp) and exon 2 (45 bp), but not exon 3 (105 bp) carrying the translation initiation codon. The entire BAC1-DNA fragment was coinjected into fertilized B6 mouse eggs with a DNA construct carrying both the minimal heat shock promoter (from pIND plasmid, Invitrogen), and the Cre cDNA with nuclear localization signal (pBS317, provided by B. Sauer). Out of eight founder lines in which both Cre and BAC1 sequences were cointegrated into the genome, the line referred to as G32-4 was crossed with the Rosa26 reporter line (S2), or the "floxed" NMDA receptor subunit-1 (fNR1) mouse line, which we reported earlier (S3) and subsequently backcrossed to B6 eight times. All procedures relating to animal care and treatment conformed to the Institutional and NIH guidelines.

Immunocytochemistry. Mice were perfused transcardially with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB). For X-gal staining, the brains were removed and post fixed in the same fixative at 4°C for 30 min. Coronal sections (50 μm thick) were cut on a Vibratome and collected in PB. Sections were then incubated in 0.1 M PB containing 0.01% SDS, 0.02% NP-40, 2 mM $MgCl_2$ at 4°C for 15 min followed by β-galactosidase reaction in 1× PBS pH 8.0 containing 0.5 mg/ml X-gal, 5 mM $K_4Fe(CN)_6/3H_2O$, 5 mM $K_3Fe(CN)_6$, 2 mM $MgCl_2$ at 37°C for 24 hours. Section were post fixed in 10% formalin for at least 2 hours, and counterstained with Nuclear Fast Red (Poly Scientific). For immunofluorescent staining in Fig. 1, the brains were post fixed in 4% paraformaldehyde overnight, and 50-μm-thick Vibratome sections were prepared. Sections were then incubated at 4°C overnight with gentle shaking in primary antibody [anti-Cre polyclonal antibody (pAb), 1/4000, BABCO; anti-GAD67 pAb, 1/1500, Chemicon; anti-calretinin pAb, 1/4000, Swant] with 0.05% Tween20 following pre-incubation with 10% normal goat serum at room temperature for 2 hours. The sections were then incubated with a biotinylated anti-rabbit IgG (1/200; Vector) at room temperature for 2 hours, and then with streptavidin-biotin/horseradish peroxidase complex (Vector) for 30 min. Cre-IR was visualized by 5-min treatment with fluorescein isothiocyanate (FITC)-tyramide at room temperature. GAD67-IR and calretinin-IR were visualized by a 5-min treatment with the Cy3-tyramide, according to the manufacture's protocol (NEN). For double immunofluorescence staining, brain sections were further incubated with anti β-galactosidase (1/2000, Cappel) at 4°C for overnight. After rinsing, the sections were incubated in Alexa488-conjugated anti-rabbit IgG (1/2000, Molecular Probes) at room temperature for 2 hours. Fluorescent images were captured with a confocal laser scanning microscope (BioRad). For immunoperoxidase staining in Fig. 2, 5-μm-thick paraffin sections were prepared as described (S4). Briefly, prior to primary

antibody incubation, sections were subjected to pepsin pretreatment (1 mg/ml in 0.2 N HCl) at 37°C for 10 min. After blocking with normal goat serum, sections were incubated overnight with anti-NR1 pAb (GluRζ1 COOH-terminus, 0.5-1.0 μg/ml), anti-PSD-95 pAb (0.5 μg/ml), anti-GluR1 pAb (GluRα1 COOH-terminus, 0.5 μg/ml), or anti-calbindin$_{D28k}$ pAb (1/10,000) followed by incubations with biotinylated anti-rabbit IgG for 1 hour and streptavidin-peroxidase complex for 30 min (Vector). Immunoreaction was visualized with 3,3'-diaminobenzidine, and photographs were taken by AX-50 light microscope (Olympus).

In situ hybridization of NR1 mRNA. Fresh-frozen brain sections (14 μm in thickness) were prepared in the parasagittal plane with a cryostat, and mounted onto pre-coated glass slides. Sections were post fixed with 4% paraformaldehyde in PBS for 15 min, and treated with 10 μg/ml proteinase K at 37°C for 30 min followed by 0.2 M HCl for 10 min. After rinsing, sections were further incubated in 0.25% acetic anhydride and 0.1 M triethanolamine for 10 min to avoid non-specific binding of the probe. Following dehydration with ethanol, hybridization was performed at 55°C for 18 hours in a hybridization buffer containing 50% formamide. For detection of the mouse NR1 mRNAs, a complementary RNA (cRNA) probe, derived from the AvrII-SphI 0.4-kb antisense DNA fragment of rat NR1 cDNA containing from exon 13 to exon 16 (S5), was labeled with [^{33}P]UTP (5×10^5 cpm), and added to the hybridization buffer. The brain sections were serially washed at 55°C with a set of SSC buffers of decreasing strength, the final strength being 0.2× and then treated with RNase A (12.5 μg/ml) at 37°C for 30 min. The sections were exposed to hyper-beta max (Amersham) for 2 days and were dipped in Kodak NTB3 nuclear emulsion followed by exposure to X-ray film for 3-4 weeks.

Slice electrophysiology. Transverse hippocampal slices (300-350 μm thick) were prepared from 18- to 28-week-old male homozygous fNR1 mice (control) and CA3-NR1 KO (mutant) mice as described previously (S6). In addition, we found that the viability of surface neurons was noticeably improved by cutting slices with freshly broken glass blades (Ralph Glass Knife Maker; Ted Pella, Inc.) placed in a Vibratome blade holder. Individual slices were transferred to a recording chamber and submerged in oxygenated (95% O_2 to 5% CO_2) bathing solution maintained at 30°-31°C and containing (in mM): 124 NaCl, 2.5 KCl, 25 $NaHCO_3$, 2-4 $MgCl_2$, 2-5 $CaCl_2$, 10 dextrose. Whole-cell patch recordings were made from visually identified CA3 and CA1 pyramidal cells with an SEC 05L amplifier (Adams and List Associates) in bridge or discontinuous voltage-clamp modes. Recording electrodes were either filled with (in mM) 120 K-gluconate, 20 KCl, 10 Hepes, 2 $MgCl_2$, 4 ATP (disodium salt), 0.3 Tris-GTP and 7 phosphocreatine phosphocreatine (osmolarity 280-290, pH 7.3, KOH), or 107 gluconic acid, 107 CsOH, 5 QX-314-Cl, 10 BAPTA, 0.2 EGTA, 5 TEA, 20 Hepes, 0.7 NaCl, 4 MgATP, 0.3 NaGTP (osmolarity 280-290, pH 7.3 CsOH). In most experiments, biocytin was added to the patch electrode solution for cellular staining and subsequent morphological confirmation. Although not systematically analyzed, no obvious differences in recorded-cell morphology were observed between control and mutant animals. Synaptic responses were evoked in either CA3, CA1, or dentate gyrus with single stimulation or brief bursts of stimulation (5 pulses at 50-100 Hz) delivered to one of three afferent

pathways (Fig. 3A): medial perforant path input to the dentate gyrus (MPP-DG), recurrent commissural/associational (C/A-CA3), or Schaffer collateral input to CA1 (SC-CA1). NMDA currents were characterized by their slow time course, insensitivity to DNQX and sensitivity to 2-amino-5-phosphonopentanoic acid (APV). Where specified, NMDA currents were isolated pharmacologically by adding the AMPA/kainate antagonist, 6-cyano-7-dinitroquinoxalline-2,3-dione (DNQX, 40 μM) and the $GABA_B$ antagonist, CGP 35348 (50-100 μM) to the bath solution. To ensure that NMDA currents were fully activated, extracellular Mg^{2+} was removed and glycine (1-10 μM) added. In some experiments NMDA currents were recorded by depolarizing the cells to a variety of membrane potentials after filling the cells with cesium. Synaptic currents were evoked by electrical stimulation with a glass or metal bipolar stimulating electrode placed in one of three afferent pathways: (i) DG molecular layer (~ 50 μm from stratum granulosum) to activate medial perforant path axons, (ii) the CA3 stratum radiatum (~200 μm from stratum pyramidale) to activate C/A axons, (iii) CA3 stratum lucidum (~50 μm from the cell body layer and ~100 μm toward the granule cell layer in the mossy fiber track) to activate the mossy fiber pathway, and (iv) CA1 stratum radiatum (~200 μm from stratum pyramidale) to activate Schaffer collaterals. NMDA currents were evoked with single pulses or a brief burst of stimulation (5-pulse bursts at 50 or 100 Hz). The presence of a synaptically evoked NMDA current was confirmed by blocking the response with the NMDA antagonist APV (50-100 μM). For LTP experiments, 4 mM $MgCl_2$ and $GABA_A$ blockers (10 μM bicuculline and 10 μM picrotoxin) were added to the bathing solution. To induce LTP, three long trains of stimulation (100 pulses at 100 Hz; 1 train per 20 sec) were delivered to either MF-CA3, C/A-CA3, or SC-CA1 synaptic pathways. In addition, a 1-s depolarizing pulse (100-500 pA) was given during the stimulation trains in an effort to elicit an equivalent number of action potentials for each of the synaptic pathways during the train.

Morris water maze. The Morris water maze tasks were carried out with male mice 18 to 24 weeks of age. All the experiments were conducted by operators who were blind to the genotypes of the mice used. The mice were kept in a temperature-controlled room on a constant 12-hour light/dark cycle. The experiments were conducted at approximately the same time each day. The facility was in a square dark room and consisted of a circular pool (160 cm diameter, 60 cm deep at the edge) filled with water at 20°-22°C that was completely covered with floating polypropylene beads (Hanna resin distribution). The pool was encircled by black curtains (90 cm from the pool periphery) on which four large illuminated objects that served as extramaze cues were hung. An overhead infrared-sensitive CCD camera tracked the movement of mice at 30 Hz and sent the feed to a VCR and a computerized tracking system. The entrance and exit for an experimenter were at NW and NE, respectively. The escape latency (platform search time), path length (the distance traveled to reach the platform), swimming velocity and wall hugging (the time spent in an 18 cm-wide ring area bordering the pool wall, a measure of the degree of the animal's thigmotaxic behavior) for each trial were measured. For the hidden platform task, the mice were trained to find a hidden circular platform (10 cm in diameter and set 1.5 cm below the surface of water) that was placed at the center (46.8 cm from the center of the pool) of a radial quadrant, which is defined as the largest circle inscribed in a quadrant. In order to balance the effects of the

environment, the mice of each genotype were divided into four groups and each group of mice were trained for a platform located in a distinct quadrant, NE, SE, SW, or NW. The data from the four groups of mice, except obvious "floater" mice (less than 5% of the mice tested), were then combined. The training was carried out in blocks of four trials per day (an inter-trial interval of about 30 min) for 12 days. During each block of trials, the mice were released from four pseudorandomly assigned start locations (N, S, E, and W) and allowed to swim for 90 s. If a mouse did not find a platform within 90 s, it was manually guided to the platform and allowed to rest on the platform for 15 s. Probe trials were performed on days 2, 7, 13, 14, and 15 under various conditions (see the text). In probe trials, the mice were released at the center of pool with a head direction that was pseudorandomly assigned, and were allowed to swim for 90 s in the absence of the platform. We measured the time (in seconds) the mice spent in the area, which corresponded exactly to the area occupied by the platform during the training session (absolute platform occupancy). We also measured the time mice spent in each of the four radial quadrants (the largest circles inscribed in each quadrant) and calculated the percent of time they spent in the target radial quadrant relative to the total time spent in the four radial quadrants (relative radial quadrant occupancy). For the partial-cue probe trial (the fourth probe trial, P4) conducted on day 14, only one of the two cues that were located more distant from the platform during the training session was kept and the other three cues were removed. Among the eight possible combinations of the remaining extramaze cue and platform position (NE, SE, SW, or NW), mice of each genotype were tested in six different combinations in order to balance the effects of the environment. The data from the six groups of mice were then combined. To evaluate the memory recall capability of each animal, we defined a relative recall index (RRI) as the ratio of the target platform occupancy of the fourth (P4) or fifth (P5) probe trial to that of the third (P3) probe trial for each animal that exhibited significant target platform occupancy (more than 0.23 sec) in P3. The values were then averaged over mice of a particular genotype.

In vivo multielectrode recording. All recordings were carried out by operators who were blind to the genotype of the mice. Male mice (18-24 weeks of age) were implanted with a microdrive array consisting of six independently adjustable tetrodes (Stereotaxic coordinates from bregma: 2.0 mm lateral; 1.8 mm posterior). Recording sessions generally consisted of one or two "Run" epochs (20-30 min each) bracketed by "Sleep" sessions in which the animal rested quietly on a small platform outside of the behavioral environment. "Run" sessions were conducted in a low walled open field arena (50-cm in diameter) placed near the center of a rectangular black-curtained chamber (1.8 m × 2.0 m). Diffuse room lighting was provided by low intensity spotlights focused onto four salient visual cues located on each of the walls of the recording chamber. As animals randomly explored the open field arena, extracellular action potentials were recorded while the animal's position was tracked using a pair of infrared diodes placed 3 cm above the animals head. Following data acquisition, action potentials were assigned to individual cells based on a spike's relative amplitudes across the four recording wires of a tetrode (S7). In order to be included for analysis, isolated cells had to satisfy three criteria:(i) fire a minimum of 100 spikes during the "Run" session, (ii) have a mean firing rate greater than 0.1 Hz, and (iii) have less than 0.5 % of the cells spikes fall within a 1

ms refractory period. For cue removal experiments cells also had to satisfy the following conditions: (i) the waveform profiles of isolated cells had to remain stable across the 2-hour delay between recording sessions, (ii) cells had to fire at least 150 spikes in the first environment and (iii) cells had to have an initial place field size greater than 10 pixels.

Place cell analysis. All isolated cells were divided into two subclasses based on waveform and firing characteristics. Putative pyramidal cells were defined as cells with relatively broad waveforms (peak to trough width > 300 μs) and a strong tendency to produce complex spike bursts (CSI > 3%) whereas putative interneurons had relatively narrow waveforms (peak to trough width < 240 μs), and few if any complex spike bursts (CSI < 3%). When comparing the cellular properties between mutant and control animals, analysis was performed separately for these two classes of cells. To determine the consequence of CA3-NR1 disruption on the firing of CA1 cells, we measured several electrophysiological properties of hippocampal activity. First, we measured the bursting tendencies of hippocampal pyramidal cells using two measures: (i) Complex spike index (CSI)-defined as the percentage of spikes with first lag interspike intervals that fall between 2 and 15 ms and whose second spike is smaller than the first and (ii) burst spike frequency-the ratio of number of spikes involved in a burst relative to the total number of spikes produced by a cell. As a measure of the intrinsic properties of pyramidal cells we measured both spike width (peak to trough width), and the degree of amplitude attenuation within a three-spike burst (S8). We also used two measures to assess the spatial tuning of individual place cells: (i) place field size, which is defined as the number of pixels in which a cell's mean firing rate exceeded 1 Hz and (ii) integrated firing rate, which is defined as the sum of average firing rates across all pixels, a measure that is less sensitive to noise than place field size. For these experiments, the coordinated firing of overlapping place fields was assessed by calculating the firing rate covariance coefficient of all cell pairs within 9 pixels of each other using a bin size of 200 ms (S9). Finally, in order to quantify relative changes in place field properties for cells recorded across experimental conditions, we calculated, for each cell, a relative change index (RCI) (defined as the difference between the cell's firing between two conditions divided by the sum of the cell's firing across the two conditions. Note that RCI does not correspond to simple percentage change). An RCI value of -0.17 is equivalent to a 30% reduction.