**Figure S1. Normal adult brain morphology and gene expression in GluN1<sup>Rneo/+</sup> mice.**

Nissl-stained sections revealed no obvious change in gross morphology in the hippocampus (a-d), cortex (e) and cerebellum (f) of GluN1<sup>Rneo/+</sup> mice when compared to their wild-type littermates GluN1<sup>+/+</sup>.

(g) Immunohistochemistry for GAD 67 positive cells in the DG.

(h) Western blot analysis on hippocampal crude homogenates from adult mice, using antibodies against NMDAR subunits (GluN2A, GluN2B), AMPA receptors (GluA1, GluA2/3), PSD 95, glutamic acid decarboxylase (GAD 67), GABA<sub>亴</sub> receptor subunit β<sub>3</sub>, and sodium channel type IIα subunit (NaCh IIα).

(i) cDNA expression profiling array experiment using 600 ng hippocampus poly(A)<sup>-</sup>-RNA, isolated from three adult (8 months old) mutant GluN1<sup>Rneo/+</sup> mice and three wild-type littermates. No significant changes in expression were observed in any of the analyzed genes (~9000 expressed sequence tags, ESTs). Expression was quantified as arbitrary fluorescence units without background subtraction. Diagonals indicate 2x and 10x differential expression.

Scale bars: (a, f) 200 µm, (b-d, g) 50 µm, (e) 100 µm. Hippocampal regions: dentate gyrus (b), CA1 (c), CA3 (d). (a-e, g) are coronal sections, (f) sagittal section. Cortical layers are indicated by roman numerals (e).
Materials and Methods, further details

Antibodies
Primary antibodies (rabbit (rb) anti GAD 67, Chemicon, Temecula, CA AB108, 1:10000; rb anti GluN1 [C-terminal], Sigma, St Louis, MO, G8913, 1:500; monoclonal anti pan-GluN1 [N-terminal], Calbiochem, San Diego, CA, 454578, 1:5000; rb anti GluN2A, Chemicon AB1555P, 1:750; rb anti GluN2B, Chemicon AB1557P, 1: 1000; rb anti GluR1, Chemicon AB1504, 1:1000; rb anti GluR2/3, Chemicon AB1506, 1:5000; rb anti GABA_A R β3, a gift from Steve Moss, UCL (Brandon et al. 2000), 1:5000; monoclonal anti PSD 95, BD Biosciences, San Jose, CA P43520, 1:1000; rb anti NaCh type II, Alomone Labs , Jerusalem, Israel, ASC-002, 1:1000; monoclonal anti α-tubulin, Sigma T9026, 1:20000 or rb anti ErbB-4, Santa Cruz Biotechnology, Santa Cruz, CA, sc-283, 1:200) were applied for one hour. Peroxidase-conjugated secondary antibodies (goat anti-rabbit, goat anti-mouse, Jackson ImmunoResearch Laboratories, West Grove, PA, 111-035-003, 115-035-003 respectively) were incubated for one hour at a dilution of 1:10000. PVDF membranes (Hybond-P) were developed with ECL Plus reagent (Amersham Pharmacia) and exposed on film (Kodak X-OMAT-AR).

Novel object recognition
Animals were tested in an arena with sloping side-walls to prevent shadows from overhead illumination (50 cm x 50 cm, 1 cm sawdust on floor). Habituation consisted of daily 10-min periods in the empty arena over a 5 day period. Mice were allowed to explore two identical objects placed into the arena at fixed locations for 10 min. The inspection time is recorded when the head of the mouse is within 1 cm of the objects. Any behavior like sniffing, sucking and biting the objects is defined as inspection. Five different sets of identical objects were used on different days. The toy
objects (for example, a model duck) measured about 10±15 cm in height and were washed in alcohol between successive tests. After the initial period of inspection, the mice and the objects were removed from the arena for the prescribed memory delay interval (1 min or 1 h spent in a transport cage in the laboratory). A third copy of the earlier identical object was then placed into the arena at one location and a new object at the other. The mice were returned to the arena and allowed to explore for 10 min. Total time spent in either the sample or choice phases was 10 minutes. Memory of the familiar object is associated with increased exploration of the new object and a performance index (PI) is calculated using the formula PI = 100 x (new object inspection time)/30. The groups did not spend significantly different amounts of time in the apparatus (F(1,17) = 3.2, p>0.05).

References
Figure S1

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