Online METHODS

Legal issues

Molecular, biochemical experiments and genetic manipulations of mouse embryos were licensed by the regional council in Karlsruhe, Germany under project licenses 37- 9185.81/G71-19 and G74-07. Electrophysiological studies were conducted according to the Norwegian Animal Welfare Act and European Union's Directive 86/609/EEC. Behavioral experiments were in accordance with the U.K. Animals, Scientific Procedures Act (1986), under project licenses PPL 30/1989 and PPL 30/2561. Efforts were made to minimize numbers of animals used.

Generation of *GluN1*$^{\Delta DGCA1}$ mice

Gene-targeted *GluN1*^{2lox} mice with (loxP5171;[46]) flanking *Grin1* exons 11 – 18 (*Grin1*^{tm1Rsp}) were generated as described previously[14]. *GluN1*$^{\Delta DGCA1}$ mice were homozygous for the floxed *Grin1*^{tm1Rsp} allele and carried the two transgenes, *Tg*^{CN12} and *Tg*^{LC1}. *Tg*^{CN12} expresses the tetracycline-dependent transcriptional transactivator, itTA, from a NRSE element modified α-CaMKII promoter[24]. Controls were *GluN1*^{2lox} littermates lacking both or either one of the transgenes of lines *Tg*^{CN12} or *Tg*^{LC1}.

Doxycycline (50 mg/l in drinking water) was given to pregnant females from the day of conception and was removed on the day the pubs were born. Offspring of *Gt(Rosa)26Sor* mice[47] and double transgenic *Tg*$^{CN12/LC1}$ mice were used for detection of Cre activity. Stereotactic rAAV-Cre virus injection (**Supplementary Fig. 1b+2a**) of *GluN1*^{2lox} mice was performed as described[51].

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In situ hybridization

[47]*In situ* hybridizations were performed as described previously[14,48] on 15 µm µcryostat sections. Anti sense oligonucleotides (*Grin1*-nr1mouseis: 5'- GAA CTG ACA GCC CCA CCA GCA GCC ACA GTG TGC TC-3' and *Gria1*-pan-A: 5'-GTC ACT GGT TGT CTG ATC TCG TCC TTC TTC AAA CTC TTC ACT GTG-3') were 3'-endlabeled with terminal deoxynucleotidyl transferase and [α-^{33}P]-dATP.

Immunohistochemistry

Immunohistochemistry was performed as described[14,49]. Brain sections (50 and 100 μm) were stained with primary antibodies anti-Cre (1 : 8000, polyclonal, BabCO), anti-neuronal-specific nuclear protein (NeuN; 1 : 100 monoclonal, Millipore) and anti-Calbindin (1 : 1000 monoclonal, Swant). Secondary anti-mouse and anti-rabbit coupled to Cy3 and FITC were used (each 1 : 200; Jackson Immuno Research). Slices were mounted on slides, air-dried, covered with cover slips using either eu-kitt (O. Kindler) or Aqua-Poly/Mount (Polysciences). Beta-galactosidase was visualized by incubating brain slices in X-gal[14, 49] in the dark at room temperature for 24 h. Subsequently, slices were washed twice in PBS, mounted and in some cases counterstained in eosine.

Electrophysiological analysis of *GluN1*$^{\Delta DGCA1}$ mice

Male and female mice (8 – 9 months old) were killed with Suprane (Baxter) and brains removed. Transverse slices (400 μm) were cut from the middle and dorsal portions of each hippocampus and prepared as described[50].

In one set of experiments orthodromic stimuli (50 μs, <280 μA) were delivered alternately through two tungsten electrodes, one in stratum radiatum and another in stratum oriens of CA1. EPSPs were monitored by two glass electrodes (filled with ACSF) placed in the corresponding synaptic layers. After obtaining stable synaptic responses in both pathways (0.1 Hz for at least 10–15 minutes), the radiatum pathway was tetanized (1 sec @ 100 Hz, repeated four times at 5 min intervals), whereas the oriens pathway served as an untetanized control pathway. To standardize tetanization strength in different experiments, the tetanic stimulation strength was set in response to a single shock at intensity just above the threshold for generating a population spike.

In a second series of experiments a stimulation electrode was placed in stratum radiatum at the border between CA1 and CA3. Stimulation at 0.1 Hz elicited synaptic responses recorded simultaneously by two glass electrodes localized to stratum radiatum in CA1 and CA3 (**Fig. 2c**). Pathways were tetanized as described above.

Synaptic efficacy was assessed by measuring the slope of the fEPSP in the middle third of its rising phase. Six consecutive responses (1 min) were averaged and normalized to the mean value recorded 4 – 7 min prior to tetanic stimulation.

Subjects for behavioral studies

All mice were bred at the Max Planck Institute of Medical Research (Heidelberg, Germany), In Oxford (U.K.) mice were maintained in groups, in a humidity and temperature-controlled environment, under a 12h light/dark cycle (lights on at 07.00). Testing took place during the light phase. All mouse lines were kept on the C57Bl/6N genetic background (Charles River) for 6 – 10 years before setting up breeding cohorts for this study. Male and female mice of at least 4 – 5 months of age were used. There were no significant main effects of sex, or interactions involving sex and genotype. Therefore sex has been removed as a factor from the analyses reported. All behavioral testing was conducted by experimenters blind to genotypes. Cytotoxic hippocampal lesion animals (**Supplementary Fig. 5**) were generated as described[52].

Spatial reference memory watermaze task

Spatial reference memory was assessed during acquisition of the fixed location, hidden platform watermaze task, as described[22,53]. Experimentally naïve Control (n=12) and *GluN1* $^{\Delta DGCA1}$ mice (n=12) were compared at 4 – 5 months of age. Mice had no prior watermaze pre-training.

Spatial reference memory radial maze task

Control (n=12) and *GluN1*^{ADGCA1} mice (n=11) were compared during acquisition of the spatial reference memory radial maze task at 8 – 9 months of age, as described previously[14]. To specifically assess spatial reference memory, three of the six arms were baited with 0.1 ml condensed milk (**Fig. 4**). Each entry into an un-baited arm was scored as a reference memory error (maximum of 3 errors per trial). Mice were only able to enter each arm once on any given trial (i.e. it was not possible to make working memory errors)[54].

Spatial reference memory Y-Maze tasks

The appetitively motivated, and the swim-escape Y-Maze spatial reference memory task (**Supplementary Fig. 6**) were done as descibed[26,53]. Both task are hippocampus dependent[52].

Appetitive visual discrimination task

Appetitively motivated, visual discrimination learning was assessed in a T- maze with a grey start arm plus two removable goal arms, as described[26]. The walls and floor of one goal arm were painted with black and white stripes, while those of the other goal arm were dark grey. Controls (n=13) and *GluN1*$^{\Delta DGCA1}$ mice (n=11) were required to associate a particular color of goal arm with reward.

Spatial discrimination beacon task

Experimentally naïve Controls (n=11) and *GluN1*$^{\Delta DGCA1}$ mice (n=12), 8 – 9 months of age, were trained on a spatial discrimination version of the watermaze task using two visibly, identical beacons.

Mice were first trained to approach a single, black, spherical, beacon (diameter 15 cm; 24 cm high), sitting on the water surface, which indicated the position of the escape platform (8 trials per day for 3 days). The spatial location of the beacon and the start position of the animal changed between trials, according to a pseudo-random sequence.

Mice were then trained to discriminate between two identical, visible beacons, depending on their spatial locations. Both beacons remained in fixed locations in space throughout testing and were in diametrically opposite quadrants of the pool. One beacon indicated the platform position (S^+ beacon), whereas the other beacon was attached to a thin metal pole, to hold it in a fixed position at the water's surface, but provided no means of escape (S^- beacon; **Fig. 6a**). The allocation of mice to particular platform locations was counterbalanced with respect to genotype. The identities of the actual physical beacons that were (i) placed on the platform and (ii) attached to the thin metal pole, were changed every 24 trials.

Mice were placed into the water facing the sidewall from one of 6 possible start locations according to a pseudo-random sequence. For half of the trials the S^+ beacon/platform was to the left of the start position and for half of the trials it was positioned to the right. In addition, two start positions were equidistant between the beacons, two were closer to the S^+ beacon/platform (~ 80 cm), and two were closer to the S^- beacon, and thus further from the S^+ beacon/platform (~ 140 cm; **Fig. 6b**). Mice received 8 trials per day. Therefore, over 3 days of testing each mouse received 4 trials from each possible start position. They received no more than 3 consecutive trials from the same start position. In total, animals received 15 days of training (5 blocks; 24 trials per block).

On top of each beacon was a circular (20 cm diameter) piece of laminated white card. On the S^+ beacon this white circle sat exactly above the position of the platform. Mice were considered to have made an error and chosen the wrong beacon when they passed under the white circle on the S^- beacon. Whether the first choice that the mouse made was correct or incorrect was recorded (first choice accuracy). In addition, we also counted the total number of errors made on a given trial. For example, if a mouse swam under the S^- beacon and then re-emerged before, again, swimming under the S^- beacon, then this was scored as two errors. In addition, two probe trials were conducted 24 hr after the previous training trial. Both beacons and the platform were removed from the pool and the mouse allowed to swim freely for 60 sec. A first probe test was conducted after 72 trials and a second probe test after 120 trials.

Visual discrimination beacon task

Experimentally naïve Controls (n=8) and *GluN1*$^{\Delta DGCA1}$ mice (n=9), 8 – 9 months of age, were trained on a non-spatial version of the beacon task. Mice were required to discriminate between two visually distinct beacons (a grey funnel vs. a black/white striped cylinder) in order to locate the hidden platform (20 cm diameter; **Fig. 7**). Mice first received 3 days of pre-training during which they were required to swim to a single black spherical beacon, in a variable spatial location, which indicated the position of the platform (8 trials per day for 3 days).

Mice were then required to choose between a grey funnel (17.5 cm high, 17.5 cm diameter at the top, 2 cm stem) and a black and white striped cylinder (17 cm high, 12 cm diameter at the top, 10 cm diameter at the bottom, 2 cm thick stripes) (**Fig. 7a**). One cue was always positioned on top of the platform while the other cue was attached to a thin metal pole and provided no escape from the water. The allocation as to whether the funnel or the cylinder was associated with the platform was counterbalanced with respect to genotype.

The spatial location of the platform was varied pseudo-randomly between the four quadrants of the pool. Therefore there was no spatial solution to the task. The incorrect (S^-) beacon was always located in the diametrically opposite quadrant. There were 6 possible start locations for each possible platform position (**Fig. 7b**). Mice were placed into the water facing the sidewall from one of the start locations according to a pseudorandom sequence. For half of the trials the S^+ beacon/platform was to the left of the start position, and for half of the trials it was positioned to the right. In addition, two start positions were equidistant between the beacons, two were closer to the S^+ beacon/platform, and two were closer to the S^- beacon, thus mimicking the spatial discrimination task. Mice received 8 trials per day. Therefore, over 3 days of testing each mouse received 6 trials with the S^+ beacon/escape platform in each of the four possible spatial locations, with one trial from each of the possible start positions for that given platform location. Mice received no more than 3 consecutive trials from the same start position. In total, animals received 18 days of training (6 blocks; 24 trials per block). Whether the first choice made by the mouse was correct or incorrect and the total numbers of errors per trial were recorded as before.

Statistical analysis

Data from behavioral experiments were analyzed using repeated measures ANOVAs. Subsequent post-hoc investigations involved analysis of simple main effects and unpaired, 2 tailed t-tests where appropriate. For analysis of time spent in each quadrant during watermaze probe tests, because the fourth quadrant data point was never independent of the other three, p values were adjusted to reflect a reduction in the degrees of freedom in both the main effect of quadrant and the group by quadrant interaction.

51. Tang, W. *et al.* Faithful expression of multiple proteins via 2A-peptide self-processing: a versatile and reliable method for manipulating brain circuits. *J. Neurosci.* **29**, 8621-8629 (2009).

52. Deacon, R.M., Bannerman, D.M., Kirby, B.P., Croucher, A. & Rawlins, J.N. Effects of cytotoxic hippocampal lesions in mice on a cognitive test battery. *Behav. Brain. Res*. **133**, 57-68 (2002).

53. Reisel, D. *et al.* Spatial memory dissociations in mice lacking GluR1. *Nat. Neurosci.* 5, 868-873 (2002).

54. Schmitt, W.B., Deacon, R.M., Seeburg, P.H., Rawlins, J.N. & Bannerman, D.M. A within-subjects, within-task demonstration of intact spatial reference memory and impaired spatial working memory in glutamate receptor-A-deficient mice. *J. Neurosci.* 23, 3953-3959 (2003).