Supplemental Material

Behavioural tests

Different behavioural test paradigms were used to examine both mGluR7a$^{AAA/AAA}$ mice and wt littermates.

Open field - Locomotor activity in the open field was examined using a brightly lit arena (50 cm × 50 cm × 50 cm, 200 lux) as described (Papadopoulos et al., 2007). Mice were adapted to the test environment for 20 min and then placed in the center of the arena for 5 min. Movements were recorded using the VIDEOMOT video tracking system (TSE-Systems, Bad Homburg, Germany), and total path length and the distance traveled in the center of the arena were determined**.**

Elevated plus-maze - The elevated plus-maze was used to further assess anxiety and exploratory behaviour of the mGluR7a$^{AAA/AAA}$ mice (Papadopoulos et al., 2007). The arena consisted of a plus-shaped maze with two arms that were closed by high side walls and two open arms without walls. The maze was located 40 cm above the table surface. Mice were placed on an open arm and allowed to explore the maze freely for 10 min whilst movements were monitored using the VIDEOMOT video tracking system. Mice were considered to be in the open region when all four paws were located on the open portion of the maze. The time spent on the open arms was used to monitor exploratory behaviour.

Tail flick - Pain sensitivity to acute thermal stimuli was assessed by placing the tip of the animal's tail above an infrared heat source (TSE-Systems, Bad Homburg, Germany) that produced heat stimuli of linearly increasing intensity. The time until the animal flicked its tail was recorded. For each animal, the response was monitored three times at distinct positions of the tail.

Rotarod - Motor coordination and balance were tested on an accelerating rod apparatus (TSE-Systems, Bad Homburg, Germany) . To this end, mice were placed on a rotating rod (3 cm diameter) and the time each animal was able to maintain its balance on the rod was determined. The speed of the rotarod accelerated from 2 to 20 rpm over a 5 min period. Mice were pre-trained for one trial on the accelerating rod prior to the real test.

Acoustic startle response and pre-pulse inhibition - Acoustic startle responses and pre-pulse inhibition of startle responses were assessed using a standard startle chamber (TSE-Systems, Bad Homburg, Germany). Mice were placed in the startle chamber and left undisturbed for 5

min prior to testing in the presence of a 60 dB background noise. Testing consisted of ten 100 dB pulses alone followed by ten pulses preceded by prepulses of 65-, 70-, 75 dB. The prepulse sounds were presented 100 msec before the startle stimulus. The average intertrial interval was 15 sec. PPI was calculated as a percentage score: PPI (%) = (1- [(startle response for pulse with prepulse) / (startle response for pulse alone)]) x 100.

Barnes maze – Mice were tested in the spatial version of the Barnes maze as described (Papadopoulos et al., 2007). The Barnes circular maze consisted of a grey platform (122 cm in diameter) elevated 90 cm above the floor. Forty holes, each 5 cm in diameter, were located 5 cm from the perimeter, and a black plastic escape tunnel was placed under one of the holes, which was connected to the home cage of the animal. One training trial was performed before the first day of testing. To this end, the mouse was placed in the centre of the maze and covered with a black start chamber for 2 min. After removing the start chamber, the mouse was guided by the investigator to the escape tunnel, and hence its home cage. One minute after this training trial, the first test session started. At the beginning of each session, the mouse was placed in the centre of the maze in the black start chamber for 2 min. After removing the start chamber, the mouse was allowed to explore the maze for finding the escape tunnel. The session ended either when the mouse had entered the escape tunnel and reached the home cage or, if not successful within 5 min, by guiding the mouse to the escape tunnel. Sessions were repeated daily, with the position of the escape tunnel being invariant throughout the experiment for each mouse. The mice were tested until they met the pre-defined criterion (4 out of 5 sessions with two or fewer errors) over maximally 32 sessions. Serial, random and spatial search strategies were defined as described (Bach et al., 1995).

Eight-arm radial maze – Prior to testing in the radial maze, the animals were singly housed and gradually reduced to 85% of their normal body weight by being fed only limited amounts of chow daily, as detailed by Minichiello et al. (1999). The mice were maintained at reduced weight throughout the experiment. The apparatus was an opaque plastic maze with eight identical arms radiating from an octagonal starting platform. The mice were pre-trained for 5 days (one 15 min trial per day) by placing the mice in the central starting platform and allowing them to explore and to consume food pellets located at the distal end of each arm. After 5 pre-training trials, actual maze trials were started. All eight arms contained a food pellet at the beginning of each trial and the baits were not replaced once they had been taken. Animals received one trial per day (14 trials in total); each daily trial terminated when 8 correct choices were made or 15 min had elapsed. A correct choice was recorded when the

mouse entered an unvisited arm and took the food pellet during the trial, while re-entering into an already visited arm from which the food had already been taken was counted as an error. ANOVA analysis was performed using GraphPad Prism software (GraphPad Inc., San Diego, CA).

References

Bach ME, Hawkins RD, Osman M, Kandel ER, Mayford M (1995) Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. Cell 81:905-915.

Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R (1999) Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24:401-414.

Papadopoulos T, Korte M, Eulenburg V, Kubota H, Retiounskaia M, Harvey RJ, Harvey K, O'Sullivan GA, Laube B, Hülsmann S, Geiger JR, Betz H (2007) Impaired GABAergic transmission and altered hippocampal synaptic plasticity in collybistin-deficient mice. EMBO J. 26:3888-99.

Supplemental Table 1. Summary of behavioural test results of the mGluR7a$^{AAA/AAA}$ mice and their wt littermates

Test paradigm used	+/+	mGluR7a$^{AAA/AAA}$
Rotarod (n = 8, each)		
Rotation speed (rpm)	16.5 ± 1.1	16.3 ± 1.6
Tail flick (n = 8, each)		
Latency (s)	3.6 ± 0.2	3.2 ± 0.2
Open field (n = 8, each)		
Total distance traveled (cm)	2019 ± 333	1605 ± 280
Distance traveled in center (%)	18 ± 3	20 ± 3
Time spent in center (%)	12 ± 3	15 ± 3
Elevated Plus Maze (n = 12, each)		
Total distance travelled (cm)	1747 ± 181	1416 ± 209
Time spent in open arms (%)	1.3 ± 0.4	2.6 ± 1.2
Acoustic startle response (n = 12, each)		
Amplitude of startle response (in AU)	43.9 ± 6.2	53.6 ± 5.9
Prepulse inhibition (%; n=12, each)		
Prepulse intensity 65 dB	19.9 ± 4.2	18.6 ± 3.2
Prepulse intensity 70 dB	43.8 ± 2.2	47.2 ± 5.8
Prepulse intensity 75 dB	57.9 ± 3.3	46.3 ± 7.7
Barnes maze (n = 10, each)		
Strategy used during the last 5 training sessions (%)		
Random	2 ± 2	2 ± 2
Serial	30 ± 11	33 ± 11
Spatial	68 ± 10	64 ± 12
Mice that acquired task (%)	70	60

Data represent means ± SEM. In none of these tests, statistically significant differences ($p<0.05$, Student's t-test or one-way repeated measure ANOVA) were disclosed between genotypes.

Legend to Supplemental Figure 1. **Western blot analysis of mGluR7-deficient mice.**

Western blot analysis of mGluR7a immunoreactivities in brain membranes prepared from wt (+/+) and heterozygous (+/-) and homozygous (-/-) mGluR7 deficient animals (top). Note strongly reduced and absent mGluR7 staining in $GluR7^{+/-}$ and $GluR7^{-/-}$ samples, respectively. Blots were re-probed with a β-tubulin antibody, and mGluR7 band intensities were quantified by densitometry and normalized to β-tubulin as internal standard. Data represent means ± SEM (n=3 per genotype; **, $p<0.01$; ***, $p<0.001$).

Legend to Supplemental Figure 1. **Immunoelectron microscopic visualization of mGluR7a.**

Overview shows several presynaptic terminals in the hippocampal CA3 region of a wt animal that are stained by the mGluR7a antibody. Note that specific immunostaining is restricted to distinct nerve terminals. Inset, enlargement with arrows pointing to labelled and unlabelled neighboring synapses. Note decrease in grain density with increasing distance from the plasma membrane and the low extent of labelling found over distant synaptic vesicles; both are consistent with vesicle labelling being due to intensification product diffusion, and hence support a predominant or exclusive plasma membrane localization of mGluR7a. Scale bars, 0.2 μm.