**Material and Methods**

*Breeding of mutants.*

Mutants with the *Ube3a* null mutation (designated as AS mice) and mutants carrying the targeted αCaMKII-T305V/T306A mutation which prevents phosphorylation at these residues (designated as CaMKII-305/6+/− mice) were developed as described previously. All experiments described in this paper were carried out using hybrid mice in the mixed 129/Sv-C57BL/6 background. To that end, male αCaMKII-305/6+/− mutants in the C57BL/6 background were crossed with female AS mutants in the 129/Sv background (weight, rotarod, watermaze) or with female AS mutants in the 129/Sv-C57BL/6 background (epilepsy, fear conditioning, LTP).

Mice were genotyped when they were 7-10 days, but the experimenter remained blind for the genotype during data collection and the initial analysis. Genotypes were again established after all experiments were done and the code was then broken to perform the final statistical analysis. All experiments were done with 2-4 month old littermates that were housed in groups of 2-3 per homecage. Genotype groups were approximately sex and age matched. Single-housed mice were excluded for the behavioral studies. The mice were kept on a 12h light/dark cycle, with food and water available ad libitum. The behavioral experiments were performed during the light period of the cycle. All animal experiments were approved by the Dutch Ethical Committee, or in accordance with Institutional Animal Care and Use Committee guidelines.

*Western blot analysis of the phosphorylation state of CaMKII.*

Lysates were prepared by quickly dissecting the hippocampus followed by homogenization in lysis buffer (10mM TRIS-HCl 6.8, 2.5% SDS, 2mM EDTA and protease and phosphatase...
inhibitor cocktails (Sigma)). Lysates were then adjusted to 1 mg/ml, and 10 µg was used for SDS-PAGE analysis and Western blotting. Western blots were first probed with antibodies directed against Thr305-αCaMKII (pB60^2, 1:5000) and after stripping re-probed with αCaMKII (MAB3119, 1:10000; Chemicon), and Actin (MAB1501R, 1:2000; Chemicon). Blots were quantified using NIH-Image using the αCaMKII signal as loading control, and normalized against wild-type. The number of mice used: WT (4); AS (4); CaMKII-306/5^+/− (4); AS/CaMKII-305/6^+/− (4).

**CaMKII Activity Measurement**

Mice were sacrificed by cervical dislocation and hippocampi were quickly isolated in ice-cold cutting solution and sonicated in cold homogenization buffer containing 4 mM HEPES, 320 mM sucrose and protease inhibitor cocktail (Sigma, Saint Louis, MO). The homogenates were centrifuged at 1500 g for 10 min. The supernatant was then centrifuged at 16000 g for 20 min to yield a pellet containing the crude synaptosome fraction. This fraction was resuspended in 200 l homogenization buffer to measure the Ca^{2+}/calmodulin dependent CaMKII activity using the assay kit from Millipore Inc. (14-217). The number of mice used: WT (6); AS (6); CaMKII-306/5^+/− (6); AS/CaMKII-305/6^+/− (6).

**Weight and seizure susceptibility**

Female AS mice started to show increased bodyweight after 2 months, whereas male AS mice started to become significantly heavier after 4 months. Number of mice used: females (8-9 weeks): WT (13); AS (7); CaMKII-305/6^+/− (10); AS/CaMKII-305/6^+/−, (13). Males
(16-18 weeks): WT (4); AS (3); CaMKII-305/6+/− (6); AS/CaMKII-305/6+/− (4). To assess seizure susceptibility we induced audiogenic seizures by vigorously scratching scissors across the metal grating of the mouse cage lid for 20 seconds, or shorter if a seizure developed before that time. The number of mice used in this paradigm: WT (11); AS (10); CaMKII-305/6+/− (12); AS/CaMKII-305/6+/− (15).

Motor performance.
We tested motor function in F1 mice using the accelerating rotarod (4-40 rpm, in 5 minutes; model 7650, Ugo Basile Biological Research Apparatus, Varese, Italy). Mice were given two trials per day with a 45-60 min inter trial interval for 4 consecutive days. For each day we calculated the average of the time spent on the rotarod, or of the time until the mouse made 3 consecutive rotations on the rotarod. Maximum duration of a trial was 5 min. The number of mice used: WT (10); AS (7); CaMKII-305/6+/− (10); AS/CaMKII-305/6+/− (8).

Water Maze.
To test spatial memory we used the Water Maze. Prior to the test mice were handled extensively (2 min/day; 5 days). Our pool is 1.2 m in diameter and has an 11 cm diameter platform submerged 1 cm below the water surface. The water is painted milk-white with non-toxic paint and water temperature is kept constant at 26°C. We use dimmed lighting, and mouse-tracking is performed using SMART version 2.0 (Panlab, Barcelona, Spain). Mice were given 2 trials per day, with 30 sec inter trial interval for 8 consecutive days. At a training session, the mouse was first placed on the platform for 30 sec. Then it was placed in the water at a pseudo-random start position and it was given a maximum of 60 seconds to find the platform. If the mouse did not find the platform within 60 seconds, it was placed
back on the platform. After 30 seconds on the platform, this training procedure was repeated once more. The platform position remained at the same position during all trials.

One hour after the training on day 6 and day 8, a probe trial was given to test spatial learning. Mice were placed on the platform for 30 seconds, after which the platform was removed from the pool and the mice were placed in the pool at the opposite side of the previous platform position. The mice were then allowed to search for the platform for 60 seconds. The number of mice used for this paradigm: WT (18); AS (11); CaMKII-306/5+/− (18); AS/CaMKII-305/6+/− (14).

The visible water maze was performed done in the same way as the hidden platform water maze, except that this time the platform was flagged with a small cue on top the platform. The number of (naïve) mice used for this paradigm: WT (6); AS (5); CaMKII-306/5+/− (4); AS/CaMKII-305/6+/− (3).

Fear Conditioning.

Fear conditioning was performed in a testing chamber (26 × 22 × 18 cm; San Diego Instruments, San Diego, CA) made of Plexiglas equipped with a grid floor via which the foot shock could be administered and a CCD camera to monitor activity. The conditioning chamber was placed inside a soundproof isolation cubicle. Training and testing occurred in the presence of white light and white noise. Each mouse was placed inside the conditioning chamber for 150 seconds before the onset of a 2 s foot shock (0.5 mA). After 150 seconds, a second foot shock was delivered, and the mouse was returned to its home cage after another
2.5 min. Testing of context-dependent fear was performed either 24 hours or 1 week after the conditioning in the same context.

For cued conditioning, each mouse was placed inside the conditioning chamber for 2 min before the onset of a conditioned stimulus (CS; an 85 dB tone), which lasted for 30 s. Training occurred in the presence of white light and white noise. A 2 s US foot shock (0.5 mA) was delivered immediately after the termination of the CS. Each mouse remained in the chamber for an additional 120 s, followed by another CS–US pairing. Each mouse was returned to its home cage after another 2.5 min. Cued fear conditioning was tested in the presence of a masked context consisting of a small Plexiglas cube attached to an opaque Plexiglas floor insert, different lighting conditions, and a vanilla odor. Each mouse was placed in this novel context for 3 min at approximately 24 h after training, and they were exposed to the CS for another 3 min.

Freezing behavior was recorded and processed by SDI Photobeam Activity System software throughout each testing session. The mouse was considered to be freezing after a lack of movement for 2 s. Number of animals used: 24 hours context: WT (6); AS (6); CaMKII-305/6+/− (8); AS/CaMKII-305/6+/− (9) ; 1 week context: (WT (7); AS (8); CaMKII-305/6+/− (7); AS/CaMKII-305/6+/− (8). 24 hours cued: WT (11); AS (11); CaMKII-305/6+/− (7); AS/CaMKII-305/6+/− (9) ;

Shock threshold was assessed by placing the animal in the conditioning chamber and by delivering foot shocks starting at 0.075 mA and increasing by 0.05 mA every 30 s. The experiment was terminated at the shock intensity sufficient to induce vocalization. There was no difference in shock sensitivity between the groups (F_{3,75}=0.53, P=0.9 ANOVA).
**Hippocampal slice preparation and electrophysiology**

The brain was quickly removed and placed in ice-cold high sucrose cutting solution containing (in mM): 110 sucrose, 60 NaCl, 3 KCl, 28 NaHCO₃, 1.25 NaH₂PO₄, 7 MgCl₂, 0.5 CaCl₂, 0.6 sodium ascorbate, and 5 D- glucose, pH 7.3–7.4. Horizontal 400 μm sections were cut in sucrose cutting solution using a Vibratome. The slices were stored in 95% O₂/5% CO₂-equilibrated artificial CSF containing (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 28 NaHCO₃, 1.0 MgCl₂, 2.0 CaCl₂, and 10 D- glucose, (pH 7.3-7.4) and kept at room temperature for at least 1 hour before switching to the interface chamber supported by a nylon mesh and allowed to recover for a minimum of 1 hour prior to recording. The chamber was kept at 32 ± 0.5 °C with a laminar ACSF flow rate of 2–3 ml/min. Extracellular field recordings were obtained from area CA1 of the stratum radiatum. Stimulations were supplied with a bipolar Teflon-coated platinum electrode and recordings were obtained with the use of a glass microelectrode field with ACSF with tip diameter about 1 μm (1–4 MΩ electrical resistance). Tetani used to evoke CA1 LTP consisted of two trains of 100 Hz stimulation for 1s with each train separated by a 20s interval. Stimulus intensities were adjusted to give pEPSPs (population excitatory postsynaptic potentials) with slopes that were <50% of the maximum determined from an input/output curve. Potentiation was measured as the normalized increase of the mean pEPSP for the duration of the baseline recording. Experimental results were obtained from those slices that exhibited stable baseline synaptic transmission for a minimum of 30 min prior to the delivery of the LTP-inducing stimulus. For recording we used an Axon 1320 Digidata data acquisition hardware operated by Axon.
pClamp 9.2 software. We used maximally two slices per mouse. The number of slices used for the experiment are: WT (11); AS (6); CaMKII-305/6\textsuperscript{+/−} (6); AS/CaMKII-305/6\textsuperscript{+/−} (6).

Statistical analysis.

StatView data analysis software was used for statistical analysis. An ANOVA or repeated measures ANOVA was used for multiple group data, to test for the effect of genotype. If this was significant, the data was further analyzed using the Fisher PLSD Post Hoc test. All figure data represents mean ±SEM. An analysis with a value of \( p<0.05 \) was considered to be statistically significant (indicated by an asterisk in the figures).

References