Supplementary Methods.

General surgical procedures. Briefly, animals were anesthetized with an IP dose of Ketamine and Xylazine (Ketaject®-Phoenix 0.12 ml/100gBW and Anased® Lloyd 0.07 ml/100gBW) after a light sedation with Halothane (Halothane® - Halocarbon). During anesthesia, animals were given a SC dose of Ketoprofen (Ketoprofen®- Dodge 0.5 mg/100g BW), as a post-surgical anti-inflammatory agent. The surgical procedure was standard and included aseptic exposure of the skull surface and the brain surface corresponding to the lesion or injection coordinates. After the lesion, sham or injection procedures, the muscle and overlying skin wounds were closed, fluid therapy was given SC, and rats were allowed to recover in a heated cage and returned to the animal facilities when fully awake.

Temporoammonic pathway and total hippocampus electrolytic lesions. Bilateral electrolytic lesions were made with stainless steel 500 µm thick electrodes (FHC®- Catalog#UESSGESEANNM) as the cathodes and silver wire, inserted in the neck muscles, as anodes. The current delivery electrode was guided to the lesion spots using a Benchmark™ stereotaxic apparatus, in which the animal was positioned according to standard procedures. For TA lesions, a current of 0.4 mA for 5 sec was passed at each spot using a current generator. The current parameters were determined in pilot experiments to optimize the destruction of the TA axons, while sparing the surrounding structures. Sites were chosen in order to optimize the disconnection between the entorhinal cortex and area CA1, based on both anatomical [1,2] and electrophysiological [3-5] findings. The lesion spot coordinates [6] were as follows:

Level 6.10 from bregma, 5.6 lateral to the midline and 6.7 and 6.0 mm ventral to the skull surface, 5.5 lateral to the midline and 7.2, 5.6 and 5.0 ventral to the skull surface, 5.3 lateral to the midline and 7.5 and 4.7 ventral to the skull surface, 5.1 lateral to the midline and 7.6 and 4.3 ventral to the skull

surface, 4.7 lateral to the midline and 8.0 and 3.9 ventral to the skull surface. Level -5.7 from bregma, 4.0 lateral to the midline and 7.8 ventral to the skull surface. Level -5.3 from bregma, 5.5 lateral to the midline and 7.6 ventral to the skull surface. All coordinates for stereotaxic surgeries were optimized to target the majority of the TA axon fibers as they enter the hippocampus, based on slice physiology experiments [3-5] and anatomical data [1,2].

Total hippocampus lesions were made with the same protocol described, but with a current intensity of 1 mA for 15 sec at the following coordinates:

Level 5.6 from bregma, 4.8 lateral to the midline and 7.0, 6.0 and 4.6mm ventral to the skull surface, 4.0 lateral to the midline and 7.6, and 3.6 ventral to the skull surface. Level 4.3 from bregma, 4.0 lateral to the midline and 3.6 ventral to the skull surface, 3.0 lateral to the midline and 3.2 ventral to the skull surface, 2.0 lateral to the midline and 3.4 and 2.8 ventral to the skull surface. Level 3.8 from bregma, 2.0 lateral to the midline and 2.8, and 3.4 mm ventral to the skull surface, 3.0 lateral to the midline and 3.2 ventral to the skull surface. Level 3.3 from bregma, 1.0 lateral to the midline and 3.6, 2.0 lateral to the midline and 3.6 and 3.0 ventral to the skull surface, 3.0 lateral to the midline and 3.6. Finally, level 2.8 from bregma, 1.0 lateral to the midline and 3.6 ventral to the skull surface, 2.0 lateral to the midline and 3.5 ventral to the skull surface. In four of the five animals lesioned, the two posterior lesion sets were not performed, only the dorsal hippocampus was lesioned. All lesions were made bilaterally in all animals. All sham animals were operated in the exact same way as the lesioned ones, except that no current was passed in the electrodes.

Lesion assessment. The effects of total hippocampal lesions were verified visually after sacrificing the animals and cutting slices from their brains followed by Nissl staining. All 5 animals in which the total hippocampus was targeted had a complete and specific hippocampal ablation, without any significant

lesions of the overlying cortex or adjacent subcortical structures. Acknowledging the inherent difficulty and uncertainty associated with electrolytic lesions, we used three different techniques to assess the extent and specificity of the TA lesions: retrograde tracing, electrophysiology, and quantitative stereological estimates. *Retrograde tracing of the projections from the hippocampus and DG to the EC*. A subset of the control (n = 4) and TA-lesioned (n = 3) animals were subject to a retrograde tracing technique. Animals were injected with a recently developed retrograde tracer, Cholera Toxin Subunit B (CT-B) conjugated with Alexa 488 ® Molecular Probes, at two distinct septo-temporal levels of the hippocampus. At each level, one spot in the CA1 area and one spot in the DG area was injected. The injections were made stereotaxically with a beveled-tip 5 µl Hamilton syringe, and 1µl was injected at each spot for a 30 sec total injection time. The coordinates for the injections were as follows:

Level 5.6 posterior to bregma, 4.0 lateral to the midline, 4.2, and 3.6 ventral to the skull surface. Level 4.30 posterior to bregma, 2.2 lateral to the midline and 3.6 and 3.2 ventral to the skull surface.

After 4-8 days, the animals were perfused with a 4% paraformaldehyde/ 1% glutaraldehyde solution in PBS 1x. The brain was extracted from the skull and stored in the same fixative for a period of 6 hours, after which it was transferred to 20% sucrose in PBS 1x solution where it stayed overnight. After this period, longitudinal sections were cut, starting at the ventral surface of the brain, and parallel to the dorsal surface. The sections were mounted onto gelatin subbed slides and allowed to dry for around 1 hr, after which they were observed under a CCD-coupled fluorescence microscope.

Electrophysiology and quantitative stereology. After behavioral testing was completed, animals were sacrificed and transverse slices were cut as previously described [3]. The sections of the hippocampus in which lesions were made (a limited longitudinal portion of the total hippocampus

corresponding to the vertical half) were allowed to recover in an oxygenated chamber after which some of them were fixed in 10% paraformaldehyde for histology and some were used for electrophysiological recordings. The fixed slices were then analyzed by an individual blind to the experimental manipulation and the behavioral data. The criteria for inclusion in the TA-lesion group was a major (> 75%) of the TA input and only minor (<15%) damage to the surrounding (DG, CA fields, subiculum) (Fig. S1). In adjacent (non-fixed) slices, electrophysiological data were collected and analysed with in-house software built in Labview, and transferred to Microcal origin for graphical display as previously published [5]. The purpose of these experiments was to determine whether other hippocampal synapses were affected by our lesions. We collected bright field images of the slices and recorded synaptic transmission at the following synapses: PP-DG, SLM-CA1 and CA1 (alveus/stratum oriens)-subiculum. Input-output synaptic relations were assessed using stimulus intensities ranging from 100-500 µA for 100 µs. The traces shown were obtained at the following intensities: PP-DG 500 µA, TA-CA1 300 µA and CA1-SUB (output) 500 µA. Based on these criteria, 10/40 animals were included in the TA lesion group for the experiment shown in Figure 2 and 5/15 animals were included in the TA lesion group for the experiment shown in Figure 3.

Another subset of animals were intracardially perfused with 5% paraformaldehyde in PBS 1x, their brain was removed and sectioned on a vibratome (100 µm slices). Every third slice was mounted on a glass slide and Nissl stained. These slices were used exclusively for a quantitative estimation of the lesion. Again, lesion estimation was done by an individual blind to the behavioral data. The same criteria were used: only those animals which possessed ablations > 75% of the TA input and < 15% of adjacent areas were included. Based on these criteria, 12 out of 33 attempted TA-lesioned animals were included in the behavioral analysis (1 in Fig. 2, 2 in Fig. 3 and 9 in Fig. 4). The animals included

had on average 8.2 +/- 1.1 % of the total hippocampal mass lesioned, and on average 90.1+/-2.8 TA ablation (Fig. S2).

References.

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