Supplemental Information for

The rodent hippocampus is essential for non-spatial object memory

Sarah J. Cohen, Alcira H. Munchow, Lisa M. Rios, Gongliang Zhang,

Herborg N. Ásgeirsdóttir and Robert W. Stackman, Jr.†

† To whom correspondence should be addressed. E-mail: rstackma@fau.edu

This file includes:

Supplemental Data:

Figure S1 depicts total object exploration of the two groups of mice for each experiment presented in Figure 2, and depicts results of an experiment that follows up from that presented in Figure 2A - C.

Figure S2 depicts results from an experiment in which hippocampal CA1 place cells were recorded from mice during the performance of an object recognition task. These results are related to Figure 3.

Supplemental Experimental Procedures:

Detailed Materials and Methods

Supplemental Information

Supplemental Data

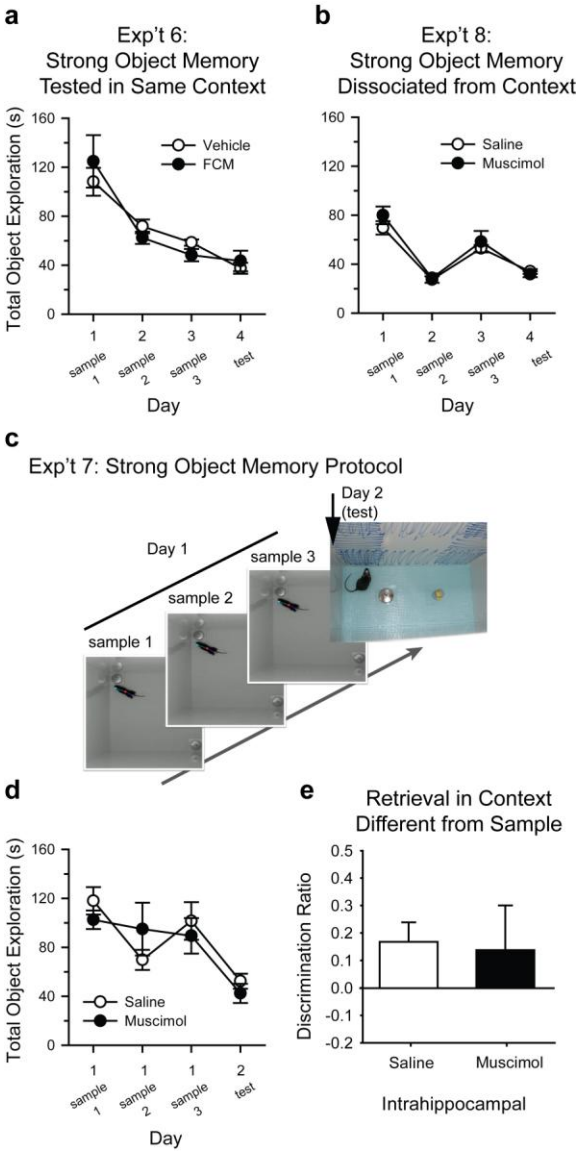

Figure S1. Data from Performance of Modified NOR Tasks related to Figure 1

a. In Exp't 6, mice explored two identical objects during each of three 10-min sample sessions (1/day), then received a test session 24 h later. All sessions were conducted in the same familiar arena. Mice received intrahippocampal saline or FCM 40 min before the test session. Total object exploration decreased over the course of the sample session, as objects became familiar. Object exploration did not differ between the treatment groups. **b.** In Exp't 8, mice explored two

identical objects during each of three 10-min sample sessions (1/day) *with each session in a novel arena*. The next day mice received intrahippocampal saline or muscimol and 40 min later received a test session in a novel arena (see Fig 2*F*). Object exploration did not differ between the treatment groups. **c.** Modified NOR task in which mice explored the same two identical sample objects during three 10-min sample sessions presented in the same context on the same day (Exp't 7). The next day (see arrow), mice received intra-hippocampal infusions of saline (n = 6) or muscimol (n = 4) 40 min prior to a test session in a novel context. **d.** Object exploration did not differ between the treatment groups across the respective sample and test sessions. **e.** Neither pre-test saline- nor muscimol-treated mice exhibited a strong preference for exploring the novel object during the test session presented in a novel context (discrimination ratios were not significantly greater than a chance score of 0: saline, $t(5) = 2.38$, *n.s.*; muscimol, $t(3) = 0.85$, *n.s.*). There was no difference in the mean discrimination ratios between the two groups, $t(8) = 0.19$, *n.s*. These results are consistent with the reports in the literature that mice encode an object-in-context memory when the three sample sessions are presented in the same context. Changing the context for the test session then impairs the retrieval of the object-in-context memory.

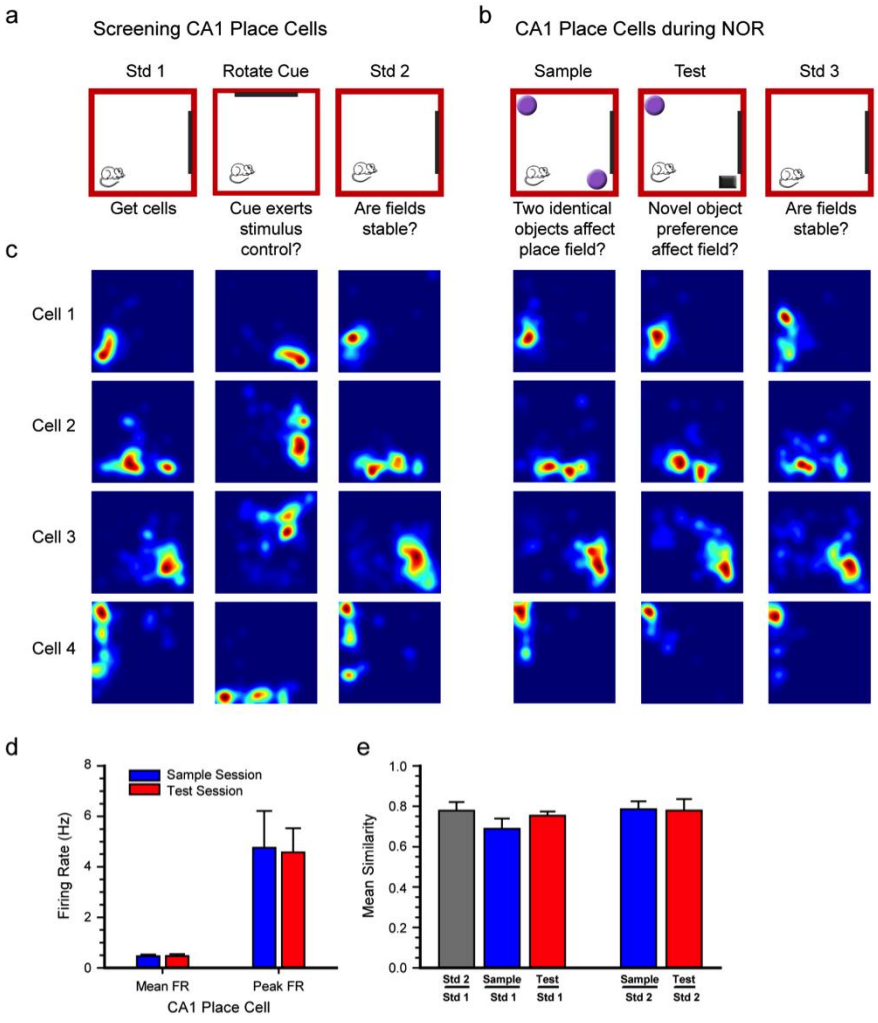

Figure S2. Place Fields of Hippocampal CA1 Neurons are Stable during Performance of the NOR Task (Exp't 10) related to Figure 3

The activity of hippocampal CA1 pyramidal neurons was recorded across the NOR sample and test sessions in a square arena containing a cue card on the East wall. **a.** Cue card stimulus control over place field location was established during sessions (three leftmost columns) in which the cue card was rotated 90° and then returned to its original position. **b.** Next, the same cells were recorded in the same arena during a sample session and again during a test session. **c.** Place fields remained stable across NOR sessions. **d**. Mean and peak firing rates of place cells did not change over the course of the NOR task. **e.** Mean similarity scores for pixel-by-pixel firing rate correlation analyses of respective pairs of firing rate maps. High similarity scores for all pairwise

comparisons indicate that the act of engaging in a hippocampal-dependent NOR task did not alter established place fields.

Supplemental Experimental Procedures

Mice and Surgery. Male C57BL/6J mice (7-10 wk old; Jackson Labs) were housed in a 12 h light/dark cycle, temperature- and humidity-controlled vivarium 4/cage with ad libitum access to food and water. Behavioral testing was conducted during the light cycle. All procedures were conducted in accordance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee.

Novel Object Recognition Task. The apparatus consisted of two open-top, high-walled square arenas made of white ABS (each: 37.5 x 37.5 x 50 cm). For all experiments except the object memory dissociated from context experiment (Exp't 8), each mouse was habituated to one of the arenas for 10 min/day for 2 consecutive days. For all other behavioral experiments, except the retrieval of strong object memory experiment (Exp't 6-7), each mouse received one sample session and one test session in the habituated arena (see Fig 1*A*). During the sample session, each mouse was returned to the familiar arena that now contained two identical novel toy objects (stainless steel cabinet leveling feet, 4.2 cm dia and 6.0 cm tall). The two sample objects were positioned on the arena floor 2 cm from opposite corners (NW and SE). The mouse was removed from the arena upon accumulating 30 s of exploration of both objects or 38 s of either object within a 10-min session. This was referred to as the sample object exploration criterion and was imposed to ensure that all mice accumulated similar exploration time with the objects. During the test session 24 h later, each mouse was given a 5 min test session in the familiar arena, which contained one of the familiar objects and one novel object (plastic toy gorilla or metal spring attached to a Plexiglas base). The objects and the arena floor and walls were cleaned with 10% ethanol after each session. All behavioral testing data was digitally acquired by the EthoVision

XT (Noldus Inc.) software package. Object exploration was scored off-line from the digital video files by experimenters that were blind to the treatment condition of the mice (inter-rater reliability, $r = 0.9$). Object memory was inferred from the discrimination ratio – calculated for each subject by subtracting the time spent exploring the familiar object from the time spent exploring the novel object and dividing the result by the total time spent exploring both objects. Discrimination ratios range from -1 to 1, with 0 indicating chance performance – a lack of preference for one object over another, and positive ratios indicating novel object preference.

Modified versions of the NOR task (see Fig. S1*C* and Fig. 2*C*) were used in Exp'ts 7 and 8, respectively to dissociate object memory from that of the context where objects were encountered. The protocol for Exp't 7 was similar to that of Exp't 6 except that each mouse received three sample sessions in the familiar arena all in the same day with a 1-min delay between each session. The test session was presented in a novel context 24 h after the final sample session. The distinct context used for the test session was a 37.5 x 18 x 50.8 cm rectangular arena constructed of three walls of white ABS acrylic sheet, and a fourth wall of white and blue-colored foam board. A pale teal plastic textured drape wiped with 10% acetic acid was placed on the arena floor). For Exp't 8 (see Fig. 2*C*), mice explored the same two identical objects on each of three 10-min sample sessions (1 session/day) in a distinct context each day, Day 1 (Context A, same ABS arena as above); Day 2 (Context B, a rectangular 30.5 cm x 24.1 cm x 21 cm chamber of brushed aluminum side walls, clear Plexiglas top, front and back walls, and the floor consisted of 36 parallel stainless steel rods 3.2 cm diameter, spaced 7.9 mm apart); Day 3 (Context C, a black 45 cm diameter polyethylene high-walled cylinder, open at the top and blue surgical drape placed on the floor). On Day 4, mice received a test session (Context D, a high-walled 37.5 x 18 x 50.8 cm rectangular arena with three walls of white ABS acrylic sheet, and the fourth of white and blue-colored foam board. A pale teal plastic textured drape wiped with 10% acetic acid was placed on the arena floor).

Intrahippocampal Cannulation and Microinfusion. Mice were implanted with chronic bilateral guide cannulae (Plastics One, Inc.) above the CA1 region of dorsal hippocampus (A/P - 2.0 mm, M/L ± 1.5 mm, D/V - 1.1 mm from bregma) [50]. Mock infusions were given each day for the 2 days prior to the actual intra-hippocampal microinfusion to habituate the mice to the microinfusion procedure. At the time of actual infusions, mice received bilateral (0.35 µl/side, 0.334 µl/min) intra-hippocampal muscimol (1 µg/µl in 0.9% saline, Tocris), fluorophore-conjugated muscimol (FCM, 1 µg/µl in PBS, BODIPY TMR-X, Molecular Probes), anisomycin (40 µg/µl dose in PBS, Sigma Aldrich) or the appropriate vehicle. For Exp't 1 (encoding), muscimol or saline was infused 20 min prior to the sample session. For Exp't 2 (consolidation) muscimol or saline was infused immediately after the sample session. Anisomycin or PBS was infused immediately and 2 h after the sample session (Exp't 3) or only 2 h after the sample session (Exp't 4) to test the dependence of object memory consolidation on protein synthesis within the hippocampus. For Exp't 5 (simulated permanent hippocampal lesion) muscimol or saline was infused 20 min before and 2 h after the sample session, and again 40 min before the test session. For Exp't 6 (retrieval of strong object memory), mice received intra-hippocampal FCM or vehicle 40 min before the test session. For Exp't 7-8 (object memory dissociated from context), mice received intra-hippocampal microinfusion of saline or muscimol 40 min prior to the test session. For all experiments in which mice received *pre-test* muscimol or FCM, mock infusions were given before daily sample sessions (Exp't 6 and 8), or before the first of the three sample sessions presented in one day (Exp't 7).

***In Vivo* Microdialysis of the Hippocampus.** Under deep isoflurane anesthesia, mice (Exp't 9) were implanted with a unilateral guide cannula over the CA1 region of dorsal hippocampus, and then recovered for 7 days. Eighteen hr after a sample session (conducted as above), the microdialysis probe (cut-off 18 kDa, 0.5 mm membrane) was inserted through the guide cannula and secured in place. The probe inlet was infused with artificial CSF (aCSF) at a flow rate of 1.2

µl/min. After 6 hr of equilibration, three 10-µl samples were collected at an interval of 10 min to obtain basal extracellular glutamate level from dorsal hippocampus. Each mouse was randomly assigned to either a *test session* as described above, or a second sample session (the control condition, *sample session 2*) in the familiar arena, during which five 10-µl dialysis samples were collected. Dialysate samples were analyzed by HPLC with electrochemical detection, and the dialysate glutamate level in each sample was estimated by comparing peak areas between the sample and a known standard. Samples were first added to 10 µl of derivation reagent (2-mercaptoethanol in 1 ml O-phthaldialdehyde solution, pH 9.5). Ten µl of derivate solution was manually injected into a reverse-phase microsphere column (2 × 100 mm, packed with a C18, 3 µm particle size, Agilent). A working electrode (MF-1000, BAS) was set at +700 mV vs an Ag/AgCl reference electrode. Mobile phase (0.1 M phosphate buffer, pH 6.0 with 5% methanol and 40 mg/L EDTA) was delivered by a LC -10AT Shimadzu pump at 400 µl/min. Glutamate was estimated by comparing peak areas between microdialysis samples and a known standard using Powerchrom V2.2 software. The mean relative recovery efficiency of the microdialysis probe was 8% during in vitro assessments.

***In vivo* Recording of Hippocampal CA1 Neurons.** Under deep isoflurane anesthesia, mice were implanted with microdrives containing four tetrodes (each a twisted bundle of four 25-µm nichrome wires, California Fine Wire; Exp't 10-11). After mice recovered for 7 days, tetrodes were advanced ventrally by 50-75 µm/day. Recording sessions began when hippocampal CA1 pyramidal cells were detected as exhibiting complex-spike bursts and spiking modulated by the theta rhythm. Neuronal activity was monitored while mice moved freely about the floor of the high-walled square arena. Spikes (filtered at 150-8000 Hz; digitized at 40 kHz) and mouse behavior were simultaneously recorded with a MAP and CinePlex system (Plexon, Dallas, TX) and analyzed by manual and automatic sorting with OfflineSorter software (Plexon v3.2.1). Only units with clear boundaries and less than 0.5% of spike intervals within a 1-ms refractory period

were included in the following analyses. The putative pyramidal neurons were included in analyses if they had: 1) low baseline firing rate (0.5-10 Hz) and irregular firing pattern; 2) dominant short interspike interval (3-10 ms); and 3) a wide waveform (>300 μsec).

Histology. Cannulae, microdialysis probe and tetrode placements were confirmed by examination of Cresyl violet stained 50-μm coronal sections with light microscopic methods. The data for any mice that were determined to have inappropriate placement were excluded from the analyses.

Data Analysis. Mice that exhibited motor impairments after muscimol infusion or inability to reach sample exploration criterion within 10 min were excluded from the analyses. To test the strength of the novel object preference during the test session, we analyzed the discrimination ratios between vehicle and respective muscimol, anisomycin or FCM group using Student's *t*-tests. To determine whether motivation to explore objects during the sample session was affected by the respective treatment, latency to reach the sample session object exploration criterion measures were also analyzed using Student's *t*-tests. Total test session object exploration measures were also analyzed using Student's *t*-tests. For Exp't 6-8, sample session object exploration was analyzed according to future treatment groups using a two-factor [treatment, sample session (1-3)] repeated measures ANOVA. *In vivo* microdialysis data were analyzed by a two-factor [group (*test session* or *sample session 2*), dialysate sample] ANOVA. Peak firing rates were determined for each CA1 neuron during each respective recording session and then analyzed by Student's *t*-test to determine whether firing rates were influenced by the behavioral condition. Similarity of place fields across sessions was determined by calculating the Pearson product-moment correlation between the location-by-firing rate arrays corresponding to the two sessions. Similarity scores were computed for pairs of place field maps determined for respective recording sessions by conducting a pixel-by-pixel correlation analysis. For all tests, differences were considered significant if $P < 0.05$.