Supporting Information

Czajkowski et al. 10.1073/pnas.1313222111

SI Methods

Histology. After completion of behavioral experiments, mice were perfused with 4% (wt/vol) paraformaldehyde. Brains were sliced coronally into 40-µm sections. For experiments involving infusion, cannula placement was confirmed. For CREB experiments, the extent of the viral infection was determined by GFP fluorescence. Only those mice that showed bilateral expression of GFP in the target region (RSC) were included in statistical analysis. Quantitative analysis of infection level was performed using ImageJ. The total number of GFP positive cells was counted bilaterally with a fixed sample window (0.04 mm^2) in six sections separated by 0.32 mm (total anteroposterior length: 1.6 mm, centered on the cannula trace). To assess the number of nuclei in the areas counted, the slices were counterstained using DAPI.

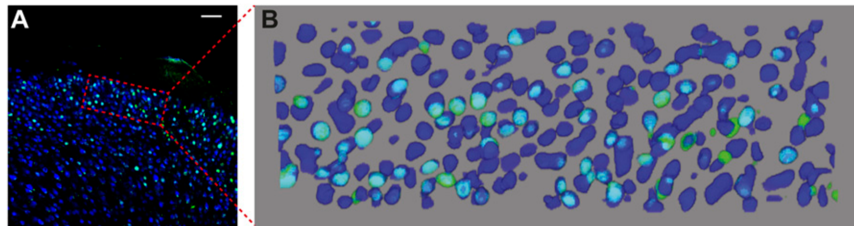

Fig. S1. Cells expressing reporter gene FosGFP in the dysgranular retrosplenial cortex (RSC-d). Immediately after the completion of the last session (5MP) of the in vivo experiment, brains were fixed, sliced, and labeled with anti-neuronal nuclei (NeuN) antibody to visualize neuronal nuclei together with FosGFP-positive cells to estimate the percentage of activated cells. (*A*) Single optical slice of the dysgranular RSC area. Green, FosGFP-expressing cells; blue, NeuN. (*B*) Three-dimensional reconstruction of area indicated in *A*. Neuronal nuclei imaged in each channel were identified and counted to obtain an estimate of percentage of FosGFP-expressing cells. NeuN-positive: 213 cells. FosGFP-positive: 101 cells. Fraction of FosGFP positive cells: 0.47.

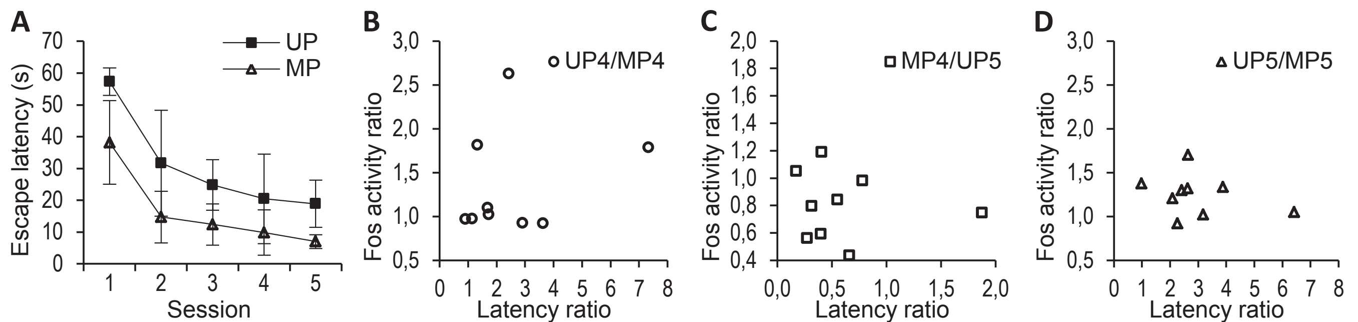

Fig. S2. Fos activity does not correlate with performance in Morris water maze. (*A*) Learning curves for unmarked platform (UP) (filled squares) and marked platform (MP) (open triangles). (*B*–*D*) Differences in FosGFP expression (measured as activity ratio) for individual animals (markers 1–8) show no correlation with differences in behavioral performance (measured as completion time ratio). (*B*) $R^2 = 0.063$. (*C*) $R^2 = 0.015$. (*D*) $R^2 = 0.085$.

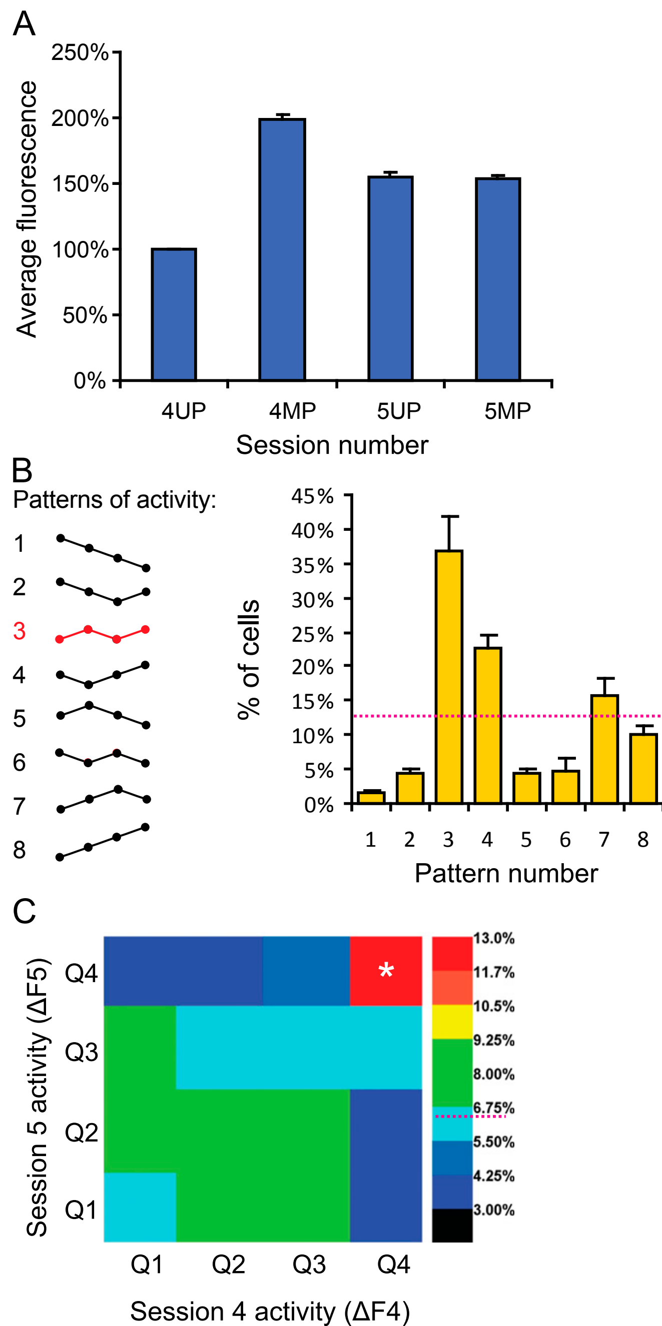

Fig. S3. Two-photon in vivo imaging of FosGFP reporter in the somatosensory cortex (SSC) during water maze. (*A*) Summary of FosGFP activation in the SSC after alternating UP and MP sessions. MP session 4 evoked the highest expression of the reporter. (*B*) Summary of activation changes displayed by SSC cells during the imaging experiment. Each cell could follow one of eight patterns of response. Approximately 35% of the cells show higher FosGFP level in both MP sessions and lower in the UP sessions (pattern 3, marked in red). (*C*) High Fos level in MP4 is a good predictor of activity in MP5. Cells that follow pattern 3 in *B* were divided into four quartiles according to the ΔF4 and ΔF5 values and a colocalization matrix was created. Cells in Q4 that show lowest ΔF4 (high 4MP with low 4UP) are twice as likely as chance to remain in the Q4 also for session 5. Matrix element [4,4] contains 12.28% of the entire population, statistically different from random distribution (*Z score = 4.30, $P < 0.001$).

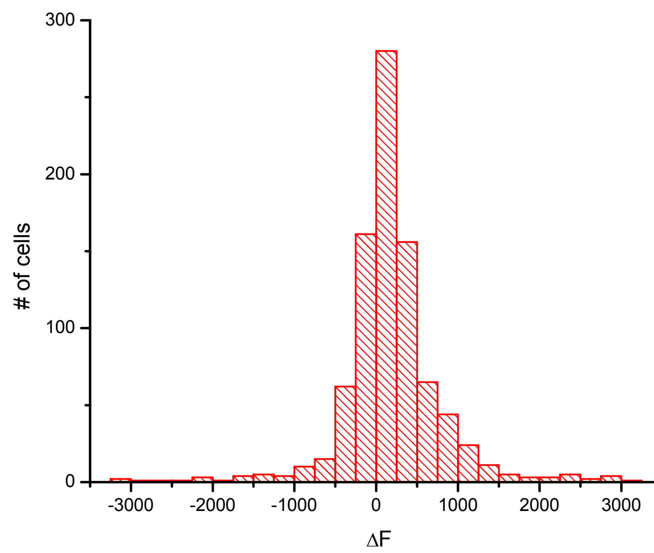

Fig. S4. Distribution of ΔF values in a typical session. No separate peak of FosGFP-expressing cells was detected.

Fig. S5. Functional estimate of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) diffusion in retrosplenial cortex using seizure-induced Fos mapping. Mice (n = 7) were implanted with guide cannuli (24 gauge) using the same coordinates as in the water maze experiment (bregma −2.9 mm, lateral ±0.25 mm, depth −0.5 mm). Animals were allowed 1 wk to recover. On the day of experiment, each mouse received an infusion of 0.65 μL of CNQX (1 mg/mL) into the right hemisphere and 0.65 μL of PBS into the left hemisphere (the infusion cannula protruded 0.5 mm beyond the guide tip). After 10 min the animals were injected with potent convulsant pentylenetetrazol (40 mg/kg), and seizures were observed in a subset of animals within 5 min. Only animals with uninterrupted flow in both cannuli and with full symptoms of seizures were included in further analysis (n = 3). Ninety minutes after the onset of seizures the animals were perfused with 4% paraformaldehyde, sliced coronally, and labeled for Fos using rabbit anti-Fos polyclonal antibody (Santa Cruz Biotechnology) and goat anti-rabbit IgG secondary antibody coupled to Alexa 555 (Invitrogen). Fos positive cells were counted on each side of the brain at the following anteroposterior coordinates: bregma −2.4 (CA1, subiculum, RSC), bregma −2.9 (subiculum, RSC), bregma −3.4 (RSC). For each identified region of the CNQX-infused side, the percentage of Fos-positive nuclei related to the saline-infused side was determined. (*A*–*C*) Fos expression in CNQX- and PBS-infused hemisphere. Each microphotograph shows Fos-positive nuclei imaged at the area corresponding to the dashed box (*Upper*). (*A*) Bregma −2.4 (0.5 mm anterior to the injection site). Dorsal subiculum and CA1 are present in this section within approximately 1 mm from the injection site, but no effect of drug on Fos expression is visible in these structures. Fos expression in the CNQX-treated rostral part of the RSC is visibly reduced. (*B*) Bregma −2.9 (injection site). Dorsal subiculum is visible in this section, but no effect of CNQX on Fos expression is detected in this structure. Fos level is strongly reduced in the RSC. Positions of cannula tips are indicated. (*C*) Bregma −3.4 (0.5 mm posterior to the injection site). Fos level in the RSC is diminished compared with control. (*D*) Summary of Fos counts from the RSC, dorsal subiculum, and dorsal CA1 at coronal levels presented in *A*–*C*. Each bar shows the percentage of Fos-positive cells detected on the CNQX-infused side related to Fos counts within the corresponding area on the contralateral, PBS-infused site. n = 3, bars show means ± SEM.

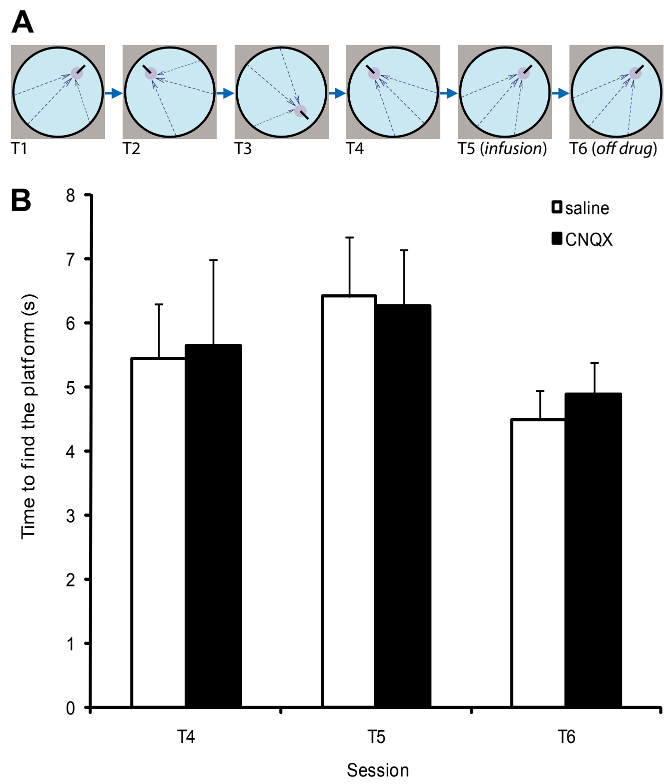

Fig. S6. RSC inactivation during visible Morris water maze. (*A*) Experimental design. Gray circles represent alternating platform location; dashed arrows show release points in each session; black line represents intramaze marker of the platform position. (*B*) Average time to reach the visible platform in the last three sessions.