SUPPLEMENTARY INFORMATION

Hippocampal Memory Traces Are Differentially Modulated By Experience, Time, And Adult Neurogenesis

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INVENTORY OF SUPPLEMENTAL INFORMATION

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Figure S1. ArcCreER^{T2} x R26R-STOP-floxed-EYFP recombination efficiency. (**A**) Mice were injected with TAM and then administered a 1- or 4-shock CFC paradigm 5 h later. Mice were sacrificed 1 h following the start of CFC and tissue was processed for Cre recombinase and Arc. (**B**) To assess recombination efficiency, confocal microscopy was then used to assess co-localization of Arc and Cre-recombinase in the DG. Over 99% of Arc^{+} cells expressed nuclear Cre recombinase, regarßdless of the CFC protocol administered. (**C**) Representative image of Cre recombinase and Arc. (**D**) Mice were injected with vehicle (Veh) and then administered the contextual fear conditioning (CFC) paradigm 5 h later. Mice were sacrificed 5 days later and tissue was processed for EYFP. (**E**) ArcCreER^{T2} x R26R-STOP-floxed-EYFP exhibit low recombination in the DG, both dorsally and ventrally (dorsal average: 4.994 EYFP^{+} cells per section; ventral average: 4.611 EYFP^{+} cells per DG section). (**F-I**) Representative images of EYFP^{+} cells in the DG following a Veh injection. Arrows indicate EYFP^{+} cells. (**J**) Binding of TAM releases CreER^{T2} from heat shock complexes in the cytoplasm, allowing for ligand-dependent translocation to the nucleus, where it can direct recombination between the loxP sites. Representative images of Cre recombinase staining in Veh-injected and TAM-injected mice. Error bars represent $\pm$ SEM. See also Figure 1.

Figure S2. EYFP expression throughout the brain following 1-shock CFC. (**A**) Mice were injected with TAM and 1-shock CFC paradigm was administered 5 h later. Mice were sacrificed 5 days following the CFC training. (**B-M**) Representative images throughout the brain of EYFP expression. S: subiculum, VC: visual cortex, AC: auditory cortex, DMC: dorsomed hypothal nu, compact, DG: dentate gyrus, CA3: Cornu

Ammonis region 3, CPu: caudate putamen, DP: dorsal peduncular cortex, IL: infralimbic cortex, PrL: prelimibic cortex, CE: central amygdaloid, BLA: basolateral amygdala. See also Figure 2.

Figure S3. Arc and c-fos expression patterns differ dramatically in CA3. (**A-B**) 10X and 20X images, respectively, of EYFP and Arc expression in the HPC. While Arc labeling in the DG is relatively somatic, the labeling becomes primarily dendritic in CA3. (**C-D**) 10X and 20X images, respectively, of EYFP and c-fos expression in the HPC. c-fos labeling is relatively somatic, not only in the DG, but in CA3 as well, making co-localization studies in CA3 much easier by using c-fos as a marker of activity.

See also Figure 3.

Figure S4. *In vivo* optogenetic inhibition of CA2 does not impair expression of initially encoded memory in ArcCreER^{T2} x R26R-CAG-STOP-floxed-Arch-GFP mice. (**A**) Genetic design. (**B**) Representative fiber tracts in the DG, CA3, and CA2 implanted mice, and their respective coordinates. (**C**) Representative trace of DG Arch-GFP^{+} neurons. (**D**) Arch-GFP expression does not differ in mice labeled in context A or in context C. (n = 3-4 mice per group). (**E**) Experimental design. Mice were implanted with fiber optics into mainly CA2 and allowed >2 weeks to recover. Mice were then injected with TAM and administered 4-shock CFC 5 h later. Two weeks later, mice were placed back into context A for 6 min. Two days later mice were placed into a novel context B for 6 min. (**F-G**) ArcCreER^{T2}(+) and (-) did not differ in fear expression during or following laser inhibition in either the training context A or novel context B during the first minute of light ON or the first minute of light OFF (minute 1 (light ON) versus minute 4 (light OFF): repeated-measures ANOVA, genotype effect, $F(1,19) = 0.002$, $p =$

0.96; light ON/OFF effect, $F(1,19) = 67.603$, $p < 0.0001$; genotype x light ON/OFF, $F(1,19) = 2.795$, $p = 0.11$) (*t*-tests for light ON and for light OFF: p's > 0.05). Minutes 1-3 (light ON) versus minutes 4-6 (light OFF) were also analyzed and gave similar results (repeated-measures ANOVA, genotype effect, $F(1,19) = 0.398$, $p = 0.54$; light ON/OFF effect, $F(1,19) = 74.947$, $p < 0.0001$; genotype x light ON/OFF, $F(1,19) = 0.224$, $p = 0.64$). (n = 9-12 mice per group). Error bars represent ± SEM. See also Figure 5.

Figure S5. *In vivo* optogenetic inhibition of CA3 impairs expression of initially encoded memory in ArcCreER^{T2} x R26R-CAG-STOP-floxed-eNpHR3.0-EYFP mice. (**A**) Genetic design. (**B-D**) Representative EYFP images of the HPC, DG, and CA3 at 2 weeks post TAM injection. (**E**) Representative eNpHR3.0-EYFP^{+} CA3 pyramidal cell. (**F-G**) In CA3, *in vitro* photostimulation resulted in complete inhibition of APs. Voltage (upper trace) and current clamp (lower trace) recording of an eNpHR3.0-EYFP^{+} CA3 neuron. *In vitro* photostimulation resulted in 94.58 ± 17.23 pA steady state current in eNpHR3.0-EYFP^{+} CA3 neurons, which corresponds to -18.01 ± 1.73 mV hyperpolarization (n = 3 cells per group). (**H**) Experimental design. Mice were implanted with fiber optics into CA3 and allowed >2 weeks to recover. Mice were then injected with TAM and administered 4-shock CFC 5 h later. Two weeks following the CFC training, mice were placed back into the training context A for 6 min. The first 3 min of context A exposure were with light ON, with the following 3 min were with light OFF. (**I**) ArcCreER^{T2}(+) mice expressed significantly less fear during laser inhibition when compared with ArcCreER^{T2}(-) mice (minute 1 (light ON) versus minute 4 (light OFF): repeated-measures ANOVA, genotype effect, $F(1,16) = 5.278$, $p = 0.04$; light ON/OFF

effect, F(1,16) = 3.912; p = 0.07; genotype x light ON/OFF, F(1,16) = 5.073, p = 0.04). Minutes 1-3 (light ON) versus minutes 4-6 (light OFF) were also analyzed and gave similar results (repeated-measures ANOVA, genotype effect, F(1,16) = 5.176, p = 0.04; light ON/OFF effect, F(1,16) = 1.360, p = 0.2606; genotype x light ON/OFF, F(1,16) = 2.523, p = 0.13; planned comparison for light ON, p = 0.0085, planned comparison for light OFF, p = 0.24). ArcCreER^{T2}(+) and (-) did not differ in fear expression following laser inhibition (p = 0.95). (n = 8-10 mice per group). * $p < 0.05$. Error bars represent $\pm$ SEM. See also Figure 5.

Figure S6. Social defeat results in a depressive-like phenotype in ArcCreER^{T2} x R26R-STOP-floxed-EYFP mice. (**A-B**) Body weight did not differ before the start of and following social defeat (SD). (**C**) In the DI paradigm, SD mice exhibited significantly less approaches to the CD-1 aggressor (p = 0.04) and significantly more approaches to the empty enclosure ($p < 0.01$). (**D**) The interaction quotient was significantly less in SD mice (p = 0.02). (**E-F**) In NOR paradigm, general activity and investigation declined across exposures 1-4 for Ctrl and SD mice. (**G**) SD mice investigated the novel object more than Ctrl mice (p = 0.02). (**H**) In the EPM, SD mice spend less time in the open arms (p = 0.02). (**I-L**) In the OF, no differences were detected between Ctrl and SD mice. (**M**) In the TST, SD mice displayed increased immobility [$F(1,18) = 8.614$, $p < 0.01$]. (**N**) TAM does not alter the 1-shock CFC impairment in SD mice. Context-elicited freezing was significantly reduced in SD mice. [$F(1,17) = 4.593$, $p < 0.05$]. (**O**) The number of BrdU$^+$ cells was significantly less in SD

mice in the most ventral DG section ($p < 0.01$). (n = 4-13 mice / group). * $p < 0.05$. ** $p < 0.01$. Error bars represent $\pm$ SEM. See also Figure 7.

Figure S7. Example fear conditioning traces during memory encoding and expression in ArcCreER^{T2} x R26R-CAG-STOP-floxed-eNpHR3.0 mice. (**A**) ArcCreER^{T2}(-) and (+) mice did not differ in freezing behavior during the training experience (genotype: $F(1,17) = 0.355$, $p = 0.56$; time: $F(1,5) = 9.853$, $p = <0.0001$; time x genotype interaction: $F(1,5) = 0.768$, $p = 0.58$). (**B**) Optogenetic inhibition of eNpHR3.0-YFP^{+} CA3 neurons impaired expression of the corresponding fear memory in context A in ArcCreER^{T2}(+) mice when compared with ArcCreER^{T2}(-) mice (Data shown as bar graph in **Figure S07**). (n = 8-10 mice per group). Error bars represent $\pm$ SEM. See also Figure 5.