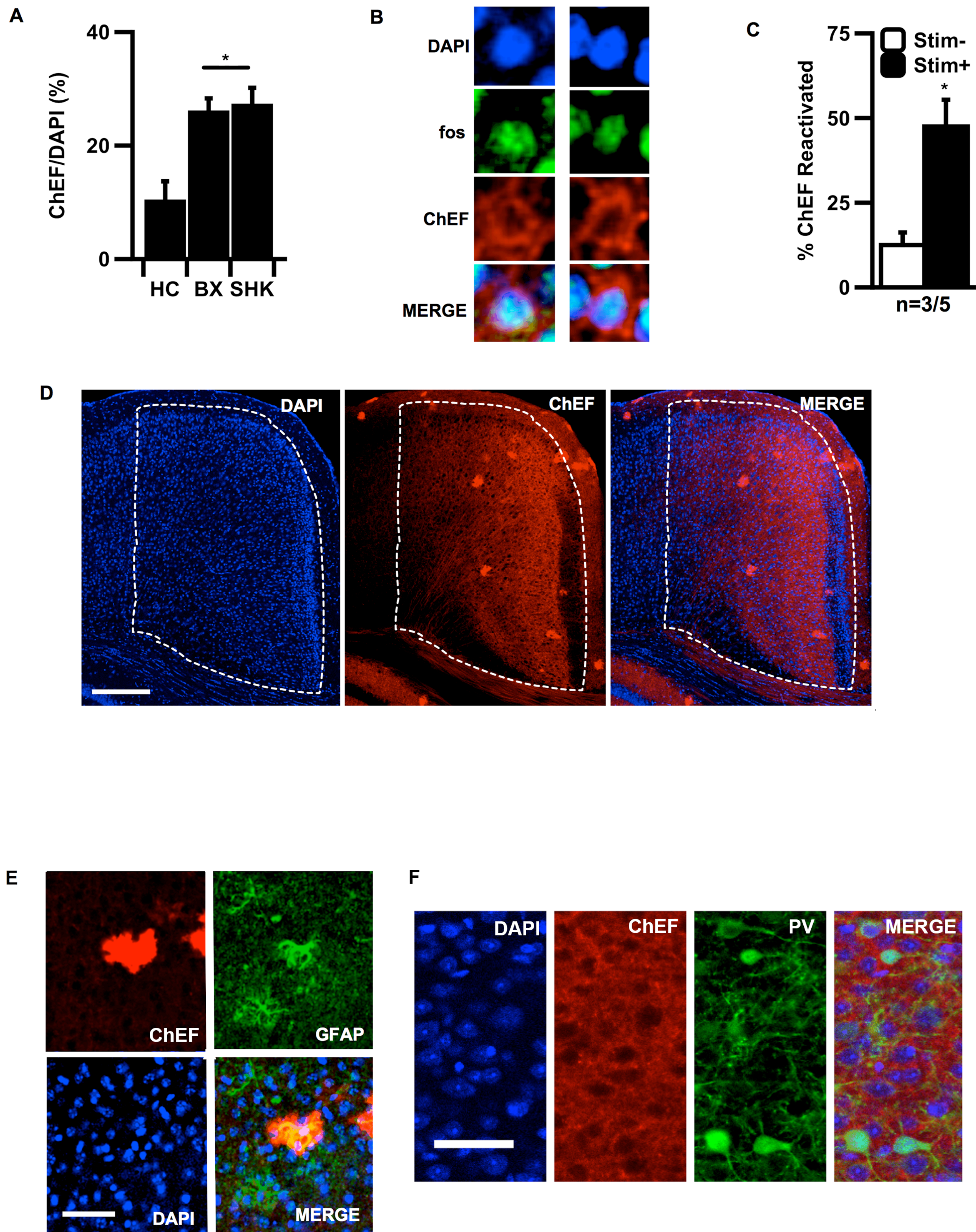
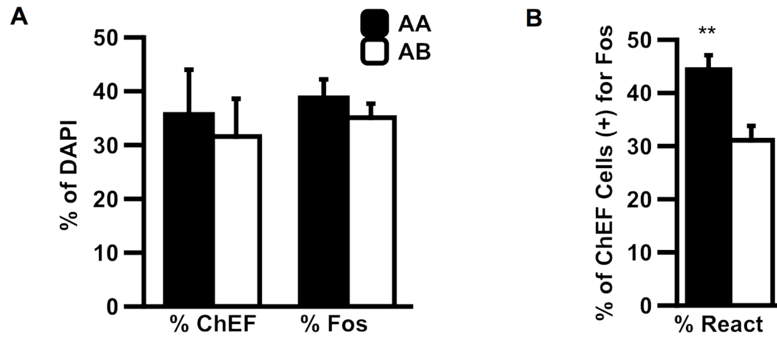
S1



S2



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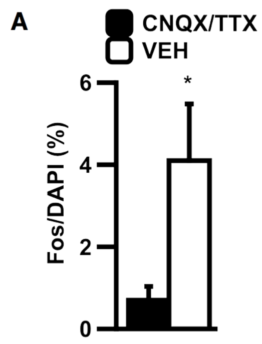

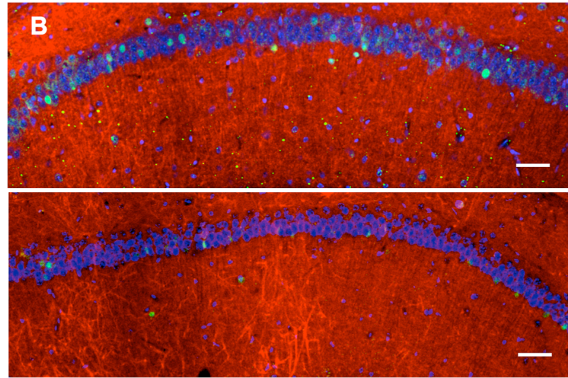

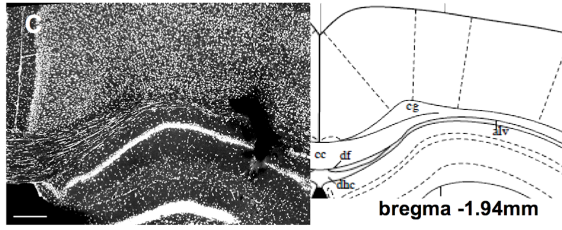

Supplemental Figure Legends

Figure S1. Characterization of ChEF transgene expression (Related to Fig. 1). A) Confirmation of ChEF quantification by Method 2 for thresholding (see Supp. Methods). Method 1 counts in Fig 1C) are obtained using a single stringent minimum intensity threshold applied globally to all images in a data set. In Method 2, images were counted at a less stringent thresholds determined on an image-by-image basis by the mean pixel intensity of the whole ROI (see Methods). This approach allowed the inclusion of low-intensity ChEF(+) cells that were excluded in Method 1. Regardless of the thresholding method, there was a significant main effect of condition on the number of ChEF(+) cells (ANOVA, $F(2,20)=6.909$, $P=0.005$), with post-hoc comparisons showing that significantly more ChEF(+) cells in FC and BX mice compared to HC mice (Tukey-Kramer, P=0.013 (HC vs BX); P=0.006 (HC vs FC); P=) but not in BX compared to FC mice ($P>0.05$). On average, cell counts for each group were similar by Method 1 and Method 2 but only Method 2 produced a significant *post-hoc* difference between BX and HC. There were no significant differences in mean cell pixel intensity between groups using either method, and all three groups included both low- and high- intensity ChEF-expressing individuals. B) Representative image illustrating individual ChEF(+) cells (Red) identified in quantifications to overlap with c-fos (green) C) Quantification of the percentage of ChEF+ cells directly reactivated (c-fos-expressing) 90-minutes after LED stimulation (Stim+, N=5) compared to ChEF+ cells that were not exposed to light (Stim-, N=3), $t(6) =3.22$, $P=0.018$. D) Anatomical expression gradient distinguishing RSC from neighboring cortical areas. White dotted line indicates approximate cortical boundary of RSC (Paxinos & Watson, 2001). Representative confocal images from fixed RSC tissue obtained from ChEF transgenic mice and immunohistochemically stained for E) the astrocytic marker, glial fibrillary acidic protein (GFAP, green) and F) parvalbumin-expressing interneurons (PV, green) overlayed with ChEF (red).

Samples were counterstained with DAPI (blue). * indicates $P<0.05$; error bars represent s.e.m. Scale bars = 50μM.

Figure S2. RSC ChEF(+) cells tagged during Box A training were reactivated by re-exposure to Box A (Related to Figs. 3). A) Mice were initially trained off dox in Box A to induce ChEF expression and were subsequently re-exposed to either training Box A or novel Box B to induce c-fos protein. Overall levels of either ChEF and c-fos protein did not differ between groups; however, B) a greater percentage of reactivated cells, determined on the basis of c-fos immunoreactivity, were colocalized to ChEF-expressing neurons in mice tested in Box B (N=5/6 per group; $t(9) = 3.60$, $P=0.006$). Quantification specifically reflects non-astrocytic labeling in the granular A subregion of RSC, the area most directly targeted during optical stimulation. ** indicates $P<0.01$; error bars represent s.e.m.

Figure S3. Infusion of CNQX/TTX significantly reduced endogenous c-fos expression in hippocampal area CA1 90min after LED stimulation (Related to Fig. 4). A) Cell counts of c-fos (+) cells confirm that CNQX/TTX had significantly reduced hippocampal activation at the time of LED stimulation. CA1 N=5/6 per group; 2-tailed t-test, $t(9) = 2.63$, $P=0.027$; scale bar = 50μM; B) Merged 20x confocal images from CA1 show endogenous c-fos expression (green) overlapping with ChEF-tdTomato (red) and DAPI counterstain (blue) in mice infused with CNQX/TTX (bot) or vehicle (top); C) Correct bilateral placement of micro-injectors was histologically confirmed for all experimental animals. Representative image counterstained with DAPI shows the entry site of an injector tip indicated by the arrow. Scale bar = 250μM. * indicates $P<0.05$; error bars represent s.e.m.

Table S1 (Related to Figure 2). Statistical output of ANOVA tests and post-hoc analysis performed on data shown in in Figure 2B. Red values indicate $P < 0.05$, indicating a statistically significant difference.

Table S2 (Related to Experimental Procedures and Fig. 4). Summary of additional statistical information based on fos-CatFISH regional mRNA overlap data presented in Figure 4B. For each region of interest, *post-hoc* tests provide a comparison between "observed overlap" and "overlap expected by chance," (intronic/DAPI * cyto/DAPI). These comparisons do not account for any underlying differences in network behavior that may intrinsically differentiate sensory from associative areas. Therefore, the comparisons in Table S2 should be interpreted separately from the main group differences shown in Figure 4B. Red values indicate $P < 0.05$, indicating a statistically significant difference.

TABLE S1

<table>
<tr><td rowspan="2">MULTIVARIATE ANOVA</td><td colspan="3">GROUP</td><td rowspan="5"></td></tr>
<tr><td>TG/SHK</td><td>TG/BX</td><td>WT/SHK</td></tr>
<tr><td>unilateral LED</td><td>7</td><td>7</td><td>6</td></tr>
<tr><td>bilateral LED</td><td>5</td><td>5</td><td>n/a</td></tr>
<tr><td>Total N</td><td>12</td><td>12</td><td>6</td></tr>
<tr><td colspan="5">UNILATERAL STIMULATION</td></tr>
<tr><td>MAIN EFFECT OVERALL</td><td>df</td><td>F</td><td>P</td><td>Sig.</td></tr>
<tr><td>All Tests/Groups</td><td>2,19</td><td>2.382</td><td>0.1225</td><td>n.s.</td></tr>
<tr><td>MAIN EFFECTS OF TEST</td><td>df</td><td>F</td><td>P</td><td></td></tr>
<tr><td>Pre x LED (within subject)</td><td>1, 20</td><td>23.62</td><td>0.0002</td><td>*</td></tr>
<tr><td>Group x Test Interact</td><td>2, 19</td><td>4.94</td><td>0.02</td><td>*</td></tr>
<tr><td>MAIN EFFECTS OF GROUP</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Pre-LED x Grp</td><td>2, 17</td><td>0.145</td><td>0.866</td><td>n.s.</td></tr>
<tr><td>LED x Grp</td><td>2, 17</td><td>3.938</td><td>0.039</td><td>*</td></tr>
<tr><td rowspan="6">post-hoc Fisher LSD: Pairwise comparisons for Test x Group</td><td rowspan="3">Pre x LED x (between)</td><td>TG/BX vs TG/SHK</td><td>0.015</td><td>*</td></tr>
<tr><td>TG/BX vs WT/SHK</td><td>0.868</td><td>n.s.</td></tr>
<tr><td>TG/SHK vs WT/SHK</td><td>0.013</td><td>*</td></tr>
<tr><td rowspan="3">LED x (within)</td><td>TG/BX vs TG/SHK</td><td>0.038</td><td>*</td></tr>
<tr><td>TG/BX vs WT/SHK</td><td>0.683</td><td>n.s.</td></tr>
<tr><td>TG/SHK vs WT/SHK</td><td>0.019</td><td>*</td></tr>
<tr><td colspan="5">BIILATERAL STIMULATION</td></tr>
<tr><td rowspan="2">ANOVA</td><td colspan="2">GROUP</td><td colspan="2" rowspan="2"></td></tr>
<tr><td>TG/SHK</td><td>TG/BX</td></tr>
<tr><td>MAIN EFFECT OF TEST</td><td>df</td><td>F</td><td>P</td><td>Sig</td></tr>
<tr><td>Pre vs LED</td><td>1,9</td><td>0.631</td><td>0.459</td><td>n.s.</td></tr>
<tr><td>WITHIN-SUBJECT</td><td>df</td><td>F</td><td>P</td><td></td></tr>
<tr><td>Box vs Shock</td><td>1, 10</td><td>10.363</td><td>0.012</td><td>*</td></tr>
<tr><td>Grp x Test interact</td><td>1, 10</td><td>5.58</td><td>0.046</td><td>*</td></tr>
</table>

TABLE S2

	O, observed; C, Chance, NR, No Retrieval; R, Retrieval						
	Brain Area		**ANOVA**			**Fisher LSD *post-hoc***	
		Factor	**df**	**F**	***P***	**Group**	***P***
AMYGDALA	**Basal**	**Group (R vs NR)**	1, 6	2.02	0.205		**O vs C**
		Overlap (O vs C)		8.40	**0.027**	**NR**	0.523
						R	**0.008**
		Int (OL x Grp)		3.33	0.118		
			df	**F**	***P***	**Group**	***P***
	LA	**Group (R vs NR)**	1, 6	0.51	0.503		
		Overlap (O vs C)		10.80	**0.017**	**NR**	0.241
						R	**0.010**
		Int (OL x Grp)		1.51	0.265		
			df	**F**	***P***	**Group**	***P***
	CeA	**Group (R vs NR)**	1, 6	2.81	0.145		
		Overlap (O vs C)		6.70	**0.041**	**NR**	0.642
						R	**0.011**
		Int (OL x Grp)		3.33	0.118		
			df	**F**	***P***	**Group**	***P***
CORTEX	**Pir**	**Group (R vs NR)**	1, 6	0.11	0.751		
		Overlap (O vs C)		16.18	**0.007**	**NR**	**0.035**
						R	**0.022**
		Int (OL x Grp)		0.07	0.793		
			df	**F**	***P***	**Group**	***P***
	Aud	**Group (R vs NR)**	1, 6	4.99	0.067		
		Overlap (O vs C)		36.70	**0.001**	**NR**	**0.050**
						R	**0.001**
		Int (OL x Grp)		4.78	0.072		
			df	**F**	***P***	**Group**	***P***
	Ent	**Group (R vs NR)**	1, 4	5.41	0.059		
		Overlap (O vs C)		11.60	**0.027**	**NR**	0.177
						R	**0.034**
		Int (OL x Grp)		1.19	0.337		

Supplemental Experimental Procedures

Immunofluroescence*.* Following completion of all behavioral procedures, mice were deeply anesthetized with isoflurane and transcardially perfused with 4% paraformadelhyde. Brains were post-fixed in PFA overnight and were sectioned by vibratome at 50uM*.* Sections were blocked in 10% Normal goat serum/0.2% Triton X and were probed with an anti-GFAP IgG (Chemicon, 1:1000) or anti-Parvalbumin (Swant, 1:5000) followed by an anti-rabbit Alexa-488 fluorescent dye-conjugated secondary antibody (Invitrogen, 1:700). Sections were mounted, counterstained with DAPI and cover-slipped before imaging (Invitrogen, Slowfade with DAPI).

Microscopy and cell counting*.* 50µM sections were imaged at 20x magnification using an A1 Nikon Confocal microscope. Whole coronal stitched images were acquired as single plane optical sections at (6.5 µM) at a scale of 0.62 pixels/µM. All imaging was done using standardized laser settings held constant for samples from the same experimental dataset. Quantifications derive from cell counts averaged across mice, where the value for each subject represents the mean of 1-2 single-plane ROIs (left and right hemisphere). The z-plane for each section was adjusted to the level where DAPI emission was highest. ROIs were determined blind to experimental group using the DAPI channel and anatomical guidelines described in Paxinos & Watson (2001). Two different methods were used to confirm estimates ChEF-tdTomato cell quantifications in RSC (as shown in Figs. 1C and S1). *Method 1 (Fig. 1C):* We first averaged the total pixel area of five individual cells with strongly visible levels of tdTomato, which were manually selected and traced in ImageJ to provide an estimate of mean pixels per cell body within the region of interest (ROI). A minimum intensity threshold (MIT) was then applied to all images from the same dataset, limiting pixels to only those of equal or higher intensity. The area of thresholded pixel area was calculated within the ROI and the total number of tdTomato(+) pixels (≥MIT) was divided by our estimated mean pixels per cell to obtain the number of ChEF(+) cells in the ROI. This value was divided by the total number of

cells in the ROI based on the number of DAPI+ cells in the image. To confirm counts obtained from Method 1, we also counted ChEF+ cells using a second procedure. *Method 2 (Supp Fig S1)*: An ImageJ macro was written to identify DAPI+ nuclei overlapping with tdTomato above one of two MITs, determined by the mean intensity of the entire ROI. This image-specific-thresholding method was used in order to permit inclusion of more weakly expressing cell populations excluded in Method 1. Thus, compared to counts from Method 1, counts from Method 2 were less contingent on expression intensity. For c-fos nuclear counts, an ImageJ macro was used to apply a standard MIT to images in a data set. An ImageJ macro was applied to count nuclei above this threshold that overlapped with in-plane DAPI+ nuclei. For counts of overlap between ChEF and c-fos (see representative example of co-localization in Supp Fig. S1), only those cells identified as positive for both ChEF and c-fos were counted as "overlapping" % overlap by chance was calculated by: [ChEF/DAPI x fos/DAPI x 100] and % overlap was calculated by: [overlap/DAPI x 100], and % overlap was normalized to % chance overlap by: [overlap/chance x 100]. Calculation of % ChEF+ cells reactivated (i.e. % of ChEF+ cells that co-express c-fos) was obtained by: [overlap/total ChEF x 100].