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Supplementary Materials for

Fear extinction relies on ventral hippocampal safety codes shaped by the amygdala

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Figs. S1 to S5

Figure S1

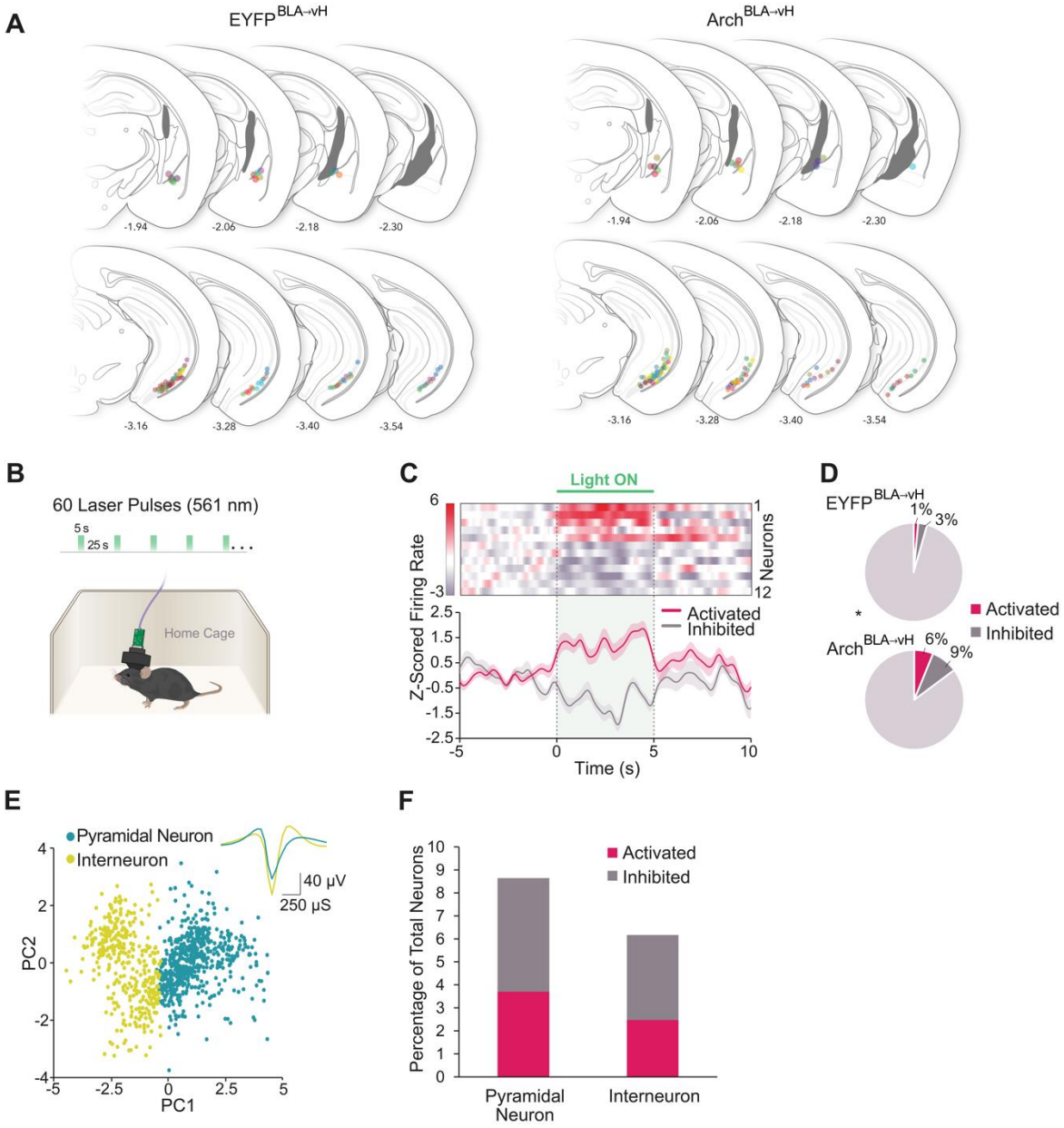

Fig. S1. **Optogenetic inhibition of BLA neurons projecting to vH alters vH neuronal activity.** (**A**) Histologically verified placements of optic fibres in the BLA and tetrodes in vH for EYFP$^{BLA\rightarrow vH}$ (left, n = 15) and Arch$^{BLA\rightarrow vH}$ (right, n = 15) mice. (**B**) Schematic of experimental protocol for laser stimulation in the home cage. Mice received 60 light pulses of 5 s duration separated by 25 s. (**C**) Top, peri-stimulus time histogram heatmaps of trial-averaged responses of laser-activated and -inhibited neurons in the vH of Arch$^{BLA\rightarrow vH}$ mice. Dashed lines mark the laser onset and offset. Bottom, Averaged responses of laser-activated and -inhibited neurons across trial time. (**D**) Percentage of laser-activated and laser-inhibited neurons out of total neurons. Difference in counts of responsive and non-responsive neurons between groups: $\chi^2_{(1,\,N\,=\,149)} = 4.42$, $P = 0.0355$, Chi-square test. C-D, EYFP$^{BLA\rightarrow vH}$ n = 68 neurons from 4 mice, Arch$^{BLA\rightarrow vH}$ n = 81 neurons from 4 mice. (**E**) Plot of principal components 1 and 2 for analysis of variation in spike waveforms. Points correspond to neurons color-coded by cluster membership assigned through K-means clustering of waveforms (K = 2). Inset: average spike waveforms of putative pyramidal neurons and interneurons. (**F**) Percentage of laser-activated and -inhibited neurons out of total pyramidal neurons or interneurons for Arch$^{BLA\rightarrow vH}$ mice. Difference in counts of activated and inhibited neurons between neuronal classes: $\chi^2_{(1,\,N\,=\,12)} = 0.01$, $P = 0.92$, Chi-square test. E-F, EYFP$^{BLA\rightarrow vH}$ n = 179 neurons from 15 mice, Arch$^{BLA\rightarrow vH}$ n = 185 neurons from 15 mice.

Figure S2

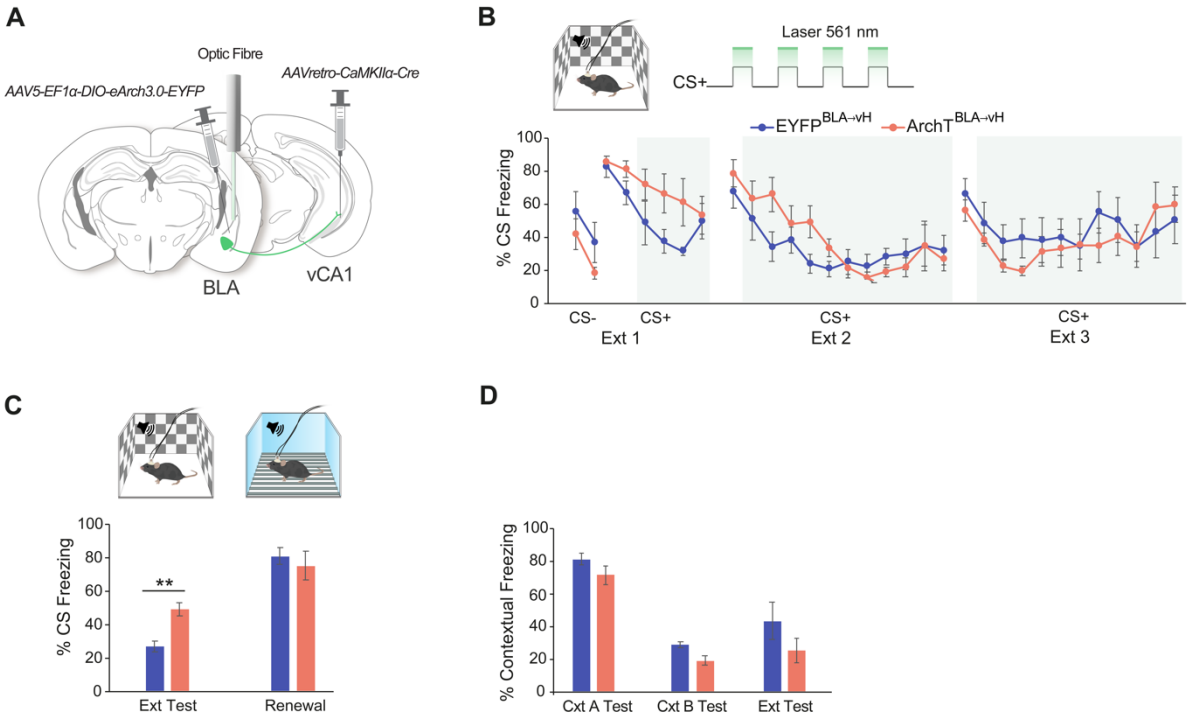

Fig. S2. Somatic optogenetic inhibition of BLA-vH neurons during extinction impairs extinction memory formation. (**A**) Schematic of optogenetic BLA-vH somatic inhibition strategy showing viral infusions in the BLA and vH, and optic fibre implantation the BLA. (**B**) Top, context B illustration and paradigm for laser light delivery during the CS-period of Ext sessions. Bottom, percent CS freezing across Ext training sessions shown as 2-trial averaged blocks. Shaded area indicates light-on trial blocks. Ext 1 $F_{(1,9)} = 0.66$, $P = 0.4$; Ext 2 $F_{(1,9)} = 0.47$, $P = 0.5$; Ext 3 $F_{(1,9)} = 0.19$, $P = 0.7$, two-way RM ANOVA per session, group effect. (**C**) Percent CS-period freezing in 4-trial averaged blocks at Ext Test and fear renewal test (Renewal), in contexts B and A (top). Ext Test: $t_{(9)} = 3.68$, $P = 0.005$; Renewal: $t_{(9)} = 0.6$, $P = 0.6$, unpaired *t*-tests. (**D**) Percent contextual freezing during first 3 minutes of context exposure in each session. Context A Test (contextual fear memory test): $t_{(9)} = 1.4$, $P = 0.2$; Context B Test (novel context fear discrimination test): $t_{(9)} = 3.0$, $P = 0.015$; Ext Test (extinction memory test in context B): $t_{(9)} = 1.24$, $P = 0.2$, unpaired *t*-tests. Mice, EYFP $^{BLA\rightarrow vH}$ $n = 6$, ArchT $^{BLA\rightarrow vH}$ $n = 5$. Data are presented as mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$.

Figure S3

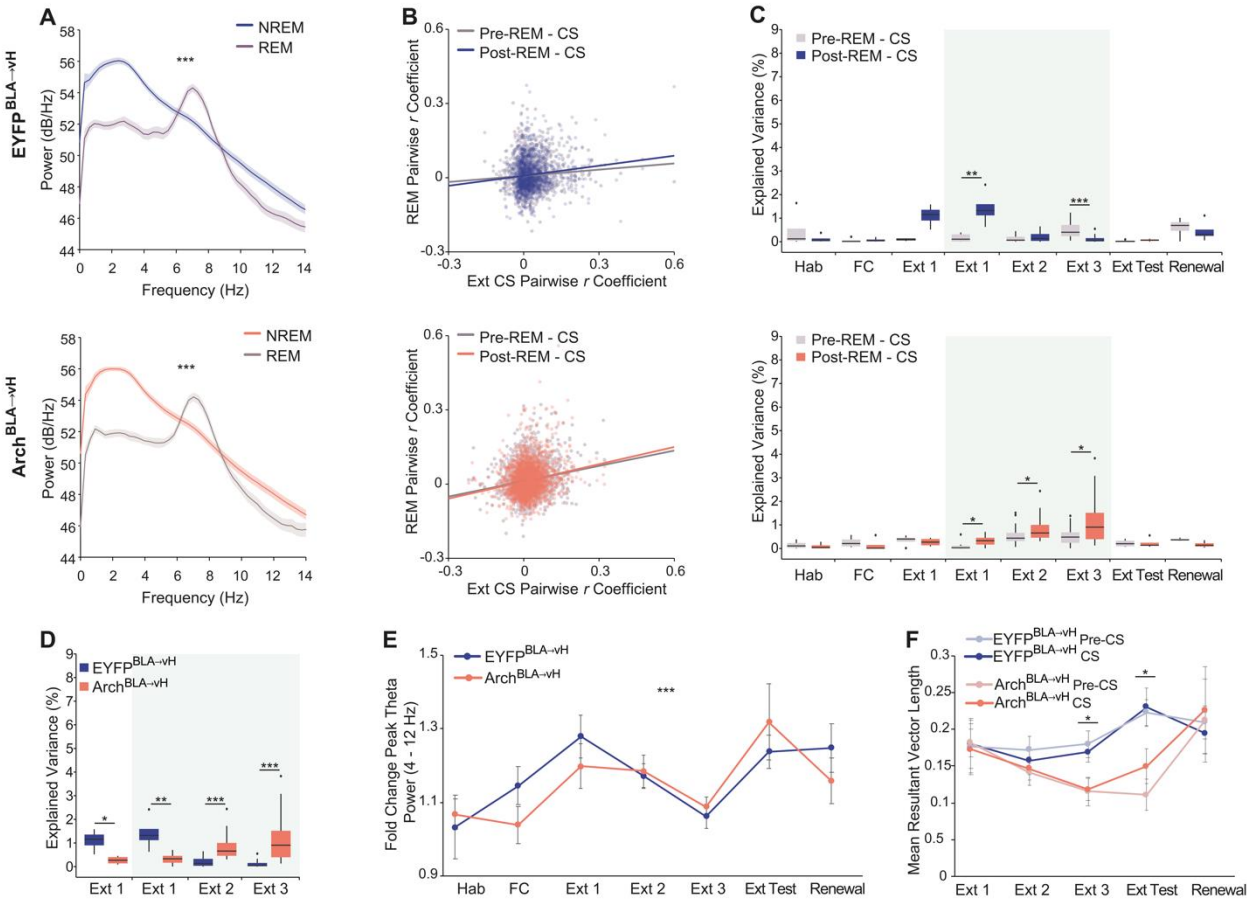

Fig. S3. Reactivation of CS-period activity during REM sleep and CS-evoked theta power across fear extinction. (**A**) Power spectral density estimates for NREM and REM sleep periods. Period effect: $EYFP^{BLA\rightarrow vH}$, $F_{(1,28)} = 17.38$, $P = 0.0003$; $Arch^{BLA\rightarrow vH}$, $F_{(1,28)} = 24.05$, $P < 0.0001$, two-way RM ANOVA. (**B**) Pairwise correlations for CS-period activity at Ext sessions versus for Pre-REM or Post-REM sleep. $EYFP^{BLA\rightarrow vH}$: $n = 1407$ pairs, Pre-REM-CS $r = 0.09$, $P = 0.0014$, Post-REM-CS $r = 0.12$, $P = 7x10^{-7}$; $Arch^{BLA\rightarrow vH}$: $n = 2649$ pairs, Pre-REM-CS $r = 0.18$, $P = 1.7x10^{-20}$, Post-REM-CS $r = 0.19$, $P = 1.3x10^{-23}$, Pearson's r. Pre-REM-CS versus Post-REM-CS: $EYFP^{BLA\rightarrow vH}$ $Z = -0.96$, $P = 0.34$; $Arch^{BLA\rightarrow vH}$ $Z = -0.59$, $P = 0.56$, Steiger's Z test. (**C**) Explained variance of pairwise correlations between CS and Pre-REM versus CS and Post-REM. $EYFP^{BLA\rightarrow vH}$: Ext 1 light trials, $P = 0.0078$; Ext 2, $P = 0.16$; Ext 3, $P < 0.00001$; $Arch^{BLA\rightarrow vH}$: Ext 1 light trials, $P = 0.0391$; Ext 2: $P = 0.037$; Ext 3, $P = 0.0177$, Wilcoxon signed-rank test. (**D**) Explained variance of pairwise correlations between CS and Post-REM comparing $EYFP^{BLA\rightarrow vH}$ versus $Arch^{BLA\rightarrow vH}$. Ext 1 No-light trials: $P = 0.0286$, Ext 1 light trials: $P = 0.0003$, Ext 2: $P < 0.00001$, Ext 3: $P < 0.00001$, Wilcoxon rank-sum test. (**E**) Fold change in peak relative power between 4-12 Hz. Session effect: $F_{(4.2,117.9)} = 5.28$, $P = 0.0005$, two-way RM ANOVA. Hab vs. Ext 1: $P = 0.0337$, Ext 1 vs. Ext 3: $P = 0.0017$, Ext 2 vs. Ext 3: $P = 0.0054$, Ext 3 vs. Ext Test: $P = 0.0119$, Tukey's post-hoc test. $EYFP^{BLA\rightarrow vH}$ $n = 15$ mice; $Arch^{BLA\rightarrow vH}$ $n = 15$ mice. (**F**) Mean resultant vector length of Ext Test CS-active theta-coupled neurons across extinction sessions and fear renewal. $EYFP^{BLA\rightarrow vH}$ vs. $Arch^{BLA\rightarrow vH}$ for Pre-CS period: Ext 3, $P = 0.0325$; Ext Test, $P = 0.0028$; for CS period (indicated by asterisk): Ext 3, $P = 0.015$; Ext Test, $P = 0.032$, Wilcoxon rank-sum test.

Figure S4

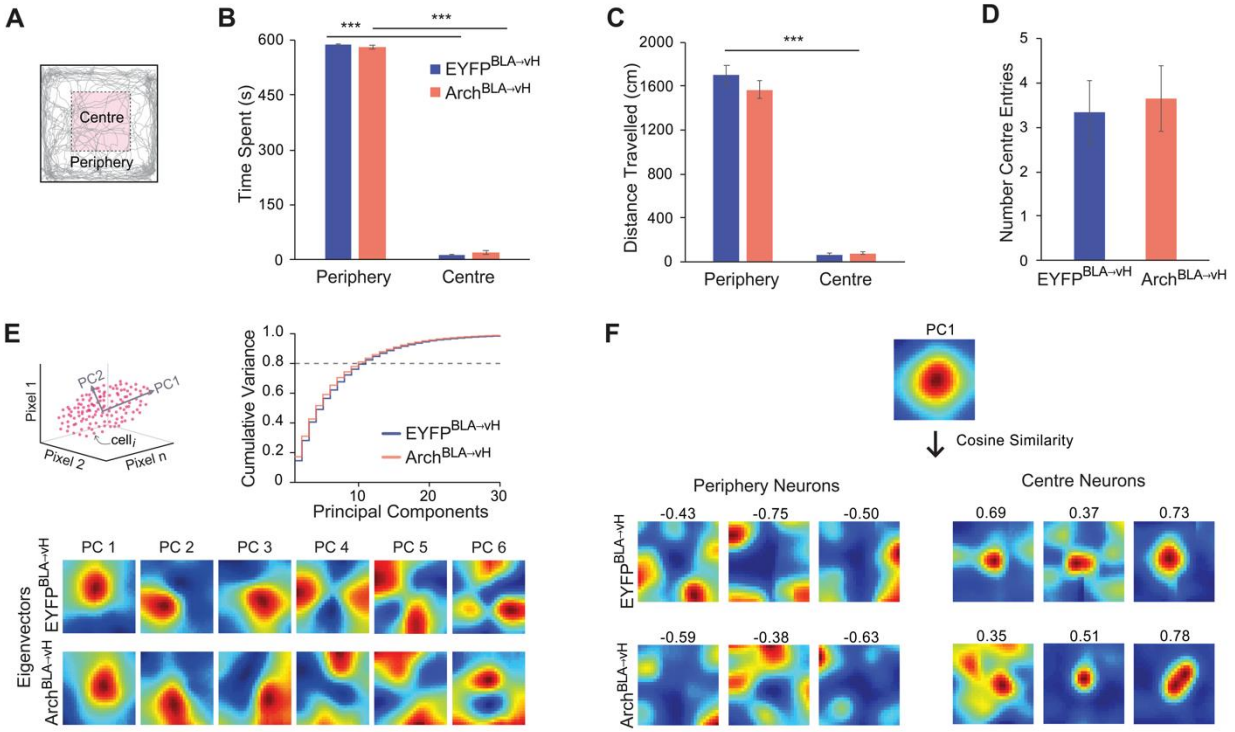

Fig. S4. Centre behaviour and categorization of zone-active neurons at extinction test. (**A**) Representative tracking plot at Ext Test. (**B**) Time spent in each zone. EYFP$^{BLA\rightarrow vH}$ vs. Arch$^{BLA\rightarrow vH}$: $t_{(24)}$ = 1.35, P = 0.19, unpaired t-test. EYFP$^{BLA\rightarrow vH}$: $t_{(11)}$ = 26.68, P < 0.0001; Arch$^{BLA\rightarrow vH}$: $t_{(13)}$ = 15.98, P < 0.0001, one-sample t-test against chance level. (**C**) Distance travelled in each zone, Zone effect: $F_{(1,24)}$ = 691.6, P < 0.0001, two-way RM ANOVA; periphery: P = 0.25, centre: P = 0.98, Sidak post-hoc test. (**D**) Number of entries into the centre zone, $t_{(24)}$ = 0.3, P = 0.77. b-d, Mice, EYFP$^{BLA\rightarrow vH}$ n = 12, Arch$^{BLA\rightarrow vH}$ n = 14. (**E**) Top left, schematic of the spatial firing rate pixel space on which principal component analysis (PCA) was performed. Top right, cumulative proportion of variance explained by principal components. Bottom, first 6 principal components of the spatial firing rate maps at Ext Test (n =364 neurons, 676 pixels). (**F**) Schematic of zone categorization approach. Top, first principal component using all sessions and neurons from both EYFP$^{BLA\rightarrow vH}$ and Arch$^{BLA\rightarrow vH}$. Bottom, examples of spatial maps from neurons categorized as periphery- or centre-active and the correlation of their firing with PC1.

Figure S5

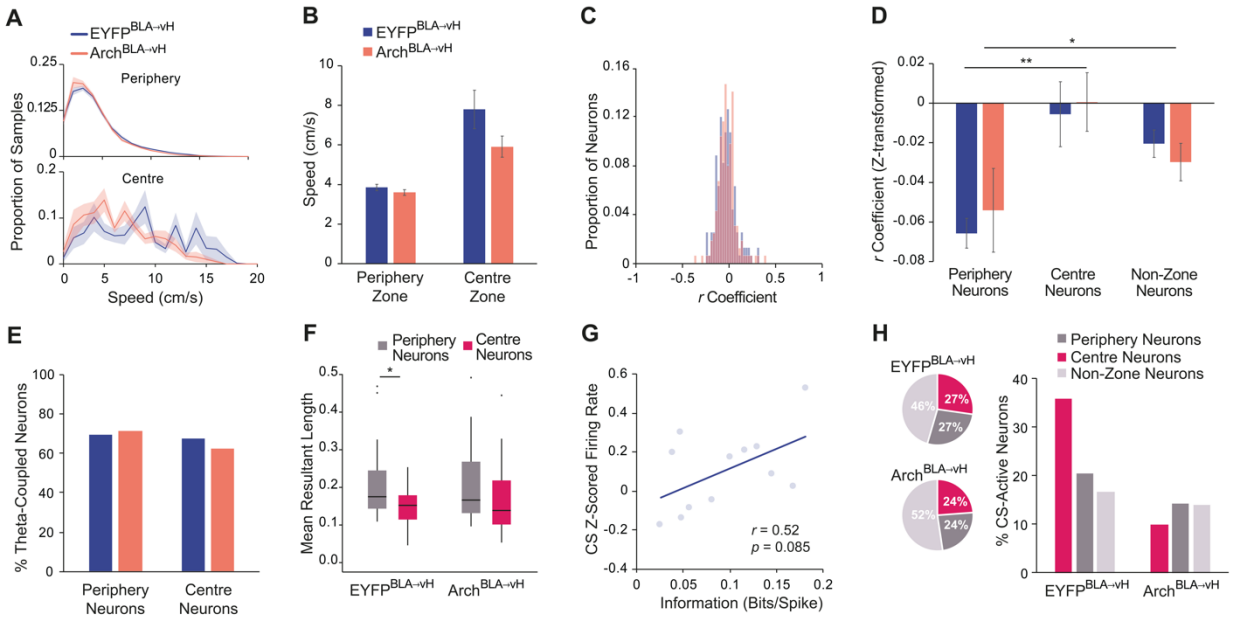

Fig. S5. Speed modulation, theta coupling, and CS-relevant activity of periphery and centre neurons. (**A**) Histogram of samples tracked across speeds in the periphery (top) and centre (bottom) zones. Group effect: Periphery $F_{(1,24)}$ = 0.7, P = 0.41; Centre $F_{(1,24)}$ = 0.14, P = 0.71; two-way RM ANOVA. (**B**) Mean speed of EYFP$^{BLA→vH}$ and Arch$^{BLA→vH}$ mice in periphery and centre. Group x Zone interaction: $F_{(1,24)}$ = 2.28, P = 0.14; two-way ANOVA. A-B, EYFP$^{BLA→vH}$: n = 12 mice, Arch$^{BLA→vH}$: n = 14 mice. (**C**) Top, percentage of speed-modulated neurons. Bottom, proportion of neurons with given Pearson's r correlation between firing rate and speed. EYFP$^{BLA→vH}$ vs. Arch$^{BLA→vH}$: P = 0.43, Kolmogorov-Smirnov. EYFP$^{BLA→vH}$: n = 158 neurons, Arch$^{BLA→vH}$: n = 163 neurons. (**D**) Mean Fisher's Z-transformed r coefficient for periphery, centre, or non-zone neurons. Group effect: $F_{(1,315)}$ = 0.05, P = 0.82; Zone effect: $F_{(2,315)}$ = 6.15, P = 0. 0024; two-way ANOVA. Periphery vs. Centre: P = 0.002; Periphery vs. Non-Zone: P = 0.032; Tukey's post-hoc test. (**E**) Percentage of periphery and centre neurons that are theta-coupled, Rayleigh's test. (**F**) MRL of theta-coupled zone neurons. EYFP$^{BLA→vH}$: P = 0.0441; Arch$^{BLA→vH}$: P = 0.16, Wilcoxon rank-sum test. (**G**) Association between spatial information content and mean normalized firing rate during CS-period of Ext Test. r = 0.52, P = 0.0851, Pearson's r. Measures averaged across all neurons from individual EYFP$^{BLA→vH}$ mice (n = 12) at Ext Test, data points are for each mouse. (**H**) Left, pie charts show proportion of CS-active neurons that were also categorized as zone neurons. Right, proportion of each zone population categorized as CS-active. E,F,H, Periphery neurons: EYFP$^{BLA→vH}$ n = 44, Arch$^{BLA→vH}$ n = 35; centre neurons: EYFP$^{BLA→vH}$ n = 25, Arch$^{BLA→vH}$ n = 50; Non-Zone neurons: EYFP$^{BLA→vH}$ n = 89, Arch$^{BLA→vH}$ n = 78.