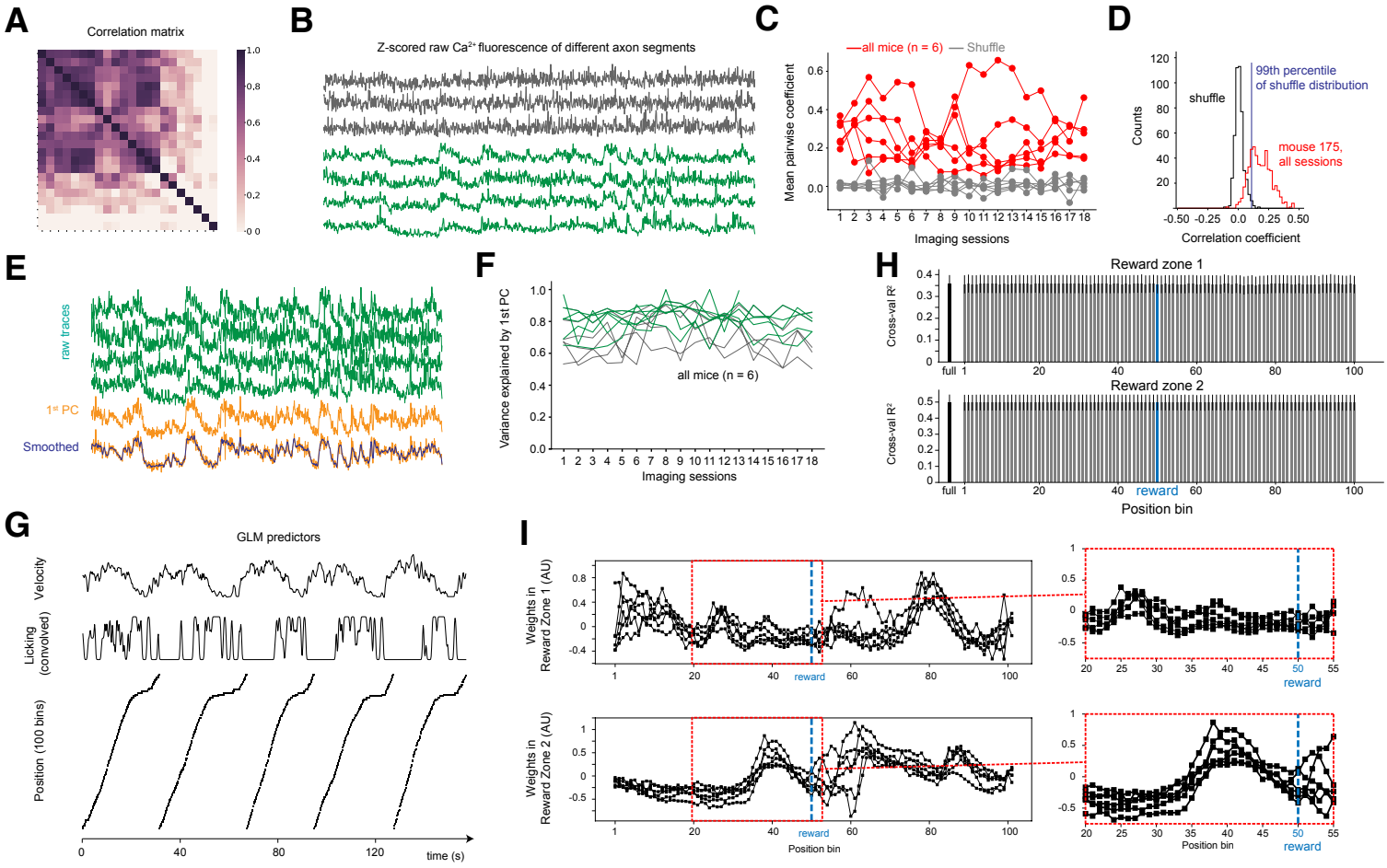
Supplemental item titles

Supplemental Figure 1 (related to Figure 2): LC axons signal processing and GLM predictors of LC activity.

Supplemental Figure 2 (related to Figures 1-2): Dynamics of LC signal development and CA1 reward enrichment in the GOL task.

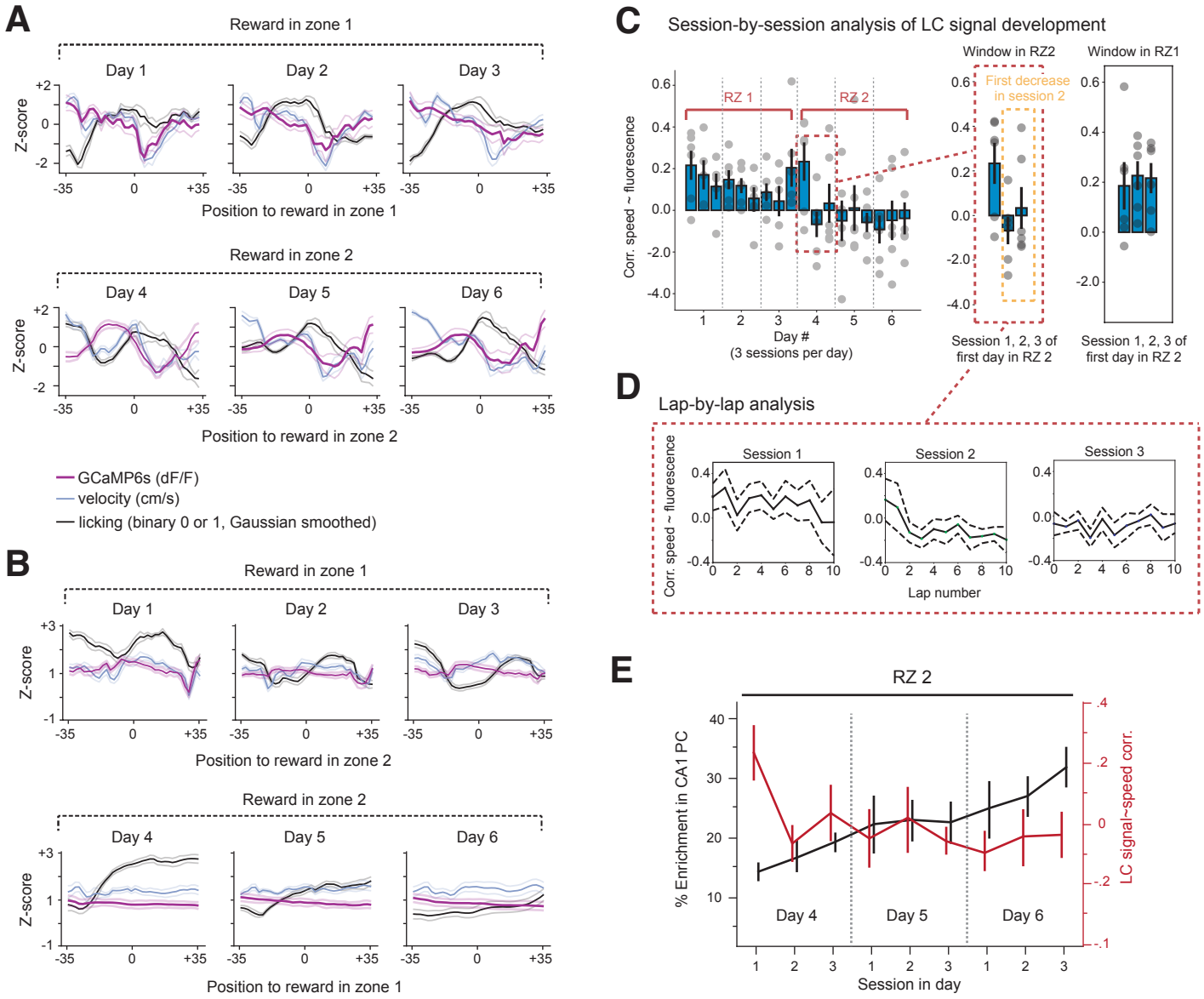
Supplemental Figure 3 (related to Figures 1-2): Locus coeruleus signal and CA1-PCs do not change in the same reward zone. Place field enrichment comes from cells shifting from outside the reward zone, and a stabilization of cells in the reward zone.

Supplemental Figure 4 (related to Figures 1-3): LED stimulation of LC axons during random foraging, random foraging paired with addition of novel cues, and random foraging with VIP interneuron stimulation.



Supplementary Figure 1 - LC axons signal processing and GLM predictors of LC activity
Related to Figure 2

A. The Pearson's pairwise correlation matrix of the calcium traces from all the axons in one session. Most axons are correlated with each other.
B. Example calcium traces from four ROIs that are highly correlated with each other, and three that are not. Traces shown in green have a good signal to noise ratio, while the axons in grey have poor signal and are not useable for analysis.
C. The mean pairwise correlation coefficient of all the axons remains consistent over days (red lines), compared to a shuffle distribution (grey). The shuffle distribution was computed using the mean pairwise correlation coefficient of a given axon in a session with all the axons taken from a different session.
D. Distribution of the mean pairwise correlation coefficient for all the axons in all the sessions (red) and the shuffle distribution as described in c (grey). The 99th percentile of the shuffle distribution (blue line) was used as the threshold to define axons with poor pairwise correlations and thus poor signal to noise ratio.
E. After discarding the uncorrelated axons, the signal from the remaining axons (green) was reduced to a single trace by taking the first principal component of the raw calcium traces (yellow), and smoothing with a Savitsky-Golay filter with a sliding window of 21 frames and polynomial degree of 6 (smoothed, blue).
F. The variance in the calcium signal explained by the first principal component was consistent across days, and consistently high. The variance explained by the first principal component shown in green do not include discarded axons, while those shown in gray include the uncorrelated axons.
G. Factors used as predictors for the GLM plotted in time. Velocity, licking and position (binned into 100 2 cm segments) were used as predictors for the calcium signal. See methods for more details.
H. The cross validated R2 value of the reduced model without each position bin, compared to the full model, left, full.
I. The weights of each position bin in reward zones 1 and 2. Each line is one mouse, and each square is one position bin plotted across the whole belt. In reward zone 2 (inset), the weights of the positions are higher 10 bins (or 20 cm), before the reward. This is not seen in reward zone 1 (top left, top inset).



Supplementary Figure 2 - Dynamics of LC signal development and CA1 reward enrichment in the GOL task

Related to Figures 1-2

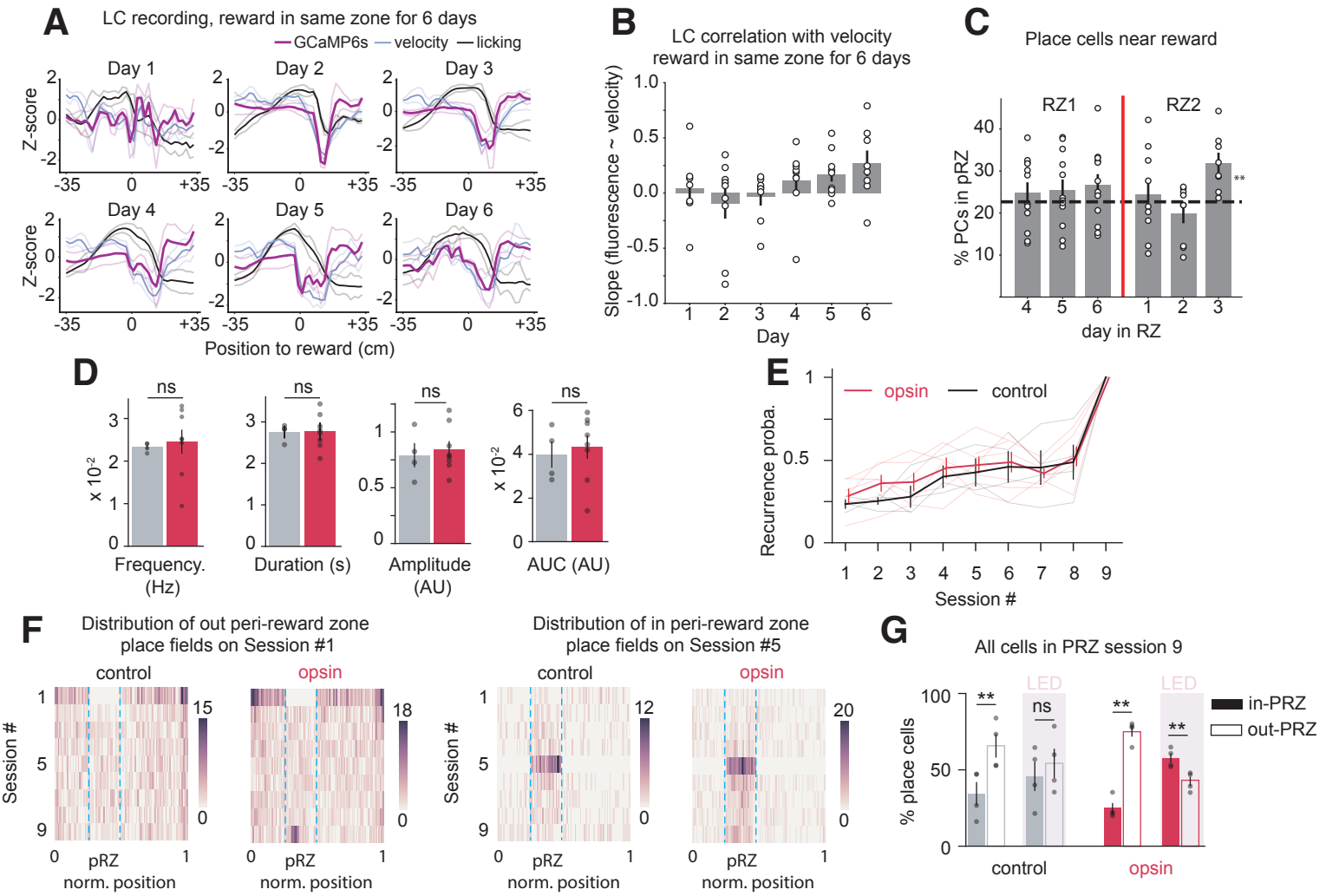
A. The GCaMP signal, velocity, and licking averaged across each day and aligned around the reward zone (0 cm). The decorrelation between velocity and GCaMP appears as a bump in the GCaMP6s signal, and is visible on days 4-6 but not on days 1-3 (corresponding to RZ2 and RZ1).

B. On days 1-3, this plot shows the GCaMP signal, velocity, and licking aligned to reward zone 2 (the future reward zone) in the first three days of the paradigm, when the reward was in zone 1. On days 1-4, reward zone 1 is set at zero, and the actual reward position is in zone 2. The GCaMP signal shows no discernible changes in reward zone 1 when the reward is in zone 2

C. Session-by-session analysis of the correlation between speed and fluorescence of LC axons.
Inset, left: first three sessions of first day in RZ2. Right, first three sessions of the location of RZ1, during the first day in RZ2 (n = 6 mice).

D. Traversal-by-traversal analysis of the LC signal versus velocity. Average of 6 mice +/- SEM on the first day of RZ2 (3 sessions per day). Laps are averaged in non-overlapping blocks of two (n = 6 mice).

E. Session-by-session analysis of reward enrichment in CA1 pyramidal cells (black) overlaid with LC signal~speed correlation (red, same data as in **C**) in the 3 days of RZ2 (day 4, 5, 6 of the GOL task) (LC, n = 6 mice; CA1, n = 9 mice).



Supplementary Figure 3 - Locus coeruleus signal and CA1-PCs do not change in the same reward zone. Place field enrichment comes from cells shifting from outside the reward zone, and a stabilization of cells in the reward zone

Related to Figures 1-2

A. LC activity, GCaMP, and velocity over 6 days of the same reward zone. Velocity and LC activity remain correlated. n= 3 mice.

B. LC GCaMP6f and velocity remain correlated over six days of the same reward zone. Mean ± SEM. across days, 0.04 ± 0.12, -0.09 ± 0.14, -0.04 + 0.08, 0.12 ± 0.10, 0.17 ± 0.07, 0.27 ± 0.12. Circles represent individual sessions. n = 3 mice.

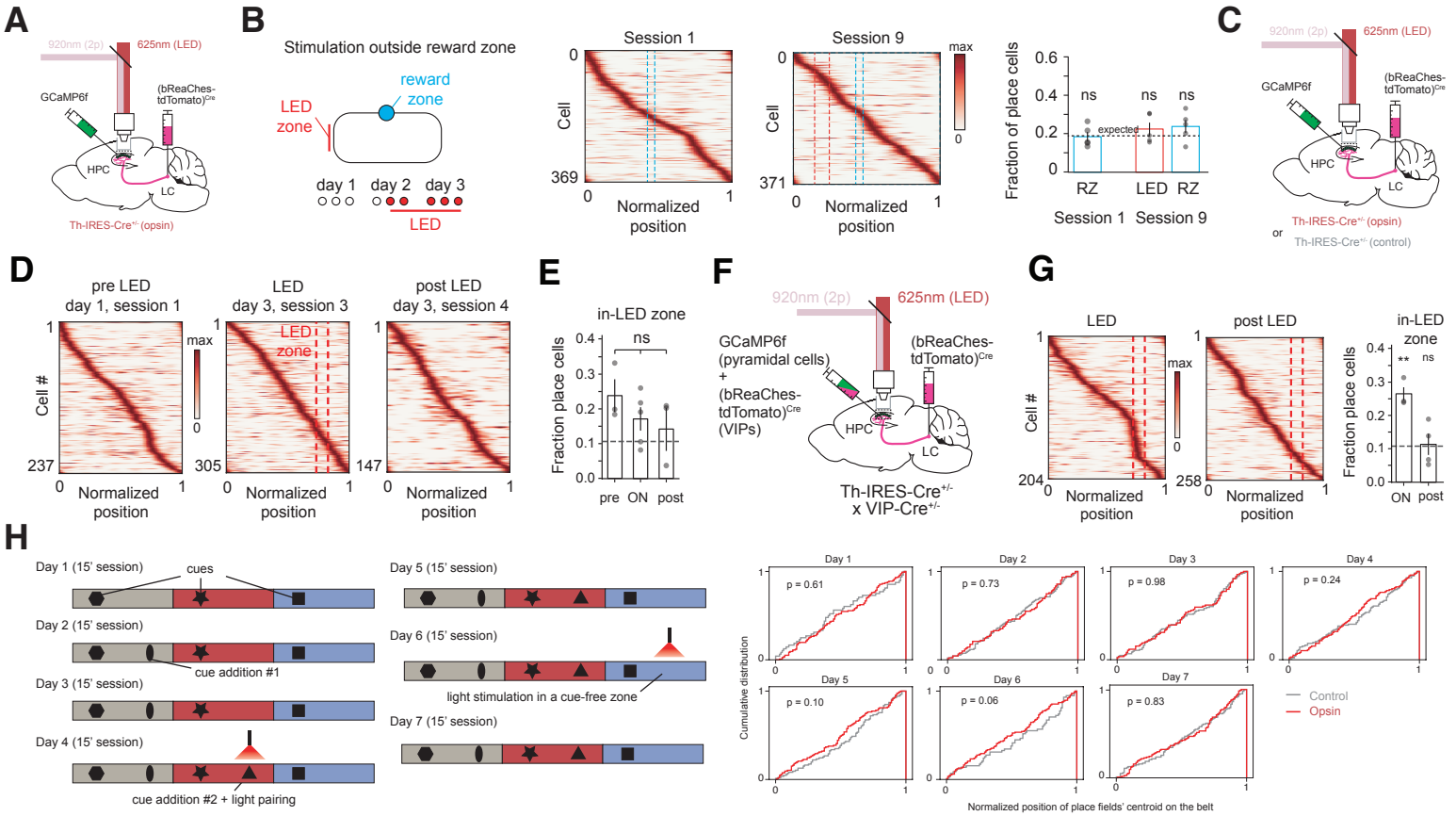
C. Place cells do not enrich over the second three days (6 total days) of the same reward zone, but are enriched around a new zone compared with a uniform distribution. Mean ± SEM, **, p = 0.0067, one-tailed t-test compared to the expected distribution. pRZ, peri-reward zone. Circles represent individual sessions.

D. Transient properties of place fields in the PRZ on session 9 in control and opsin mice (Mean ± SEM and compared with Mann-Whitney U tests. Frequency, control: 0.023 ± 5x10-4; opsin: 0.024 ± 0.0028; z(11)= 8.0, P=0.101. Duration, control: 2.75 ± 0.103; opsin: 2.77 ± 0.146, z(11)= 14.0, P=0.399. Amplitude, control: 0.792 ± 0.108; opsin: 0.846 ± 0.074, z(11)=13.0, P=0.336. AUC, area under the curve, control: 0.039 ± 0.006; opsin: 0.043 ± 0.005, z(11)=13.0, P=0.336; control n = 4; opsin, n = 8 mice).

E. Recurrence probability of place cells with fields in PRZ on session 9. There is no difference between opsin and control groups (Holm-Sidak method, with α = 0.05. Computations assume sessions are from populations with the same standard deviation. Control, n = 4 mice; opsin, n = 8 mice. None of the days were significantly different, all p-values > 0.9).

F. Distribution of place fields along the belt across different sessions. The same cells were tracked over all the imaging sessions. Left, distribution across sessions 1-9 of cells outside the PRZ on session 1. Right, of cells with fields inside the PRZ on session 5.

G. Origin of PRZ place cells. We restricted this analysis to place cells in the PRZ on session 9 and determined where their place fields were on previous sessions - inside (in-PRZ), or outside the PRZ (out-PRZ). The number of cells was averaged across pre-LED (sessions 1-4) and LED-on sessions (LED, sessions 5-8). Pre-LED stimulation, in-PRZ session 9 cells for both groups had place fields outside the PRZ. After LED, cells maintained fields in-PRZ in opsin animals (Mean ± SEM, compared with Mann-Whitney U tests. Control mice, n = 4. pre-LED, in-PRZ: 0.34 ± 0.08, out-PRZ: 0.66 ± 0.08, in-PRZ vs out-PRZ, z(4) = 0.0, p = 0.01. LED on, in-PRZ: 0.46 ± 0.09, out-PRZ: 0.54 ± 0.09, in-PRZ vs out-PRZ, z(4) = 6.0, p = 0.33. Opsin mice, n = 8; pre-LED, in-PRZ: 0.25 ± 0.04, out-PRZ: 0.75 ± 0.04, in-PRZ vs out-PRZ, z(7) = 0.0, p = 0.01. LED on, in-PRZ: 0.57 ± 0.04, out-PRZ: 0.43 ± 0.04, in-PRZ vs out-PRZ, z(7) = 0.0, p = 0.01).



Supplementary Figure 4 - LED stimulation of LC axons during random foraging, random foraging paired with addition of novel cues, and random foraging with VIP interneuron stimulation
Related to Figures 1-3

A. Labeling protocol with bReaChes expressed in the LC, and pyramidal neurons labeled with GCaMP.
B. LC fibers were stimulated outside the reward zone, using the same behavioral paradigm as for LC stimulation in the reward zone (left). Heatmaps of place fields shows that there was no place field enrichment near the LED stimulation zone or near the reward (center), quantified as the fraction of place fields in session 1 and session 9 (right) (mean ± sem, reward session 1: 0.18 ± 0.02, session 9: 0.22 ± 0.03, LED session 9: 0.24 ± 0.04). Percentage of place fields around the reward zone and LED zone were not different on any sessions from an expected uniform distribution, showing no enrichment around those 2 zones (Reward session 1: , $t_{(3)}$=0.32, p = 0.4; session 9, $t_{(4)}$ = 0.76, p = 0.24; LED session 9, $t_{(4)}$ = 1.28, p = 0.13) `
C. Labeling protocol with bReaChes expressed in the LC, and pyramidal neurons labeled with GCaMP.
D. The rewards moved each lap, and the LED zone was fixed. Mice ran 3 x 10-minute sessions per day, and the LED was present from the third session of day 1 to the third session of day 3. The mice ran one more session to determine whether any potential enrichment remained. Heatmaps of place fields before the LED (n = 3 mice), on the last LED session (n = 5 mice), and post LED (n = 3 mice).
E. There was no difference across days in the proportion of place fields in the LED zone (mixed-effects model F(2, 8) = 1.022, p = 0.4024). Data presented in mean ± sem; pre-LED: 0.29 ± 0.047, LED-ON: 0.22 ± 0.034, post-LED: 0.19 ± 0.062.
F. Labeling strategy to stimulate LC axons and VIP interneurons while imaging CA1 pyramidal cells. In a Th-IRES-Cre+/- crossed with a VIP-Cre+/-, the LC and the HPC were injected with a rAAV expressing bReaChes-tdTomato [rAAV2/9:EF1a-(bReaChes-tdTomato)Cre] in a Cre-dependent manner, and a non-Cre dependent GCaMP6f (rAAV2/1:CaMKII-GCaMP6f) was also injected in dorsal CA1.
G. Left, Heatmaps of place fields during the last LED stimulation session (n = 3 mice), and post LED (n = 4 mice). Enrichment occurs during VIP stimulation, likely due to direct disinhibition of pyramidal cells, but it is not present in the session after stimulation. Right, quantification of place fields number near the LED zone. Data presented in mean ± sem; LED-ON: 0.26 ± 0.02, post: 0.11± 0.032 (one-sample two-tailed t-test for expected value derived from a uniform distribution of 0.11, control: t(2) = 9.8, p = 0.01, opsin: t(3) 1.62, p = 0.24).
H. Pairing cues with LED stimulation. Mice ran one 15-minute session per day for 7 consecutive days, and the reward delivery positions were randomized each lap (random foraging).The belt contained a few sparse cues. First, we habituated the mice to the belt for one day (day 0, not shown). We then added a succession of stimuli, including cues and LED light to stimulate the axons (Day 2, cue 1, day 3 cue 1 post, day 4 cue 2 + LED, day 5 cue 2 alone, day 6 LED, day 7 post LED). The cumulative proportion of place field centroids on the belt on each day was not different between opsin mice with bReaChes and control mice without bReaChes (opsin, n = 5 mice; control, n = 5 mice for each day. Day 1: Two-sided Kolmogorov-Smirnov test, n1 = 48, n2 = 117, z(164) = 0.126, p = 0.617. Day 2: Two-sided Kolmogorov-Smirnov test, n1 = 94, n2 = 91, z(184) = 0.098, p = 0.735. Day 3: Two-sided Kolmogorov-Smirnov test, n1 = 103, n2 = 84, z(186) = 0.0067, p = 0.983. Day 4: Two-sided Kolmogorov-Smirnov test, n1 = 97, n2 = 97, z(193) = 0.144, p = 0.244. Day 5: Two-sided Kolmogorov-Smirnov test, n1 = 86, n2 = 106, z(191) = 0.173, p = 0.102. Day 6: Two-sided Kolmogorov-Smirnov test, n1 = 58, n2 = 116, z(173) = 0.207, p = 0.063. Day 7: Two-sided Kolmogorov-Smirnov test, n1 = 102, n2 = 115, z(216) = 0.082, p = 0.836).