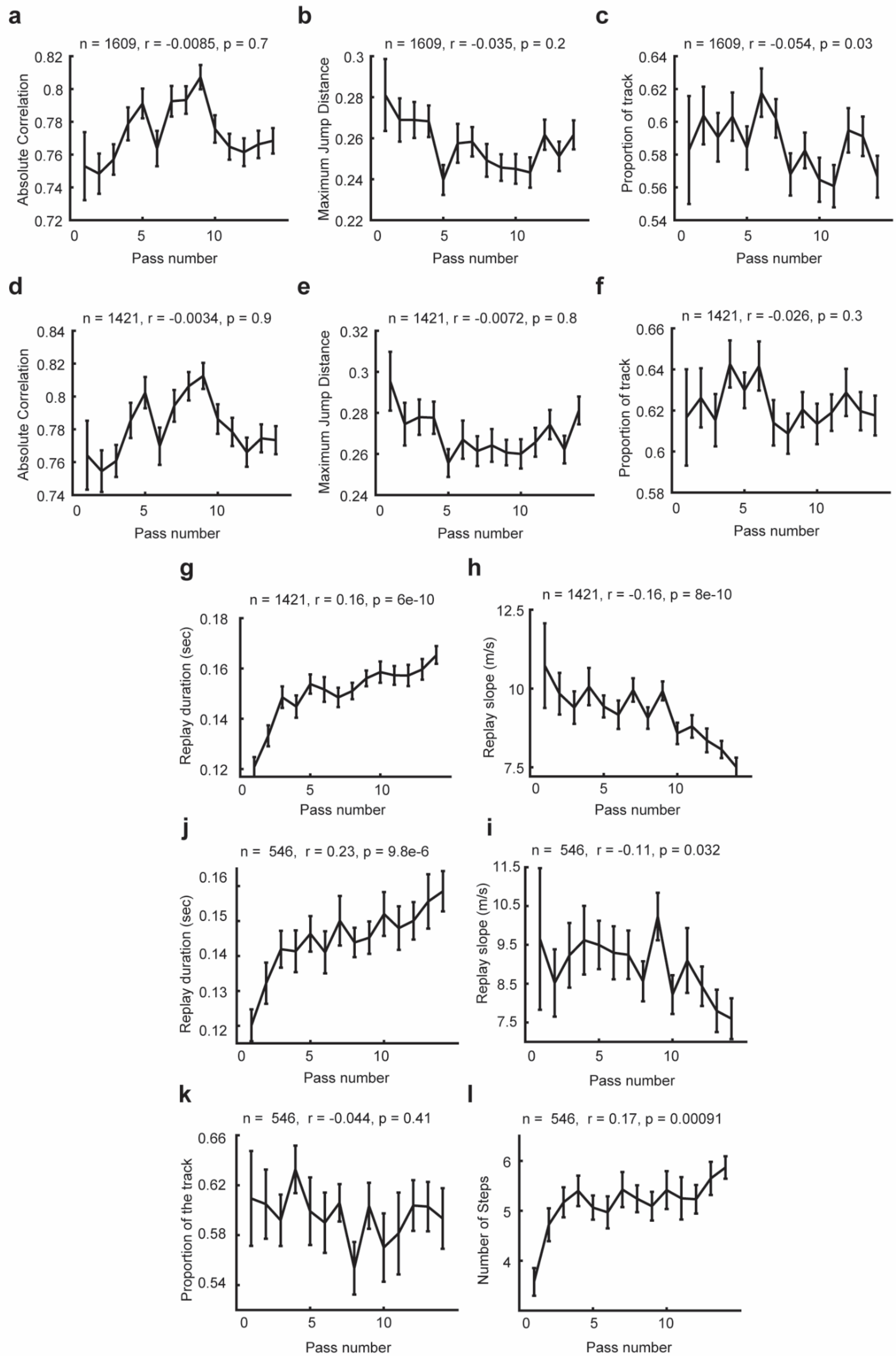


a
n = 1609, r = -0.0085, p = 0.7
Absolute Correlation
Pass number
b
n = 1609, r = -0.035, p = 0.2
Maximum Jump Distance
Pass number
c
n = 1609, r = -0.054, p = 0.03
Proportion of track
Pass number
d
n = 1421, r = -0.0034, p = 0.9
Absolute Correlation
Pass number
e
n = 1421, r = -0.0072, p = 0.8
Maximum Jump Distance
Pass number
f
n = 1421, r = -0.026, p = 0.3
Proportion of track
Pass number
g
n = 1421, r = 0.16, p = 6e-10
Replay duration (sec)
Pass number
h
n = 1421, r = -0.16, p = 8e-10
Replay slope (m/s)
Pass number
j
n = 546, r = 0.23, p = 9.8e-6
Replay duration (sec)
Pass number
i
n = 546, r = -0.11, p = 0.032
Replay slope (m/s)
Pass number
k
n = 546, r = -0.044, p = 0.41
Proportion of the track
Pass number
l
n = 546, r = 0.17, p = 0.00091
Number of Steps
Pass number

Figure S1 As replays slow down, other aspects of replay remain constant across experience. Related to Figures 2 and 5.

(a) Weighted correlation, and **(b)** maximum jump distance (as a proportion of the track) did not change across passes.

(c) The proportion of the track covered changed slightly. To correct for this, we imposed an additional criterion that replays must cover at least 40% of the track.

Under these criteria the **(d)** weighted correlation, **(e)** the maximum jump distance, and **(f)** proportion of the track covered, remained constant, however **(g)** durations increased and **(h)** slopes decreased across passes.

When we assessed the correlation of replays across passes across sessions [averaging all replays in each session (N = 39) for each pass (1-14)] instead of across individual replays, **(k)** the proportion of the track covered did not change.

However, the **(i)** duration, **(j)** slope, and **(l)** number of steps significantly changed.

All data show mean ± S.E.M. Correlations are Pearson's correlation values.

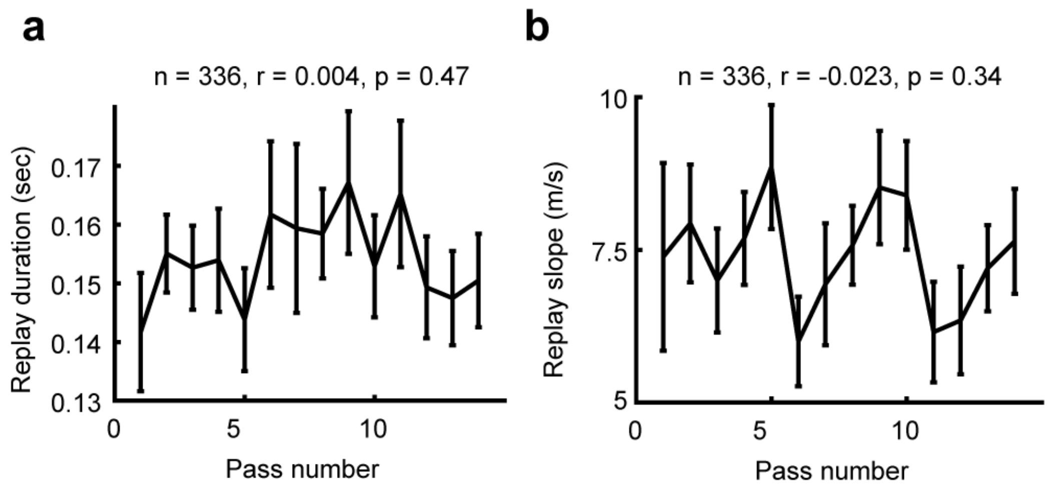


Figure S2 Replays in familiar environments do not slow down. Related to Figure 2.
(**a**) The duration and (**b**) slope of replay as a function of passes on familiar linear tracks. Two-way ANOVA of replay duration between novel and familiar environments across passes: main effect of novelty: $F_{(1,1944)} = 8.9$, $P = 2.9e\text{-}3$, main effect of pass: $F_{(1,1944)} = 6.3$, $P = 0.012$, interaction: $F_{(1,1944)} = 5.6$, $P = 0.018$; two-way ANOVA of replay slope between novel and familiar environments across passes: main effect of novelty: $F_{(1,1944)} = 19$, $P = 1.3e\text{-}5$, main effect of pass: $F_{(1,1944)} = 11$, $P = 1.1e\text{-}3$, interaction: $F_{(1,1944)} = 6.1$, $P = 0.014$. All data show mean ± S.E.M. Correlations are one-tailed Pearson's correlation values.

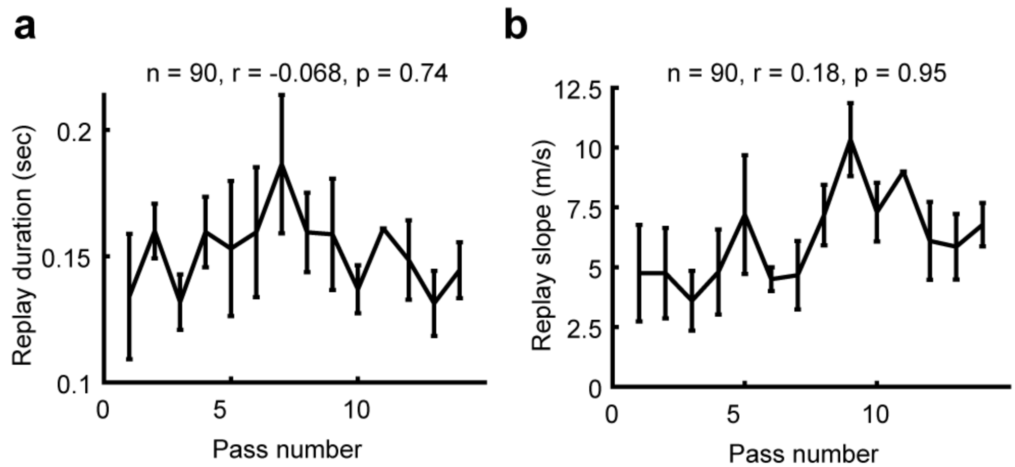
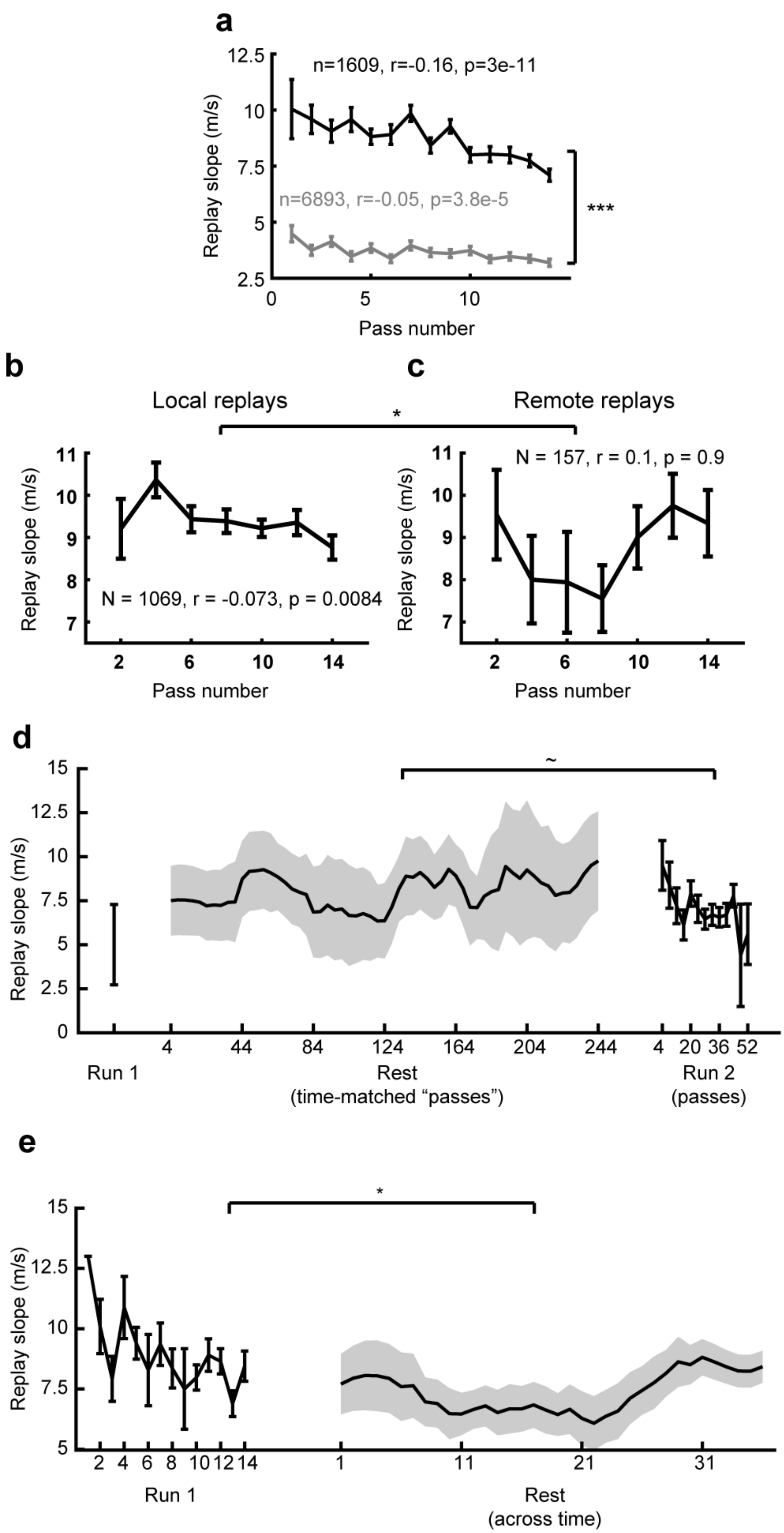


Figure S3 Reward changes do not produce rapid replay. Related to Figure 2.
(**a**) The duration of replays did not increase and the (**b**) slope of replays did not decrease across passes on a familiar track in a task with an increased or decreased reward. Two-way ANOVA between novel tracks and reward change tracks for duration: main effect of group, $F_{(1,1698)} = 3.9$, $P = 0.049$, main effect of pass $P = 0.49$, interaction: $F_{(1,1698)} = 4.4$, $P = 0.037$. Two-way ANOVA for slope: main effect of group, $F_{(1,1698)} = 25$, $P = 6.6e-7$, main effect of pass $P = 0.98$, interaction: $F_{(1,1698)} = 11$, $P = 1.1e-3$. All data show mean ± S.E.M. Correlations are one-tailed Pearson's correlation values.



a
n=1609, r=-0.16, p=3e-11
n=6893, r=-0.05, p=3.8e-5
***
Replay slope (m/s)
Pass number
b
c
Local replays
Remote replays
*
N = 1069, r = -0.073, p = 0.0084
N = 157, r = 0.1, p = 0.9
Replay slope (m/s)
Pass number
d
~
Replay slope (m/s)
Run 1
Rest
(time-matched "passes")
Run 2
(passes)
e
*
Replay slope (m/s)
Run 1
Rest
(across time)

Figure S4 Replay slopes. Related to Figures 3 and 4.

(**a**) The duration of replay across passes for all replays (black) and all non-replay candidate events (gray), as in Figure 3b. Pearson's correlation: N = 6893, R = 0.05, P = 3.8e-5. Two-way ANOVA between group and lap, main effect of group: $F_{(1,8501)} = 57$, P = 4.4e-14, main effect of pass: $F_{(1,8501)} = 428$, P = 2.5e-92, interaction: $F_{(1,8501)} = 17.2$, P = 3.2e-5.

(**b**) The slope of local replays (as in Figure 3d) decreased across passes on Track A (One-tail test: N = 1069, R = -0.073, P = 0.0084).

(**c**) The slope of remote replays of Track A did not decrease across passes on Track B (related to Figure 3e; One-tail test: N = 157, Duration: R = 0.1, P = 0.90). Two-way ANOVA between **b** and **c**: Two-way ANOVA of replay slope: main effect of group P > 0.6, main effect of passes: $F_{(1,1225)} = 7.58$, P = 0.0060, interaction: $F_{(1,1225)} = 4.74$, P = 0.030. Additionally, the slope of Track A replays at the end of session 1 in **b** was not different than at the beginning of session 2 in **c** (Session 1 local replays > 10 passes N = 856, Session 2 remote replays passes 1-4 N = 33; Wilcoxon rank-sum test, slope: P = 0.2).

(**d**) Slope data for replay during rest after a single pass on a novel track (Related to Figure 4a-b). Replays did not decrease in slope during rest (One-tail test: N = 228, R = -0.013, P = 0.42), but did with subsequent experience on the same novel track (One-tail test: N = 280, R = -0.11, P = 0.033). Two-way ANOVA: main effect of group $F_{(1,507)} = 0.091$, P = 0.76, main effect of pass $F_{(1,507)} = 2.1$, P = 0.14, interaction $F_{(1,507)} = 1.9$, P = 0.17.

(**e**) Slope data for replay after a full session on a novel track (related to Figure 4c-d). Replays of the novel track (Run 1) decreased in slope with continued experience on the track (One-tail test: N = 216, R = -0.17, P = 0.0057), but did not continue to decrease in slope during subsequent rest (One-tail test: Rest; N = 79, R = -0.072, P = 0.26). Two-way ANOVA: main effect of group: $F_{(1,294)} = 5.8$, P = 0.017, main effect of pass: $F_{(1,294)} = 6.4$, P = 0.12, interaction: $F_{(1,294)} = 3.9$, P = 0.049.

All data show mean ± S.E.M. Correlations are Pearson's correlation values. *** P < 0.001, * P < 0.05, ~ P < 0.2.

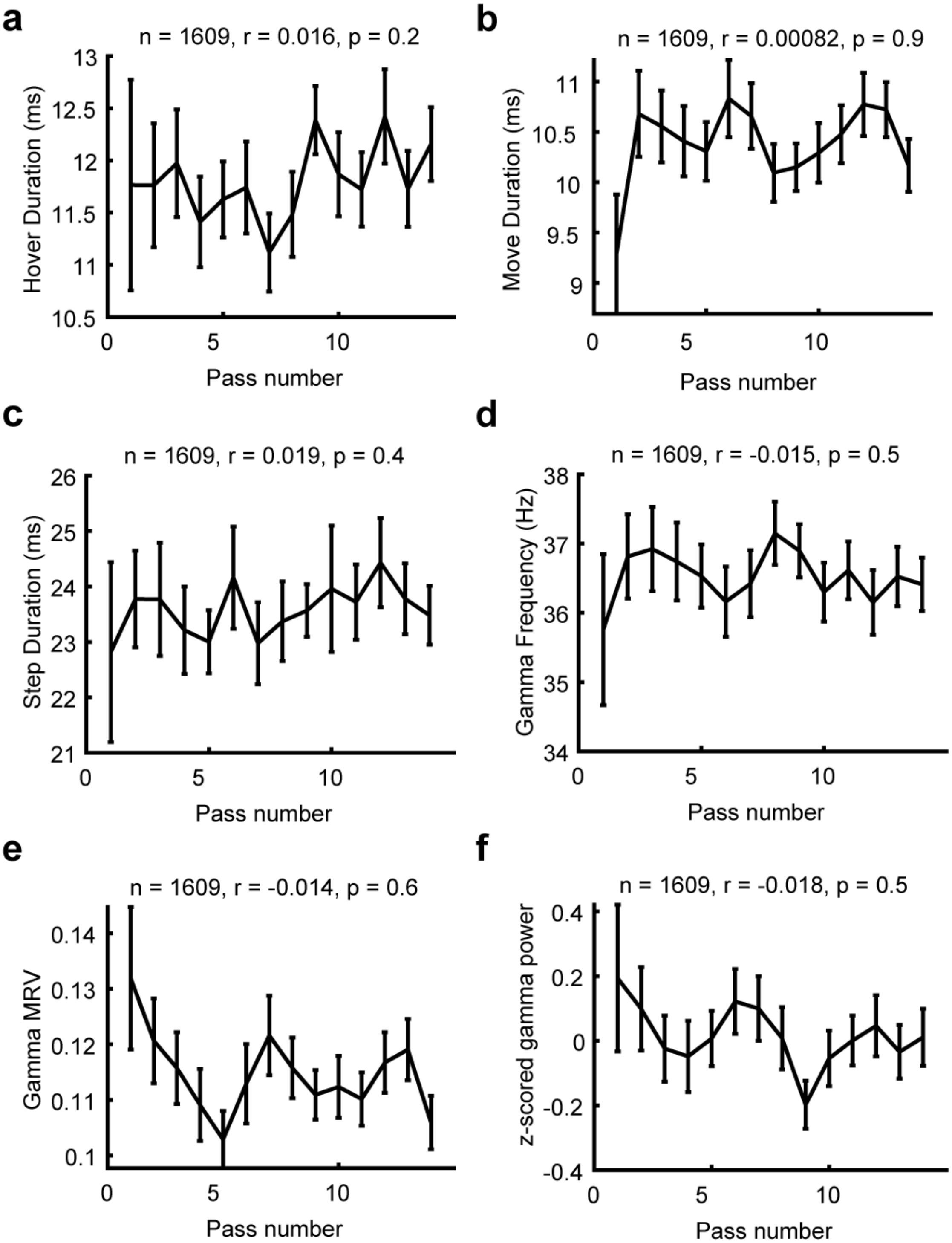


Figure S5 Temporal aspects of hover-and-jump dynamics do not change with experience. Related to Figure 5.
(**a**) Hover durations,
(**b**) move durations,
(**c**) step durations,
(**d**) slow gamma frequency,
(**e**) HP neuron gamma mean resultant vectors (MRV)
and (**f**) z-scored gamma power within replays did not change across experience on a novel track. All data show mean ± S.E.M. Correlations are Pearson's correlation values.

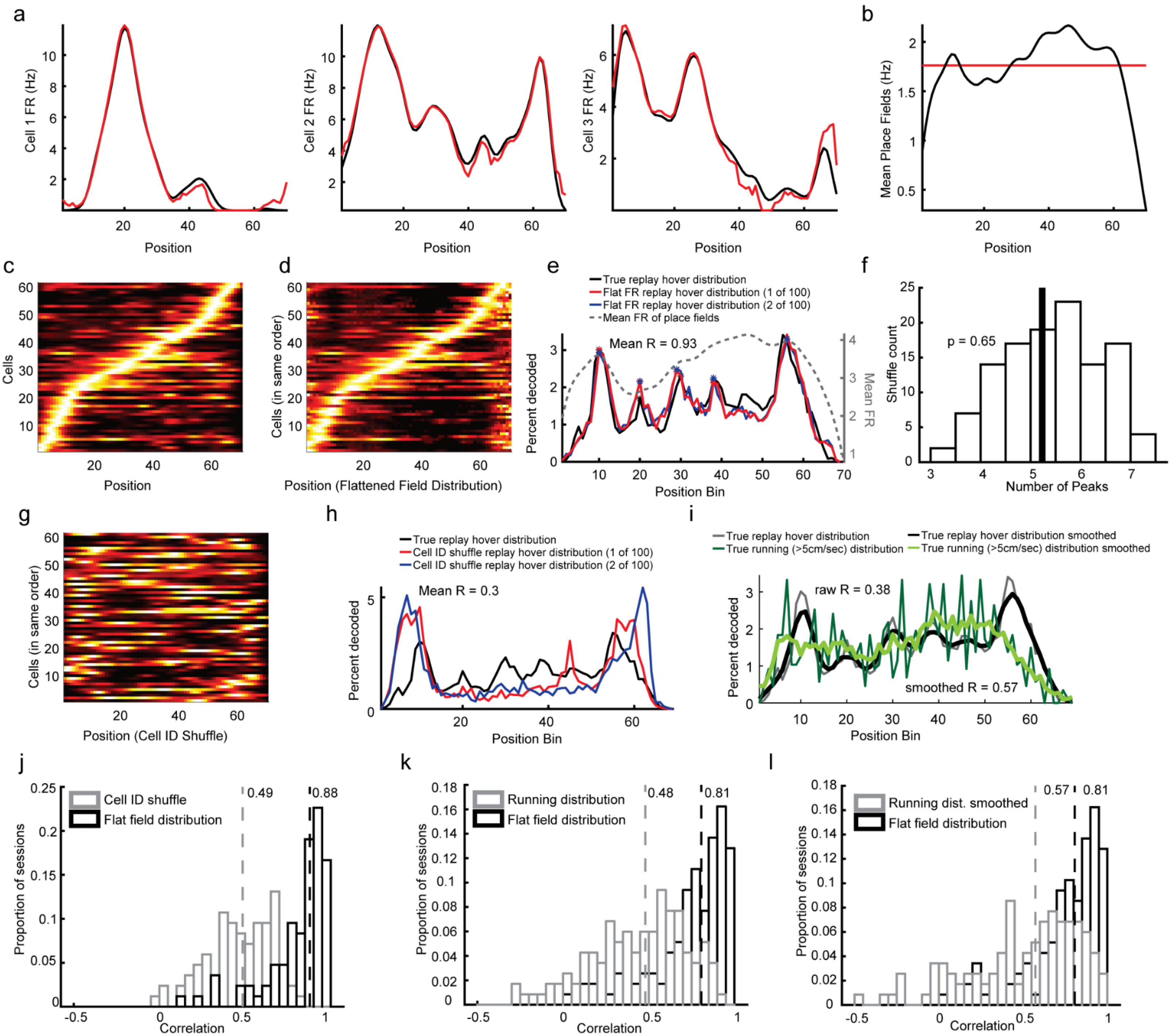


Figure S6. Controls for hover location analysis. Related to Figure 6 and STAR Methods.

(a) Three example neuron's firing rates before [black] and after (red) adjusting to flatten the distribution of fields.

(b) The mean place fields in this example session before (black) and after (red) artificially flattening the distribution. The flattening procedure was done by adding small amounts of noise to each cell's place field in a systematic (i.e. non-random) manner (shown in **a**) such that the population firing rate (FR) was flattened.

(c) Ordered place fields before **(d)** and after the artificial flattening.

(**e**) Left axis: Two example replay hover distributions made from fields using the flattened procedure (red and blue) and the true replay hover distribution (black). The mean Pearson's correlation across all 100 iterations of flattening for this example session is shown. Right axis: The mean FR of HP neurons' firing during running (their place fields).

(f) The number of peaks (asterisks in **e**) were not different between the real (vertical line, average across sessions) and flattened control distributions (histogram, average across sessions, Monte-Carlo test P = 0.65).

(g) Fields ordered the same as **c** and **d** after a cell ID shuffle.

(h) Two example distributions made from cell ID shuffles (red and blue) and the true distribution. The mean Pearson's correlation across all 100 iterations of flattening is shown.

(i) The distribution of decoded locations when performing the same timescale decoding on periods of running (> 5cm/sec; dark green) compared with the replay hover distribution (gray). The Pearson's correlation coefficients for this analysis is labeled "raw". The moving average (5 position bins) is depicted for the running (light green) compared to the replay hover distribution (black) and the correlation between these two is labeled "smoothed".

(j) Across all sessions with 60 or more neurons, the distribution of the mean Pearson's correlation for each session between the artificially flattened procedure's distributions with the true distribution (as in **e;** Black; N = 84 sessions, median = 0.88, mean = 0.81 ± 0.021) as well as a distribution of the mean Pearson's correlation for the same sessions between the cell ID shuffle procedure's distribution with the true distribution (as in **h**; gray; N = 84, median = 0.49, mean = 0.47 ± 0.021. Wilcoxon signed-rank test: Z = 7.5, P = 9.2e-14). The medians of both distributions are marked with vertical lines.

(k) Across all sessions, the distribution of the mean Pearson's correlation for each session between the artificially flattened procedure's distributions with the true distribution (as in **e;** Black; N = 117 sessions, median = 0.81, mean = 0.74 ± 0.021) as well as the distribution of the mean Pearson's correlation for each session between the true running distribution with the true replay hover distribution (as in the raw traces in **i;** gray; N = 117 sessions, median = 0.48, mean = 0.44 ± 0.026 Wilcoxon signed-rank test: Z = 7.4, P = 1.2e-13). The medians of both distributions are marked with vertical lines.

(l) Across all sessions, the distribution of the mean Pearson's correlation for each session between the artificially flattened procedure's distributions with the true distribution (as in **e;** Black; N = 117 sessions, median = 0.81, mean = 0.74 ± 0.021) as well as the distribution of the mean Pearson's correlation for each session between the true running distribution smoothed with the true replay hover distribution smoothed (as in the smoothed traces in **i;** gray; N = 117 sessions, median = 0.57, mean = 0.48 ± 0.033 Wilcoxon signed-rank test: Z = 5.5, P = 2.9e-8). The medians of both distributions are marked with vertical lines.

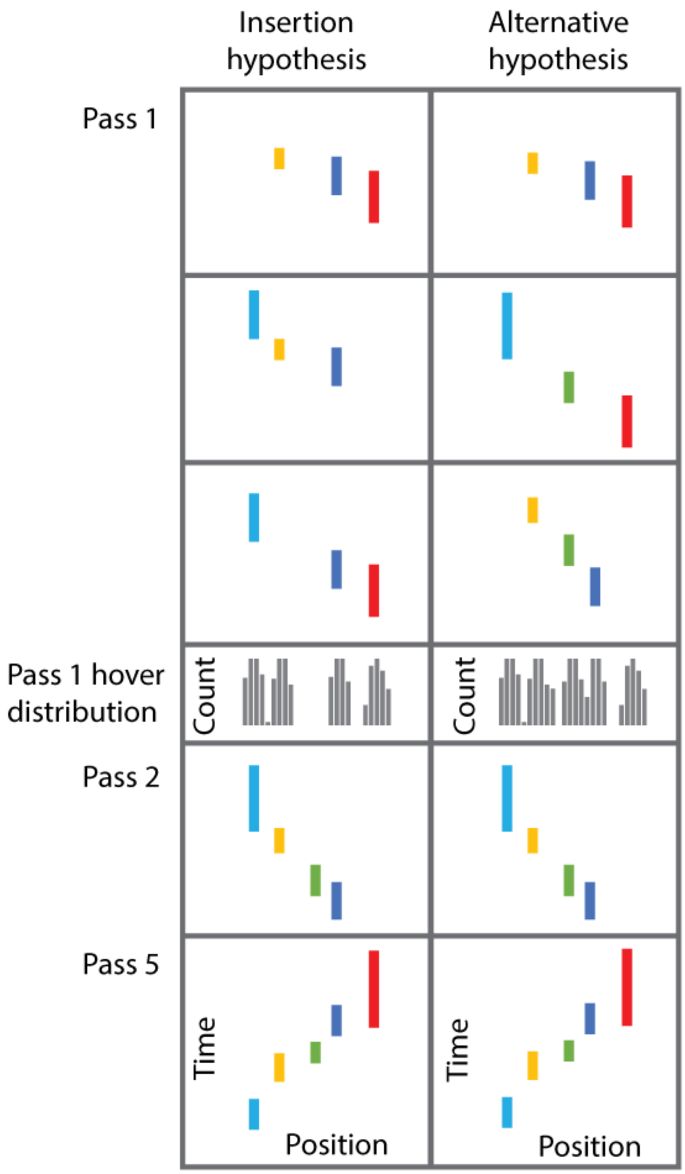


Figure S7 Two hypotheses of experience-dependent aspects of hover additions. Related to Figure 6.

Left: the "Insertion hypothesis" whereby replays that occur on pass 1 are all different, but they form four hovers, shown in the "Pass 1 hover distribution". No replay hovered in the eventual green hover location.

Right: the "alternative hypothesis" shows three different replays, none of which hover in each hover location, but together the hover location distribution shows all the hover locations ("Pass 1 hover distribution"). Eventually both scenarios produce replays that hover in each location, but the Insertion hypothesis reflects that the insertion of the hovers is experience-dependent.