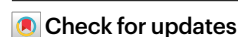


Lateral entorhinal cortex subpopulations represent experiential epochs surrounding reward

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During goal-directed navigation, ‘what’ information, describing the experiences occurring in periods surrounding a reward, can be combined with spatial ‘where’ information to guide behavior and form episodic memories. This integrative process likely occurs in the hippocampus, which receives spatial information from the medial entorhinal cortex; however, the source of the ‘what’ information is largely unknown. Here, we show that mouse lateral entorhinal cortex (LEC) represents key experiential epochs during reward-based navigation tasks. We discover separate populations of neurons that signal goal approach and goal departure and a third population signaling reward consumption. When reward location is moved, these populations immediately shift their respective representations of each experiential epoch relative to reward, while optogenetic inhibition of LEC disrupts learning the new reward location. Therefore, the LEC contains a stable code of experiential epochs surrounding and including reward consumption, providing reward-centric information to contextualize the spatial information carried by the medial entorhinal cortex.

Episodic memories of rewarding experiences include information about where episodes occurred and also nonspatial information about what occurred (event context)¹. For example, a dinner out is an experience that consists of clear spatial navigation components but also different experiential epochs: hunger and anticipation before arriving at the restaurant, eating and drinking during consumption of the dinner, contentment and satiation after departing the restaurant. These experiential epochs are not obviously aligned to sensory inputs but are important components in forming episodic memories. Here, we refer to such nonspatial components of these epochs as reward experience epochs.

The integration of spatial and experiential information is thought to occur in the hippocampus², where place cells encode not just locations in an environment but also additional nonspatial information^{3,4}, as evidenced by the context-dependent firing of place cells^{5–7}. Context dependence is especially prominent in reward-guided navigation, which leads to an overrepresentation in the number of place cells encoding the region around reward^{8,9} and distinct signaling of goal

approach and goal departure^{10,11}. How the ‘what’ information about reward experience reaches the hippocampus is poorly understood however. In particular, such information should not just encode the discovery of a reward but also represent information from the periods leading up to and after reward (Fig. 1a)^{12–14}.

The medial entorhinal cortex (MEC)—a main source of input to the hippocampus¹⁵—contains spatially modulated cells such as grid cells¹⁶, border cells¹⁷ and head direction cells¹⁸ that convey spatial information. The MEC could also be a potential source of reward experience information to the hippocampus. As such, several studies have investigated the role of the MEC in reward-guided navigation^{19–21}. Bulk MEC activity is influenced only moderately by the presence of rewards²¹ and, while a rewarded location could distort nearby firing fields of grid cells, these changes were not shown to be specific to the trajectory¹⁹. Thus, MEC has not been shown to represent different reward experience epochs²¹.

Alternatively, neuromodulatory systems could provide such reward experience information to the hippocampus²². Most notably, the locus coeruleus (LC) provides bursts of noradrenaline and

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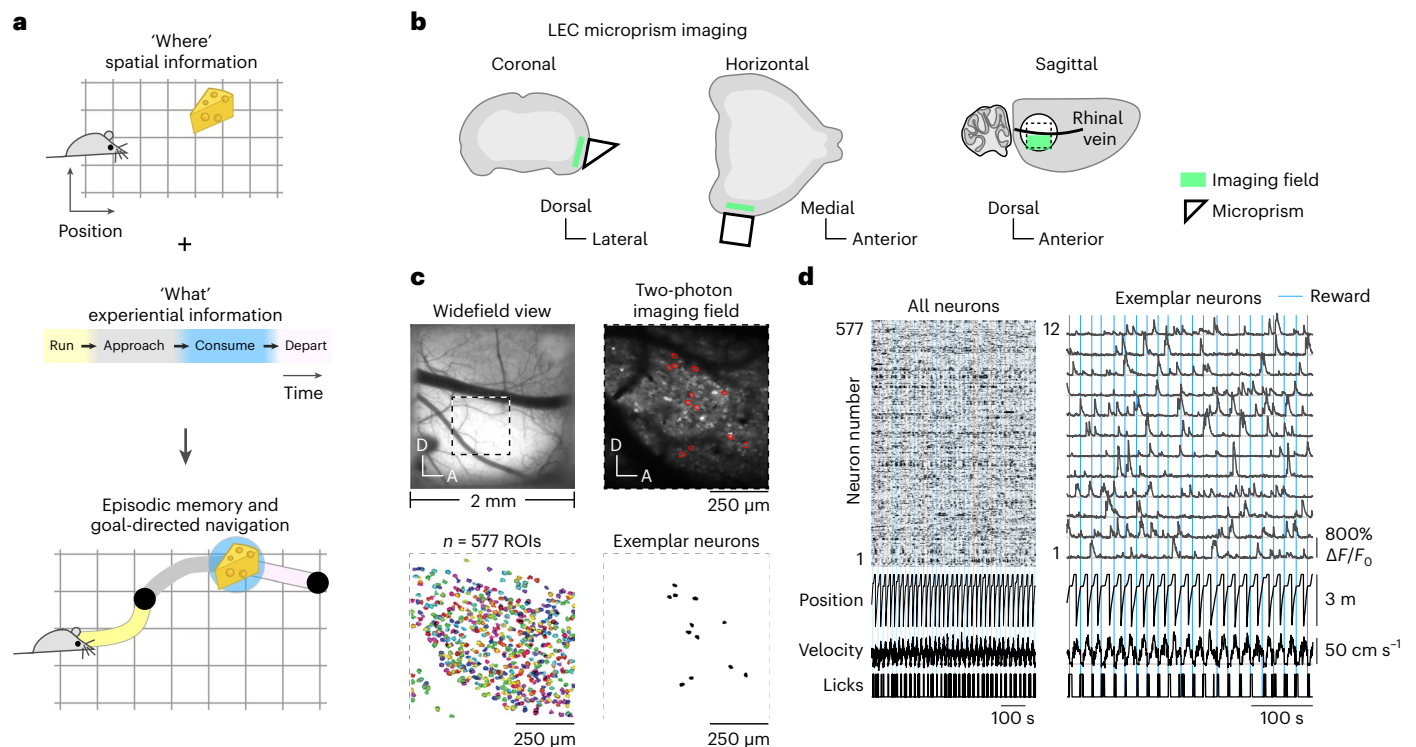


Fig. 1 | Two-photon imaging of LEC. **a**, Schematic of goal-directed navigation requiring a spatial map along with a reward experience representation. In this model, episodic memories of goal-directed navigation combine spatial 'where' information with experiential 'what' information during the experience. **b**, Schematic of LEC imaging preparation, with a 3 mm No. 0 glass coverslip (circle) bonded to a 45° 2 × 2 mm microprism (square/triangle). **c**, Exemplar field viewed through microprism using widefield and two-photon fluorescence

imaging of neurons expressing GCaMP6s. Below, segmentation of individual cells and cell masks for all 577 neurons and 12 exemplar neurons shown in **d**. **d**, Fluorescence traces for all detected neurons (sorted using k-means clustering) and expanded view for 12 exemplar neurons after neuropil subtraction and baseline correction. Behavioral signals are shown underneath. A, anterior; D, dorsal; ROI, region of interest.

dopamine at a new reward location during navigation^{23,24}. Inhibition of these inputs prevented the formation of place cell overrepresentation of reward locations. However, activation of these inputs alone was not sufficient to drive the overrepresentation, as concurrent rewards were still required²³. Thus, LC inputs to the hippocampus provide a learning signal that can open a plasticity window for spatial information to be associated with reward experience information but, crucially, the reward experience information seems to reach the hippocampus from a separate and currently unknown source.

Finally, the LEC, which also provides substantial excitatory input to the hippocampus¹⁵, could possibly provide the reward experience information. Whereas previous studies have uncovered a variety of roles for the LEC across a range of behaviors, including object coding^{25,26}, timing²⁷, olfaction²⁸ and nonspatial associative learning²⁹, its role in representing rewards and reward experience has not been addressed directly. Critically, previous studies did not disentangle rewards from objects, making it unclear whether LEC was encoding the object salience itself or valence related to the reward experience. Therefore, here we developed a two-photon imaging technique that allowed for functional recordings of large populations of LEC neurons and made use of a virtual reward-guided navigation task, which added valence to an otherwise unmarked location, thus dissociating object salience and reward experience.

Results

LEC firing segregates into pre- and postreward populations

As two-photon functional imaging has not been used previously in the LEC during behavior²⁸, we first developed a microprism-based method^{30,31} for two-photon imaging of the LEC (Fig. 1b and Methods). This approach enabled us to capture firing from ~500 cells in a typical

imaging field while head-fixed mice ran on a treadmill to traverse a one-dimensional virtual track (Fig. 1c,d, Extended Data Fig. 1 and Supplementary Video 1). To examine how LEC may represent reward experience epochs, we designed the track to be visually cue rich but the reward location itself not marked by any visual feature or object. This design helped isolate visual object coding from reward coding, which we exploit in later sections to move the reward without changing other aspects of the environment. Mice learned over several training sessions to decelerate and lick in anticipation of the reward location, where a drop of water was delivered (Fig. 2a). We quantified the firing of neurons in LEC along the track during this behavior using spatial information since reward experience is expected to change along the track. We also performed two-photon Ca²⁺ imaging of populations of neurons in MEC and in the CA1 region in separate groups of mice implanted with microprisms³² or cannulas³³, respectively (Fig. 2b). Analyses of changes in reward location are left to later sections; here, we examine coding of a learned reward location. Three different reward locations were used in our experiments. We first show data from the most used reward location (2.3 m) and pool data from the other reward locations (0.7 m and 1.5 m) where appropriate.

Among active LEC neurons, we selected those with significant spatial information along the track³⁴ (Methods). From 17 fields of view (FOVs) across seven mice with the reward located at 2.3 m, 2,016 such spatial cells were found, representing 24.9% of active cells (Extended Data Fig. 2f). Across all reward positions, we identified 3,956 spatial cells out of 14,489 active neurons from 32 FOVs (Extended Data Tables 1 and 2). We plotted their spatial tuning, with neurons sorted by where their firing peaked along the track. Nearly half of spatial cells in LEC were active near the reward location, with such reward cells defined as spatial cells whose mean firing peaked within 40 cm of the reward location

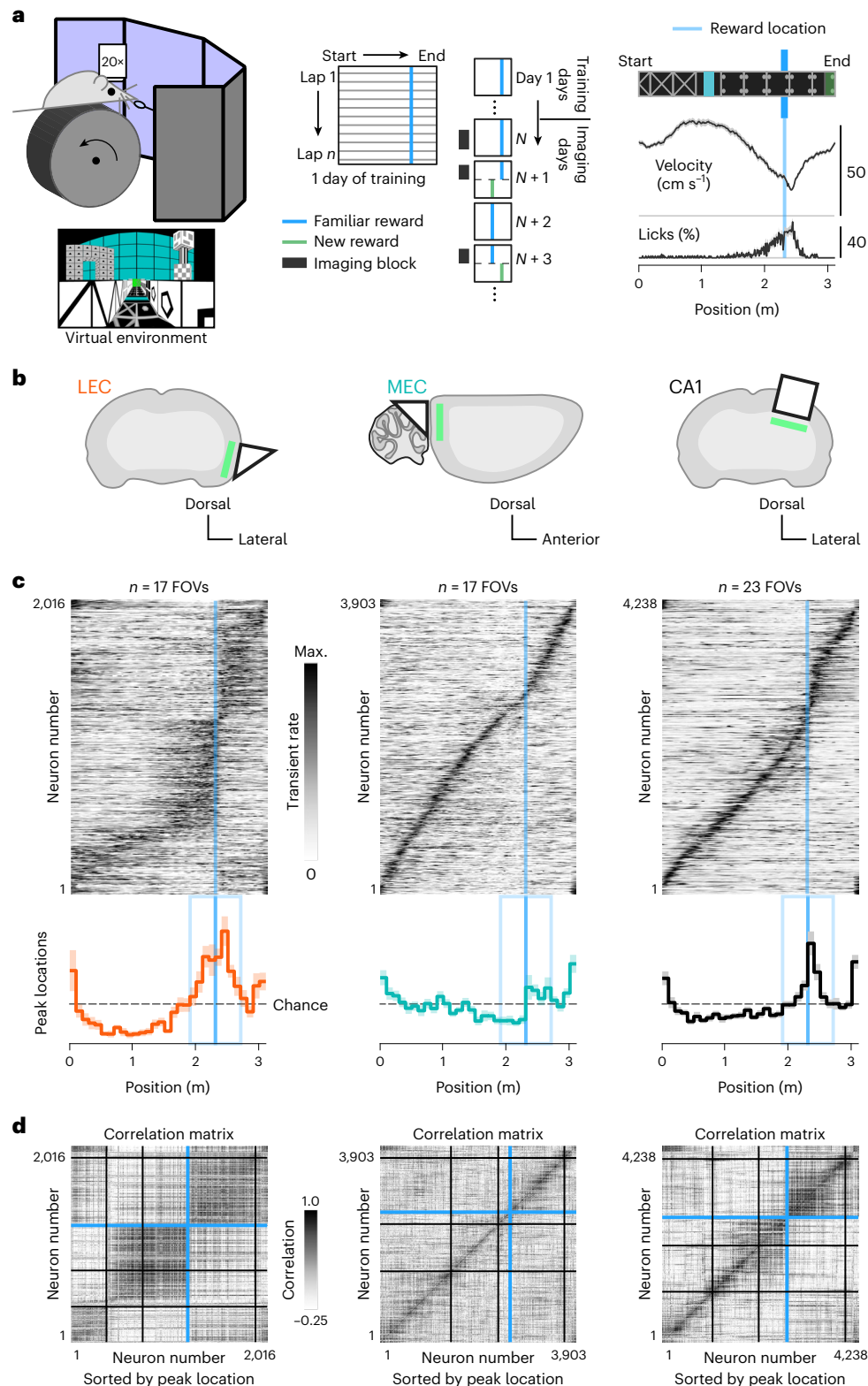


Fig. 2 | Prominent reward clustering in LEC with segregation of pre- and postreward populations. **a**, Head-fixed mice traverse a linear track in virtual reality (VR) for water rewards. Once behavior reached criteria (more than two laps per minute over a ~40-min training session along with anticipatory licking and deceleration for reward), imaging sessions began. On subsequent days, reward location was moved to 0.7 m or 1.5 m, with further moves only after new reward location became familiar (assessed by anticipatory behavior of reward). Treadmill velocity and detected licks (averaged over 43 traversals) shown for example session. **b**, Schematics of imaging approaches using implanted microprisms (LEC, MEC) or cannula (CA1). **c**, Cross-validated population spatial firing patterns for LEC, MEC and CA1 mice. Spatial cells (neurons with significant

spatial information; Methods) sorted by where their firing peaked along the track on even laps. Each row of the image is the firing of one neuron averaged over odd laps (1-cm binning after 170-ms Gaussian filter), normalized to its maximum (Max.) value. Histograms show proportion of peak locations for neurons in 10-cm bins, calculated for individual sessions (FOV), with mean $\pm$ s.e.m. shown as dark line with light shading; 'chance' calculated as expected number in each bin if uniformly distributed (1/31 bins). 'Reward cells' are those that peak within the blue rectangle (± 40 cm from reward). **d**, Correlation matrix formed from sorted heatmaps in **c**, calculated between average firing on even and odd laps. Each pixel is the Pearson correlation coefficient between pairs of neurons. Blue lines divide prereward and postreward neurons. Black lines indicate 1, 2 and 3 m locations.

(Fig. 2c and Extended Data Fig. 2f). By comparison, MEC spatial cells were active nearly uniformly across the track, and the fraction active around reward was as expected by chance, whereas in CA1 an enhanced fraction of cells, but less than in LEC, was active near reward (Fig. 2c and Extended Data Fig. 2a,f). Track position could still be decoded from nonreward spatial cells in LEC, but the decoder error was higher for LEC than MEC or CA1 (Extended Data Fig. 2c). Cells from all three regions also tended to cluster near track start and end, but we focused our analysis around the reward location.

LEC spatial cells had wide spatial fields and were separated by whether they were active before or after the reward location (Fig. 2c). These features are clear in plots of the correlation matrix of the sorted mean spatial firing maps (Fig. 2d). While MEC shows a clear diagonal band structure, indicating narrow spatial fields across all track locations, LEC shows a block-like structure, indicating wide spatial fields (Extended Data Fig. 2e) that largely segregate into pre- and postreward-active neurons—a division confirmed by k-means clustering (Extended Data Fig. 2d). To quantify the enrichment of spatial cells near reward, we divided the number of cells that peak in each reward zone (either pre- or postreward) by the expected number of cells if peaks were distributed uniformly. This reward clustering ratio was enhanced in LEC for prereward compared with CA1 and MEC and in both LEC and CA1 for postreward (Extended Data Fig. 2g).

Superficial layers of LEC differ in cell type and connectivity¹⁵, so we next explored whether laminar differences relate to reward clustering. In particular, LEC layer II fan cells receive dopaminergic inputs from the ventral tegmental area (VTA)^{29,35}, suggesting a potential locus where reward information may enter the system. We imaged at depths targeting two layers: 80–150 μm below dura for layer II (23 fields) and 200–250 μm below dura for layer III (9 fields) (Extended Data Figs. 1 and 2b). Although both regions show preferential firing near the reward location, the enhancement of firing in the prereward region was significantly larger in layer II versus layer III (Extended Data Fig. 2h). Importantly, such a difference did not exist for MEC, with a paucity of reward neurons across depths.

Therefore, LEC, unlike MEC, was highly active around the reward location, with populations of neurons segregated by their firing during either goal approach or goal departure and the goal-approach population particularly enriched in LEC layer II.

Location-invariant representation of reward in LEC

We next asked whether pre- and postreward populations were dedicated to encoding particular epochs around reward irrespective of the location of the reward. To disentangle whether reward cells are encoding spatial information (track position) or reward itself, the reward location was moved in the middle of a session (after ~40 laps) without any cue, indication or other change to the environment. Mice learned to anticipate the new reward location by shifting their deceleration along the track (Fig. 3a). Imaging was performed throughout the session.

Some LEC neurons maintained their firing field with respect to track position (LEC stable spatial cell; Fig. 3b) and others with respect to the reward location (LEC stable pre- and postreward cells; Fig. 3b). We plotted all spatial neurons before and after this change in reward location from 2.3 m to 1.5 m, sorted by their firing peaks with respect to the familiar reward location (Fig. 3c). Reward clustering again developed around the new reward location. Indeed, relatively few LEC neurons continued to fire at the same track location, especially compared with MEC and CA1 (Fig. 3d and Extended Data Fig. 3a,b). Instead, over half of the cells active in the reward zone for the familiar location shifted their firing fields to the new reward location (stable reward cells)—more than expected by chance (Fig. 3e).

When examining pre- and postreward cells as distinct groups (Fig. 3c,f), we found that most prereward cells for the familiar reward location remained prereward cells relative to the new reward location

and, similarly, postreward cells remained postreward relative to the new reward location (Fig. 3f–h). The probability of a prereward cell remaining a prereward cell was 2.7 $\times$ chance and 2.4 $\times$ for postreward cells (geometric mean of ratio for each session; Fig. 3i). This dedication was largely absent in MEC (Fig. 3g–i). Cells in CA1 showed a mixture of the strong dedication from LEC and the weak dedication from MEC (Fig. 3g–i). When we teleported mice to new environments with unmarked reward locations, we found that LEC reward dedication was similarly maintained (1.5 $\times$ for prereward and 2.3 $\times$ for postreward cells; Extended Data Fig. 3c–h). Thus, LEC neurons consistently maintained their firing patterns relative to a new reward location, indicating robust representations of pre- and postreward epochs that are largely invariant to location and environment.

State transitions underlie LEC prereward ramping

The prereward period, during which mice may be anticipating reward, is a particularly interesting reward experience epoch. Both behavioral changes (slowing; Fig. 2a) and the firing in prereward neurons in LEC (ramping; Fig. 4b) precede the reward, which led to two questions: first, what are the dynamics of population activity of the prereward cells, which may give insight into the state of the network?; second, does this LEC state relate to behavior in a way that might help precisely define the prereward epoch?

On average, mean firing in prereward LEC neurons ramped up until the reward was delivered (Fig. 4b), similar to other brain regions such as VTA³⁶ and orbitofrontal cortex³⁷. We first asked what underlying population dynamics could give rise to this observed mean ramping signal. Various models have been proposed^{38–40}. For example, an increasing number of neurons might be recruited or firing in individual neurons could increase as the animal approaches reward, in which case ramping activity would be observed across the population on both individual trials and the trial averages (recruitment model; Fig. 4a). Alternatively, the prereward population might undergo coherent state changes at different times or positions with respect to reward on each trial, in which case discrete changes in activity, not ramping, would be observed across the population on individual trials and ramping would be observed only in trial averages (state model; Fig. 4a). To examine these possibilities, we quantified the number of prereward neurons active in the period leading up to reward on individual trials. We often observed step-like increases in the active number of neurons (Fig. 4d,e). Indeed, when we used a hidden Markov model (HMM) to identify such transition times from inactive to active population states (Extended Data Fig. 4a) and then plotted the firing rate of the prereward neurons aligned to these transition times (with each ‘epoch’ spanning the time between transitions using linear interpolation), we observed a clear step-like increase across the population of individual prereward neurons (Fig. 4b,c). We calculated that 44% of the variance in single-lap activity was explained by a step increase, with some ramping activity observed on top of the step-like change (Fig. 4b). Thus, part of the increasing ramp in average LEC prereward neuron activity could be explained by a discrete switch in firing state occurring at variable times and positions with respect to reward on different traversals, but some contributions from an increasing number of active neurons (or an increase in firing in individual neurons) were also present.

Next, we asked whether the state transitions in LEC related to behavior. Interestingly, on individual trials we also observed behavior state changes: mice switched from fast running to a much slower speed ahead of the reward location and maintained this slow speed until the reward was encountered (Fig. 4d and Extended Data Fig. 4b–c), with the timing and position of this deceleration varying across trials (Fig. 4e). To investigate whether the discrete behavior changes correlated with the LEC population state changes, as suggested by individual example trials (Fig. 4d), we examined the relationship between the HMM state transition times with the deceleration times on each trial (Fig. 4e). We found these times were highly correlated, both for

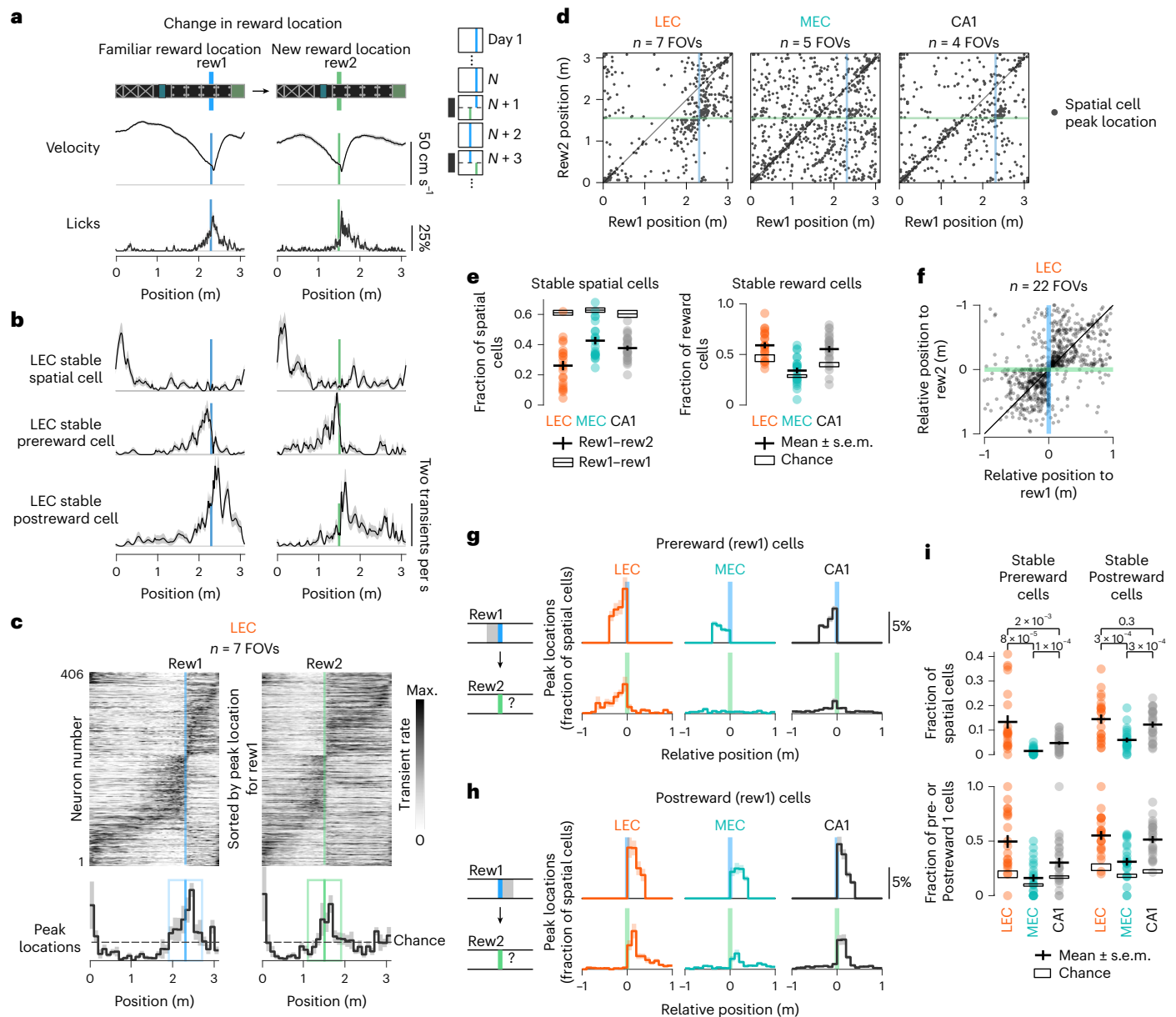


Fig. 3 | Dedication of pre- and postreward populations in the LEC. **a**, Licking and velocity before (66 laps) and after (49 laps) change in reward location; $\pm$ s.e.m. in light shading. In this example session, reward was moved earlier on the track, from familiar (rew1, 2.3 m) to new (rew2, 1.5 m) location. Training paradigm shown in scheme on the right. Two-photon imaging was performed spanning >20 laps with both familiar (blue) and new (green) reward locations. **b**, Spatial firing (deconvolved Ca²⁺ transients) for exemplar neurons (same session as **a**); $\pm$ s.e.m. in light shading. Imaging performed for a subset of laps (56 before and 44 after reward move). **c**, Spatial firing patterns along the track and histogram of firing peaks for spatial cells, sorted by firing peaks with familiar reward location. **d**, Dot raster of peaks for spatial cells. Cells on diagonal maintain their firing positions (stable spatial cells) whereas cells at intersection of reward locations adjusted their firing positions relative to reward locations (stable reward cells). **e**, Stable spatial cells quantified as fraction of spatial cells that are stable after reward move (peak of spatial tuning curve changes <40 cm; rew1-rew2). For comparison,

same quantification is applied to difference in peak location for even and odd laps with familiar reward location (rew1-rew1). Stable reward cells quantified as fraction of reward cells (for familiar reward location) that remain reward cells relative to new reward location. **f**, Peak locations relative to reward locations for LEC neurons that were spatial cells for both familiar (x axis) and new reward conditions (y axis). **g**, Peak locations for new reward location (rew2) for cells that are spatial in both conditions and prereward relative to familiar reward location (rew1). Histograms calculated for each imaging session; mean $\pm$ s.e.m. shown as dark line with light shading. **h**, Same as **g** but for postreward cells. **i**, Quantification of stable pre- or postreward cells at both reward locations, either as fraction of all spatial cells or of pre- or postreward cells for familiar reward location. **e–i**, Two-sided two-sample *t*-tests for comparisons between regions, indicated by horizontal line with *P* values. *n* = 22, 22 and 25 FOVs for LEC, MEC and CA1, respectively; each dot represents average for one imaging session and black cross represents mean $\pm$ s.e.m. across sessions.

the example session (Fig. 4f) and across all LEC sessions (Fig. 4g). A similar effect was observed for CA1 prereward cells, but no correlation was observed for MEC prereward cells (Fig. 4g). Thus, discrete transitions in population firing state in LEC prereward neurons correlate with slowing behavior.

Location-invariant representation of reward consumption

Pre- and postreward periods are separated in time by a period of reward consumption, during which the mouse is stationary and consuming water. Signaling in this epoch (during discovery and consumption of reward) is necessary to forming a representation of the experience

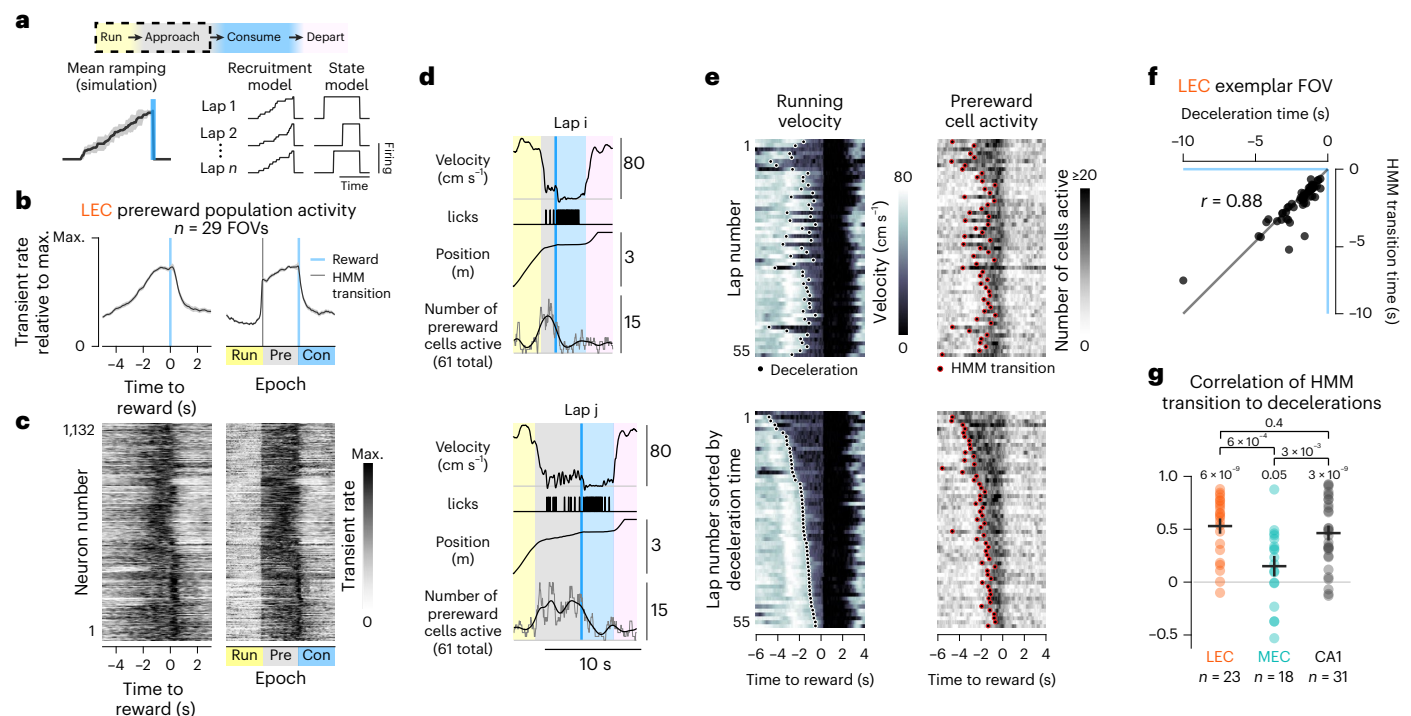


Fig. 4 | Prereward cell activation is composed partly of state changes linked to behavior. **a**, Prereward population activity exhibits a ramp up until reward delivery. Two proposed models: ‘recruitment model’, where cell activity or number of active cells increases as reward location is approached on individual trials; ‘state model’, where the whole population activates together at some location before reward, but this location varies across trials. **b**, Mean activity of LEC prereward population as a function of time relative to reward or by epoch (three epochs defined by start of traversal, HMM-detected transition of prereward cells to an active state as in **e**, and reward delivery time; intervals within each epoch linearly interpolated across time between transitions). Interpolation calculated for individual sessions (mean of all prereward cells) and normalized to their maximum values; mean $\pm$ s.e.m. shown as dark line with light shading. **c**, Transient rate for individual neurons by time or by epoch. Each row is one LEC prereward neuron, grouped by session, averaged across laps in a

session and normalized to the maximum. **d**, Representative sample of behavior and prereward activity during two traversals on virtual track. The number of prereward cells active shown as raw value (gray) and with 2-s Gaussian filter (black). **e**, Running velocity and number of prereward cells active on each lap. Detected deceleration and HMM-detected state transition times shown with dots. Top plots sorted by lap number for this session. Bottom plots sorted by deceleration times on each lap. **f**, Times of deceleration and state changes for exemplar session from **e**. Each point represents data from one lap; r indicates Pearson correlation coefficient. **g**, Pearson correlation coefficients, calculated as in **f**, for fields with at least ten prereward cells. Each point represents one imaging session; black cross represents mean $\pm$ s.e.m. across sessions. Statistical tests performed between each pair of regions (two-sided two-sample t -test) and to zero as well (one-sided one-sample t -test), with P values indicated; $n = 23, 18, 31$ FOVs, respectively.

of the reward. Since this epoch is typically excluded from analysis of spatial coding properties of cells (which excludes times when velocity is zero), we examined LEC firing relative to reward time, focusing on the period of reward delivery and consumption. At the time of reward delivery, LEC population firing increased dramatically and transiently—a signal not prominent in either MEC or CA1 (Fig. 5a). Nearly one in eight LEC neurons peaked within the first second after reward delivery. Using a shuffle test for significance of this firing peak, we developed criteria for ‘reward consumption active’ (RCA) neurons¹⁴ that contain such a peak in firing at reward. Across all sessions, 13.7% of active LEC neurons (1,978 of 14,489) qualified as RCA cells (Fig. 5b,c); this proportion was similar between LEC layers II and III (Extended Data Fig. 2b). The fraction of RCA cells was much lower in MEC (299 of 9,488, or 3.1%), while CA1 was intermediate (816 of 11,564, or 7.1%) (Fig. 5d).

We next asked whether and how the RCA cell population overlapped with spatial cells in LEC. Similar fractions of spatial and nonspatial cells were RCA cells (Fig. 5d). The nonspatial ‘pure’ RCA cells, such as the exemplar shown (Fig. 5c), were robustly active only during reward consumption. As for spatial cells, RCA cells were more common in the prereward cell population of LEC, but were also found in the postreward population (Fig. 5d). When reward location was moved, the reward consumption signal occurred at the new reward (Fig. 5c,e), with one-third of RCA cells in LEC maintaining their firing pattern with respect to reward time—higher than in CA1 and MEC (Fig. 5e). When

the environment was changed, again RCA cells maintained their firing pattern (Fig. 5e). Therefore, LEC represents the epoch of reward delivery and consumption with a dramatic transient increase in firing that is generated by a population of neurons with a significant level of dedication to providing this signal.

To investigate whether the signals in LEC surrounding reward delivery were due to reward itself or to the experiences surrounding reward, in separate experiments we measured the reward-triggered signal in LEC for randomly delivered rewards outside of the context of VR-based spatial navigation. In these sessions, we still observed a dramatic and transient increase in firing immediately after reward delivery (for 1 s) but did not observe the increasing ramp in firing leading up to reward delivery nor the later increase in firing 2–4 s after reward delivery (Extended Data Fig. 5). Thus, the signal carried by RCA cells seems to be a generalizable reward signal while the prereward and postreward signals are specific to a navigation task.

We applied principal component analysis to our population of 9,554 active LEC neurons (across 22 imaging sessions with a change in reward location) and plotted the first two principal components (Extended Data Fig. 6), similar to previous analysis²⁹. Jumps in the neural activity trajectory occurred during transitions between the previously defined experience epochs (postreward/running to prereward approach to consumption), quantified as the magnitude of the difference between successive points in state space (Extended

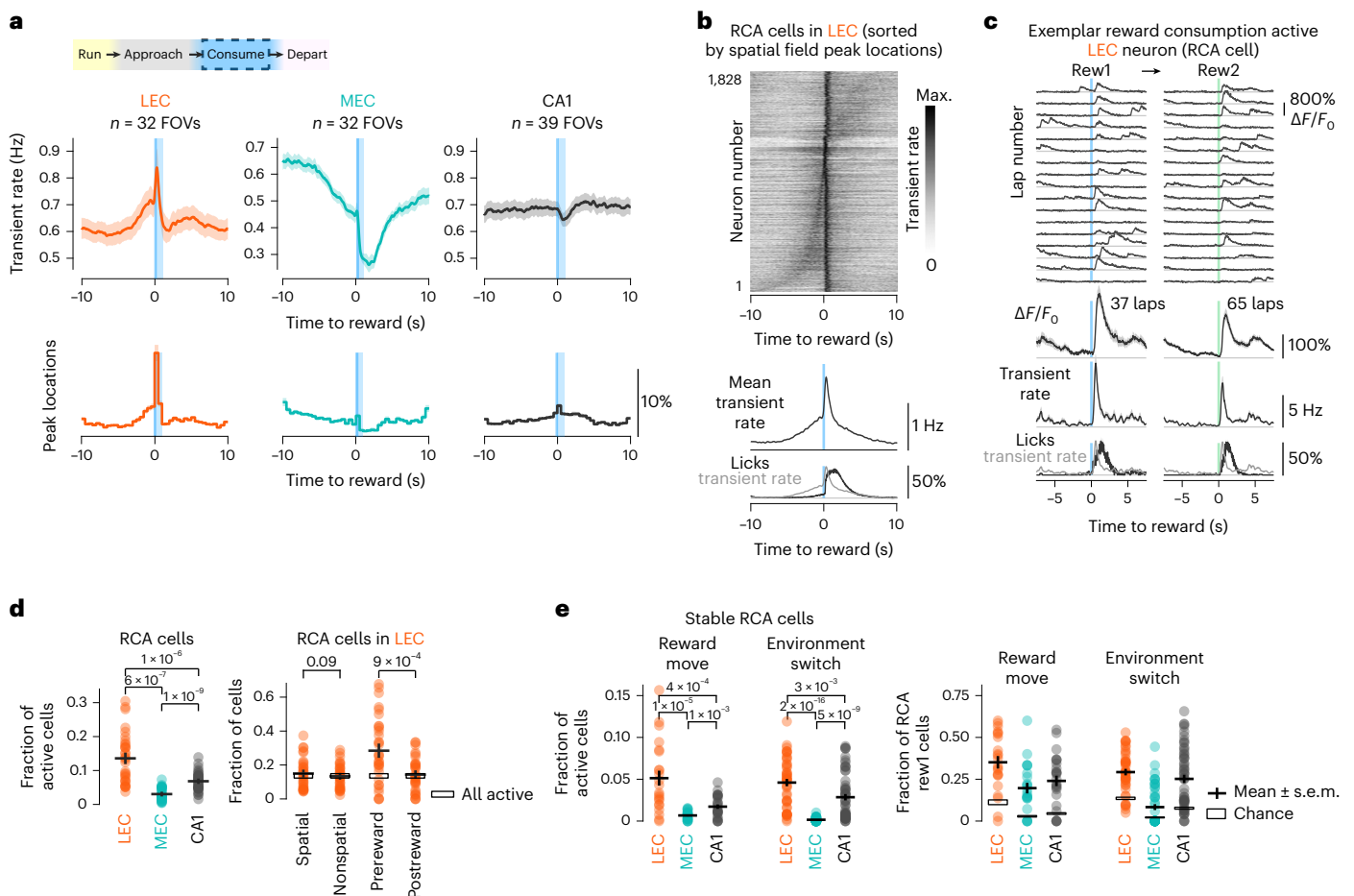


Fig. 5 | A population of LEC neurons signal reward consumption. a, Mean transient rate relative to time of reward delivery for LEC, MEC and CA1 imaging fields, averaged by session. Histogram shows timing of peak firing for individual cells from each session. All active cells included. Mean $\pm$ s.e.m. shown as dark line with light shading. Blue bar highlights first second after reward delivery. **b**, RCA LEC neurons from all imaging sessions sorted by peak firing location along the track, averaged over all laps for a session. Each neuron (one row) is normalized to its maximum firing rate in this time window. Mean transient rate (across all 1,828 RCA cells in LEC) and licking (32 sessions) shown below, with mean transient rate reproduced as gray trace overlaid on licking average. **c**, Exemplar nonspatial RCA cell in LEC. Fluorescence traces for subset of laps shown, with averages of all laps underneath for both fluorescence and inferred transient rate following deconvolution relative to familiar (rew1) and new (rew2) reward location in the

same imaging session. Licking shown along with overlay of mean transient rate (gray). Mean $\pm$ s.e.m. shown as dark line with light shading. **d**, Fraction of cells that are RCA cells in each region ($n = 32, 32, 39$ FOVs), further subdivided as a fraction of spatial, nonspatial, prereward and postreward cells in LEC. **e**, Fraction of stable RCA cells (RCA for both familiar and new reward location), either as a fraction of all active cells or as a fraction of RCA cells for the familiar reward location (rew1). Fractions shown for both a reward move (same environment) or for an environment switch (nine pairs of comparisons per session); $n = 22/5, 22/5$ and $25/6$ FOVs for LEC, MEC and CA1, respectively (reward move/environment switch). In **d** and **e**, two-sided two-sample t -tests for comparisons between regions or two-sided one-sample t -test for comparisons within LEC, indicated by horizontal line with P values; each dot represents one imaging session or pair of environment switches and black cross represents mean $\pm$ s.e.m. across sessions.

Data Fig. 6b). This pattern was largely unperturbed after the reward was moved, following a similar trajectory as for the familiar reward (Extended Data Fig. 6a). Thus, the LEC population robustly tracks reward experience epochs.

Stability of LEC reward representation during learning

We next investigated lap-by-lap changes in the LEC populations following a reward location change to determine whether cells quickly and stably encoded the new reward or whether they slowly formed their representations, as previously seen for CA1 place cells^{21,41}.

During learning of a new reward location, mice adapted their behavior by initially slowing down for a longer time and distance before the reward (Fig. 6a,b); then, over subsequent laps, they slowed closer to the new reward location so that their running behavior looked similar to the stable pattern observed for familiar rewards (but relative to the new reward location). While behavior adapted over several laps, prereward and postreward populations continued to fire

relative to the new reward location on each lap (Fig. 6a). In particular, the prereward population was initially broadly active across a large portion of the track but gradually sharpened to be active only in locations just before the reward (Fig. 6a). These transition times, fit with an HMM on individual laps as in Fig. 4, captured this evolution (Fig. 6b) and were highly correlated to slowing behavior on individual laps for both the familiar and the new reward locations (Fig. 6b). Indeed, when reexamining cell firing with respect to the approach epoch, defined by the time from deceleration until reward delivery (as in Fig. 4c), the stability of the prereward representation across the switch becomes evident (Fig. 6c). When mean activity is evaluated for this prereward epoch, no detectable change in firing rate across the prereward population was observed during learning of the new reward location (Fig. 6c).

Surprisingly, both the RCA population and the postreward population began encoding the reward consumption epoch immediately on the first traversal after the switch (Fig. 6c). Even though the

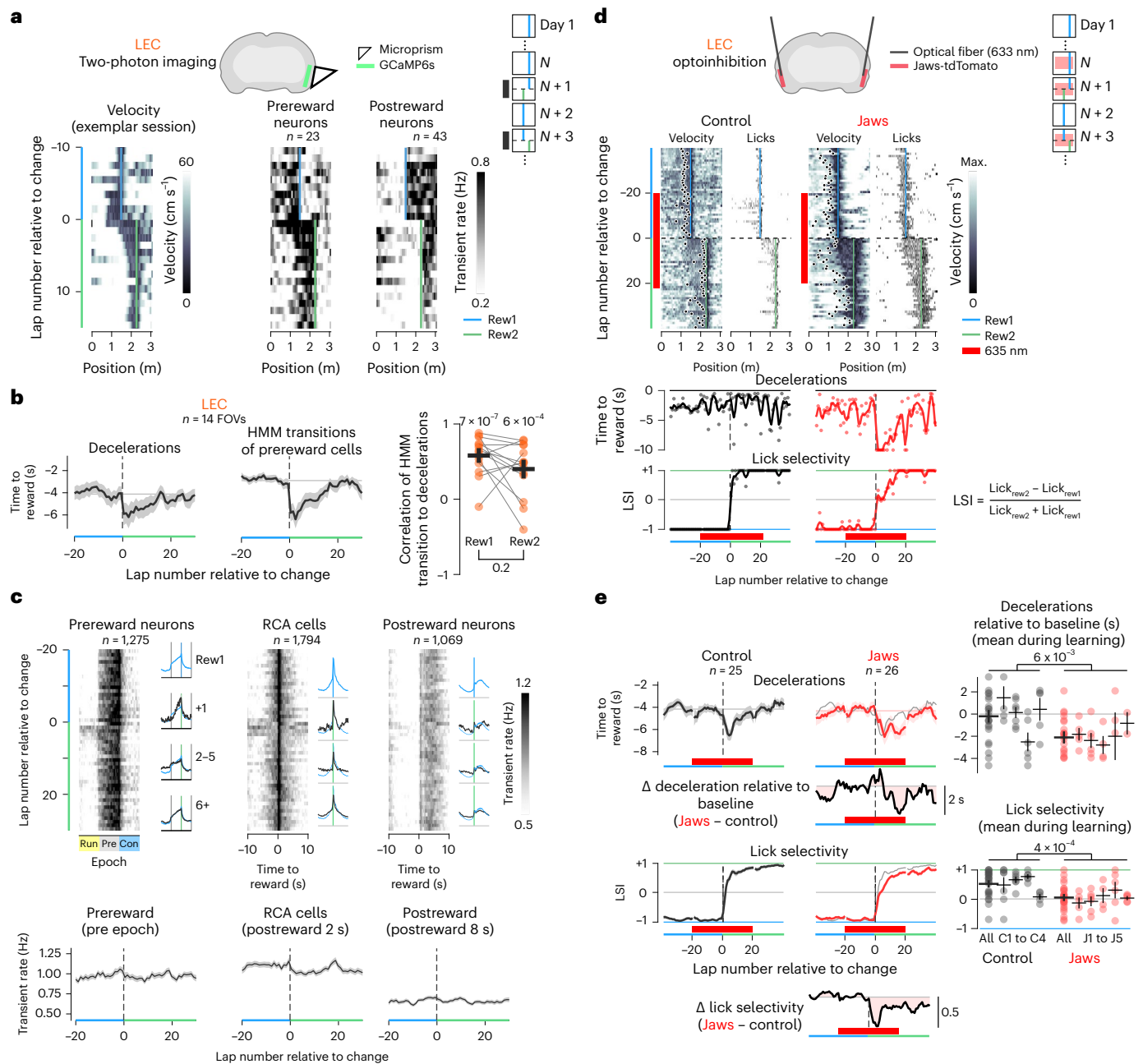


Fig. 6 | LEC stably represents reward experience during learning while inhibition of LEC disrupts learning. **a**, Velocity (1-cm bins), prereward and postreward population firing (averaged over neurons) shown for final 10 laps with familiar and first 15 laps with new reward location for exemplar session. **b**, Deceleration and HMM transition times during learning, averaged across imaging sessions with at least ten prereward neurons; first move session excluded. Mean $\pm$ s.e.m. shown as dark line with light shading. Pearson correlation coefficients between prereward HMM transition and decelerations on each lap, shown for familiar (rew1) and new (rew2) reward location. Each point represents one imaging session; black cross represents mean $\pm$ s.e.m. Statistical tests between rew1 and rew2 conditions (two-sided Wilcoxon signed-rank test) and to zero (one-sided one-sample t -test); P values indicated; $n = 14$ FOVs. **c**, Mean firing for each reward population. For prereward cells, firing resampled as a function of behavioral epoch. Insets, firing averaged over subsets of laps (rew1, last 20 laps before reward move; +1, first lap after reward move; 2–5, next 4 laps; 6+, next 25 laps). Underneath, mean transient rate during ‘pre’. For RCA

or postreward cells, mean firing relative to reward delivery time, with mean transient rate averaged over first 2 s (RCA) or 2–10 s (postward) after reward delivery; $n = 1,275$; 1,794 and 1,069 neurons. **d**, Velocity and licking behavior for exemplar control and Jaws sessions. Red bar, laps with optogenetic inhibition; 633-nm light delivered for final 10–20 laps with familiar (rew1) and first 20 laps with new (rew2) reward location. Maximum velocity, 30 (control) and 50 (Jaws) cm s⁻¹. Underneath, lap-by-lap measures of deceleration time relative to reward and LSI; line is five-point moving average. **e**, Session averages of deceleration times relative to reward and lick selectivity. Mean control trace reproduced in gray; difference between Jaws and control data underneath. ‘Mean during learning’ mean deceleration times on laps 13–20 relative to baseline (mean deceleration times on 10 laps before move) and mean value of lick selectivity on laps 2–6. Each point represents one imaging session, both for all mice (‘all’) and broken up by individual mice (controls, C1–C4; Jaws, J1–J5). Black cross represents mean $\pm$ s.e.m. across sessions ($n = 25$ controls and 26 Jaws). P values: two-sided, two-sample t -test.

receipt of reward was unexpected at the new location, the amplitude of the transient increase in firing in the RCA population did not change across the reward switch laps (Fig. 6c). The postreward population

rapidly shifted to fire at the new reward location (Fig. 6a), thus encoding the new postreward epoch from the first traversal after the switch and with no detectable change in firing rate (Fig. 6c).

Overall, the prereward, postreward and RCA populations in LEC shift their firing immediately to the new reward location during learning. Given such stable location-invariant reward representations, even while reward location and animal behavior dramatically changed, the firing patterns of these neurons provide reliable experiential information of the epochs surrounding reward.

Inhibition of LEC disrupts learning of a new reward location

We then asked whether LEC was causally involved in the behavioral changes that occur after reward switch. We expressed *Jaws*⁴², or *mCherry* as a control, in LEC and, using 633 nm illumination delivered bilaterally through chronically implanted fibers in LEC, inhibited LEC activity in the 10–20 laps before moving the reward and through the first 20 laps during learning of the new reward location (Fig. 6d and Extended Data Table 3). We found no change in behavior when we inactivated LEC before moving the reward location, as assessed by anticipatory deceleration and licking selectivity index (LSI; Methods) at the reward location. When the reward was moved, control mice quickly adapted to the new reward location, shifting their decelerations and licking within around the first ten laps (Fig. 6d,e), similar to the behavior observed during two-photon imaging of the LEC (Fig. 6b). However, mice expressing *Jaws* took longer to adapt their behavior to the new reward location (Fig. 6d,e). Both deceleration behavior and licking selectivity adapted more slowly when compared with control mice. These results indicate that LEC is necessary for the learning of new reward locations but not for already learned reward locations, consistent with past studies in the hippocampal formation^{43,44} and the role of LEC in associating objects with location⁴⁵.

Discussion

The original proposal of a cognitive map⁴⁶ described not just a representation of spatial position but information to guide goal-directed navigation, a dichotomy exemplified by the context dependence of hippocampal place cells^{3,4}. In particular, a representation of reward experiences provides information needed to contextualize a spatial code^{12,13}. How this reward experience information reaches the hippocampus has been uncertain. Here, we find that the LEC contains cell populations that signal goal approach, the reward consumption period itself and goal departure; these populations are largely invariant to spatial location or environment (Figs. 3 and 5) and maintain stability during learning; inhibition of LEC significantly slows learning of a new reward location (Fig. 6). We further find that these signals differ drastically from the MEC, which shows little change around reward and does not represent reward experience epochs in an obvious way.

Our findings revisit the discussion about the dueling identities of MEC and LEC. These differences have been cast in terms of ‘where’ versus ‘what,’ spatial versus nonspatial, self and nonself^{47–49}, and, more recently, allocentric versus egocentric⁵⁰. During open-field navigation, neurons in LEC encoded egocentric coordinates relative to items in the environment, which contrasted with allocentric coding by neurons in MEC. A parsimonious explanation of our findings is that prereward, RCA and postreward cells encode egocentric coordinates, but relative to an abstract object, which is the reward itself. This internal behavioral space we describe broadly as experiential epoch coding, consistent with a past proposal that LEC encodes the ‘content of an experience’⁴⁷ and with our finding that prereward populations are activated in a behaviorally dependent manner. Interestingly, along with the finding of goal-related egocentric tuning⁵⁰, older research that had found a prominent trajectory encoding signal in rat entorhinal cortex during reward navigation⁶ was probably sampling LEC, not MEC, which is consistent with our findings. Moreover, a recent study found that CA1-projecting axons from layer III of LEC represented reward but also strongly coded spatial position during a reward navigation task in virtual reality (VR)⁵¹. Together our methods provide a more comprehensive picture of how information from LEC reaches the hippocampus

during reward navigation behaviors, with layer II providing the strongest prereward information (Extended Data Fig. 2b) that is probably routed to DG and CA3, whereas layer III is providing stronger spatial information that is routed to CA1.

During goal approach, activation of the prereward population coincides with deceleration of the animal (Fig. 4). These cells are enriched in LEC layer II, which contains the dopamine-recipient fan cells²⁹ alongside CA3-projecting pyramidal cells and thus may be modulated by direct dopamine release during reward anticipation³⁶. Further research may delineate whether fan cells themselves are enriched in the prereward population and, if so, what role dopamine plays in modulating firing in this population⁵². The LEC population decorrelates during goal approach (Extended Data Fig. 7) along with an increase in overall firing (the opposite of the decreased firing we observed in the MEC), consistent with increased information coding^{53,54} that may, in combination with slower running speed, be used to gather more information about the environment for optimal foraging^{55,56}. Together, this evidence points to the goal approach epoch and especially the LEC prereward population as playing an important role for the hippocampus and other downstream regions in forming memories of the experience and improving predictions that could guide future behavior^{57,58}. Intriguingly, similar reward-anticipation cells have been described in LEC during non-navigation tasks, for example, ‘hold-type’ cue-active cells during a lever pull to receive reward⁵⁹ and cue-active cells during an odor cue-reward association task²⁹. The latter study found these cells in layer II fan cells of LEC, similar to our finding of an enrichment of prereward cells in LEC layer II. Thus, the same cells in LEC may generalize to perform a similar function such as encoding expectation or value, perhaps in cooperation with signals from dopaminergic cells⁶⁰. By using a spatial navigation task with variability in deceleration behavior, we further discovered that behaviorally linked state transitions in prereward firing contributed to observed ramping activity (Fig. 4), which is relevant not just for reward navigation signals in the hippocampus but may inform studies of ramping signals in the dopamine system^{38,61}.

During reward consumption, firing in LEC increased transiently and dramatically (Fig. 5 and Extended Data Fig. 5). This signal was observed in a population of neurons we have termed RCA cells. Although, on average, CA1 did not exhibit a similar increase, a fraction of CA1 neurons qualified as RCA cells, which perhaps receive stronger inputs from LEC than from MEC⁶². Thus, a large burst of excitation may be sent from the LEC to the hippocampus during the initial moments of reward consumption and could drive behavioral timescale synaptic plasticity⁶³ or dendritic spikes⁶⁴ in CA1 pyramidal neurons, in turn leading to an overrepresentation of reward locations²¹. RCA cells do not seem to be modulated by licking itself as most RCA cells (~70%) are not prereward cells (Fig. 5d) and are thus largely inactive during anticipatory licking. Further, firing in RCA cells subsided rapidly after reward delivery (within ~0.5 s) but licking typically continued for 2–4 s (Fig. 5b,c). Rather, RCA firing seems to correlate most strongly with reward discovery or consumption.

During goal departure, postreward cells in LEC became active. Interestingly, MEC and CA1 were also enriched in the number of postreward cells (Fig. 2). Along with signaling the goal departure experience itself, neurons active in the postreward epoch might coordinate across all three regions to provide a trace signal, which could be used to associate reward with recent locations^{65,66}. An alternative interpretation is that the postreward signal is due to reward consumption or satiation and has no special role in spatial navigation; however, the absence of a prominent postreward signal for randomly delivered rewards outside of a task structure (Extended Data Fig. 5) supports the notion that postreward firing is specific to encoding information that relates to predicting the goal location during future behavior and may thus be unique to spatial navigation tasks. Dopamine may also play a role during goal departure. Dopaminergic neurons that project to the

tail of the striatum signal goal departure following interactions with a new object⁶⁷. Perhaps postreward cells in the LEC are signaling several aspects of a recent reward or experience that incorporate such novelty signals from the dopamine system.

By providing stable representations of reward experience in LEC in parallel to spatial information in MEC, the entorhinal cortex could provide a computationally efficient and flexible factorization for learning to associate these two components of episodic memory^{68–70}. For example, a critical component of the successor representation model of the hippocampus⁵⁷ is the presence of a source of reward prediction information¹². In parallel, neuromodulatory systems such as the LC^{23,24} or VTA⁷¹ could signal novelty and open a window for plasticity when encountering a new environment or unexpected reward.

Our findings also provide support for the hypothesis of functional specialization of LEC subcircuits. Many prereward, postreward and RCA cells maintained their reward coding across reward and environment switches, so perhaps they are recruited from largely nonoverlapping pools of LEC neurons, each with its own specialized circuitry and dedicated function. However, the LEC is known to be involved in many tasks and behaviors beyond what was studied here, such as in olfaction²⁸ and timing on the order of minutes²⁷. Indeed, the spatial and reward-encoding populations we identified represent only a third of neurons active during our spatial navigation task. Thus, it is yet to be determined precisely how these diverse roles are supported by the same brain region or what the remaining two-thirds of neurons are encoding.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41593-023-01557-4>.

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Methods

All animal procedures were approved by the Northwestern University Institutional Animal Care and Use Committee. For imaging of the medial and lateral entorhinal cortices (MEC and LEC, respectively), mice expressing GCaMP6s were generated by crossing tetO-GCaMP6s mice⁷² (JAX catalog no. 024742) with Camk2a-tTA mice (JAX catalog no. 007004). For imaging of CA1 or optogenetic inhibition of LEC, wild-type offspring of these crossings were injected with adeno-associated virus (AAV) as detailed below. Sex used is indicated in Extended Data Tables 1 and 3. Female/male: LEC, four/three; MEC, two/four; CA1, three/five; optogenetic inhibition, zero/five; optogenetic control, one/three.

Surgery

Mice were anesthetized with isoflurane (4% for induction, 1–2% for maintenance in 0.5 l min⁻¹ O₂, temperature maintained at 37 °C and ointment applied to the eyes). Dexamethasone (5 mg kg⁻¹, intramuscular) was given for inflammation, buprenorphine-SR-LAB (1 mg kg⁻¹, subcutaneous) for pain and normal saline (0.5–1.0 ml, intraperitoneal) for dehydration. Details of the surgeries to implant prisms to access the MEC and the LEC, to inject virus and cannulate to access CA1 or to inject virus and implant fibers to optically inhibit LEC are provided in the next sections. In all cases, after implantation, a titanium headplate was attached to the skull with dental cement (Metabond, Parkell). Mice were monitored closely for 24 h and given 3–5 days to recover before water restriction and behavioral training were begun.

LEC surgery

As it is situated ventral to the rhinal fissure⁷³, the LEC is a lateralized structure in rodents, and direct access with a microscope requires approaching from an angle greater than 90 degrees to the horizontal plane. A further complication is the surrounding anatomy: the pinna, nearby vasculature such as the petrosal squamosal sinus and protrusions of the skull (zygomatic process) each impinge upon optical access with a microscope⁷⁴. Previous work used a lateral approach in anesthetized animals and achieved two-photon imaging field sizes of 200 μ m and up to 50 neurons at a time²⁸. To overcome these limitations, we developed surgical methods to implant a cranial window with an attached microp prism to rotate the imaging plane 90 degrees^{30–32} (Fig. 1b). A 3 mm craniotomy was made over the right lateral surface of the skull and positioned so that the posterior edge aligns with the ventral portion of the transverse sinus and the anterior edge with the insertion of the zygomatic protrusion from the squamosal bone, centered at -3.5 mm caudal to Bregma. The dorsal edge was 1–2 mm dorsal of the rhinal fissure and the ventral edge was extended as far ventral as possible without incurring large amounts of bleeding or damage to soft tissue structures. Once the brain was exposed, any soft tissue overlying dura was removed. A 3 mm round No. 0 coverslip (CS-3R-0, Warner Instruments) was lowered and held in place with a 1.0 mm diameter pipette (Q100-70-7.5, Sutter Instrument) positioned by a micromanipulator while the top and side edges of the coverslip were cemented (Metabond, Parkell). Then, a small dab of ultraviolet (UV)-cured adhesive (NOA81, Norland) was placed on the outer surface of the coverslip and a 2.0 mm microp prism (MPCH-2.0, Tower Optical) placed against the coverslip and positioned as far ventral as possible before UV-curing the adhesive (CS20K2, Thorlabs), thus fixing the microp prism in place. Dental cement was then used to fill in the remaining gaps while leaving the dorsal face of the prism clear for optical access. In a typical imaging field of 700 $\times$ 700 μ m, ~500 active cells were observed during behavior (mean across 47 imaging fields: 496 cells; range: 150–843 cells).

The location of the window over the LEC was confirmed in three ways (Extended Data Fig. 1). First, expression of GCaMP6s is enriched in the entorhinal cortex in the tetO-GCaMP6s $\times$ CamKIIa-tTA transgenic mouse line⁷². We confirmed this overlap through retrograde labeling of CA1-projecting neurons. Labeled neurons in layer III of the LEC and the

MEC coincided with increased brightness of GCaMP6s fluorescence in the same regions as visualized in histological slices. Second, this expression and the location of the LEC is found ventral to the rhinal vein. As this is a prominent structure seen during surgical implantation of the window and during subsequent *in vivo* imaging, it provided a reliable landmark for locating the LEC and, under epifluorescence imaging, matched the area of increased GCaMP6s fluorescence. Finally, the drastic difference in the location and angles of our MEC and LEC prisms (they are perpendicular to each other, with the MEC prism facing anterior and the LEC prism facing medial) ensures that they are targeting different regions of cortex, and this was confirmed by the presence of a lamina dissecans between layers II and III of cortex in only the lateralized and not the posteromedial portions of entorhinal cortex⁷⁵.

MEC surgery

This surgery is a modified version of the MEC prism implant surgery previously described³². A 2–3 mm craniotomy was performed over the right cerebellum with the anterior edge positioned along the transverse sinus, just posterior of the lambdoid suture and the medial edge 2 mm lateral of midline. The craniotomy was extended posteriorly and laterally to where the skull begins its ventral descent. To aid drilling, the mouse was rotated to bring the edge of the craniotomy in plane (counterclockwise roll of 20–30 degrees and downward pitch of 5–10 degrees). Next, a 2 mm incision was made in the dura over cerebellum along the posterior edge of the transverse sinus and the flap of cerebellar dura reflected posteriorly away from the sinus. A portion of cerebellum was then suctioned until the caudal surface of the cortex was visible and expanded to yield a ~2 mm opening. A 45-degree 1.5 or 2.0 mm microp prism (MPCH-1.5 or MPCH-2.0, Tower Optical) was mounted onto a custom stainless-steel mount with UV-cured adhesive (NOA81, Norland) and wedged with the front surface of the prism abutting the MEC and the back surface against the caudal portion of the skull. Once inserted, this prism assembly was adjusted gently to achieve maximal exposure of the MEC, angled to match the natural surface of the MEC (typically a clockwise roll of 10 degrees and upward pitch of 10–15 degrees). Dental cement was then applied around the prism and surrounding skull to hold the prism in place; gentle anterior pressure was applied against the posterior edge of the prism assembly to provide some mechanical stability against the MEC.

CA1 cannulation

A small craniotomy (~0.5 mm) was performed at 2.3 mm caudal and 1.8 mm lateral (right hemisphere) relative to Bregma. pAAV.Syn.GCaMP6f.WPRE.SV40 (Addgene catalog no. 100837-AAV1, diluted -10 $\times$ from 2 $\times$ 10⁻¹³ GC ml⁻¹ stock into PBS), was injected by a beveled glass micropipette at a depth of 1.3 mm beneath dura. Typically, two injections, each 60 nl, were performed at spots ~500 μ m apart within the same craniotomy. Then, 2–4 days later, a stainless-steel cannula with a glued (NOA81, Norland) 2.5 mm No. 1 glass coverslip (Potomac Photonics) was implanted above the hippocampus.

Retrograde labeling

CA1-projecting neurons were labeled by injection of rAAV2-Retro-CAG-tdTomato (Janelia, diluted 20 $\times$ from 1.8 $\times$ 10⁻¹² GC ml⁻¹ stock into PBS) into the right CA1 using the same procedures for injection of virus as detailed above ('CA1 cannulation'). For *in vivo* imaging, an LEC prism was implanted on the same day using the procedures as above for 'LEC surgery,' and two-photon imaging was performed 3 weeks later. For histology, injection of virus into CA1 was performed in a transgenic mouse expressing GCaMP6s. After 3 weeks, the mouse was anesthetized with 5% isoflurane and perfused with 4% paraformaldehyde. After leaving the extracted brain in paraformaldehyde at 4 °C overnight, it was moved to 30% sucrose in PBS for a few days. Using a freezing microtome, 50 μ m horizontal slices were cut and placed on slides. Images were taken with a slide scanner microscope (VS120, Olympus).

Implantation of optical fibers for optogenetic inhibition of LEC

Inhibition of LEC was achieved by bilateral expression of the inhibitory opsin Jaws⁴² followed by implantation of a pair of tapered optical fibers⁷⁶. First, a craniotomy was performed over right LEC as detailed in 'LEC surgery.' The head was rotated 40 degrees (counterclockwise roll), allowing direct access to LEC using a pipette from above. The pipette was positioned ~3.5 mm caudal to Bregma and punctured dura just ventral to the rhinal vein and advanced ~0.5 mm for the first injection and another 0.5 mm for a second injection. Either AAV2/8-hSYN-JAWS-tdTomato-ER2 (Neurophotronics, diluted 2.5× from 9.8×10^{-2} GC ml⁻¹ stock into PBS) or AAV8-hSyn-mCherry (Addgene catalog no. 114472, diluted ~6× from 2.6×10^{-13} GC ml⁻¹ stock into PBS) was injected for a set of Jaws mice and a set of control mice, respectively. The pipette was withdrawn after 5 min. After another 10–15 min, the head rotation was reduced to 15 degrees (counterclockwise roll) and a fiber cannulae (Lambda-B Fiber, 0.39 numerical aperture, 200 µm with a slotted 1.25 mm ceramic ferrule, 1.5 mm active length plus 1.5 mm tapered implant length; Optogenix) was inserted. The fiber was positioned at ~3.5 mm caudal to Bregma and punctured dura 1.5 mm dorsal to the rhinal vein before being advanced 3 mm and then secured in place with dental cement. The procedure was then repeated for the left LEC. After experiments were completed, horizontal brain slices were taken as detailed above under 'Retrograde labeling.'

Behavior

Water-restricted mice received 1.0 ml of water per day. Weights and health were monitored daily. Training and behavior were performed in VR (ViRMen⁷⁷). Head-fixed mice running on a one-dimensional treadmill moved through a VR environment displayed on a set of five monitors covering a 225 degree FOV (horizontal axis)³². Water rewards of 4 µl were delivered at a fixed location on the virtual track. Water volume was controlled by a solenoid that was calibrated based on open duration (typically 20 ms). Once the end of the track was reached, a 4 s 'time-out' period was included, during which the mice were kept at the end of the virtual track before returning to the start. Licks were monitored by a capacitive sensor attached to the lick spout. During imaging experiments, we also monitored the face of the mouse using a camera (Zelux CS165MU1, Thorlabs) synced to the two-photon microscope frame times.

Imaging sessions were performed once the behavior reached a satisfactory level (usually after 5–10 training sessions), judged by (1) number of laps per minute (more than two laps per minute in a 40-min session) and (2) anticipation of reward (deceleration and licking before reaching the reward location seen in reward-triggered average of behavior traces). On the first 1–3 days of imaging, the reward location was fixed (at 2.3 m). Then, in the middle of an imaging session, the reward was moved, and this new position was used for the remainder of the session and the next session as well (Fig. 2a). For the next couple of weeks, we interleaved more reward location moves between three possible positions: 0.7 m, 1.5 m and 2.3 m. Where appropriate, we pooled data from the three reward positions (Extended Data Table 2). We considered the possibility that the direction the reward location was moved (either earlier or later along the track relative to the familiar reward) may influence our results. We repeated the analysis for Figs. 3 and 6 for LEC imaging sessions stratified by the direction in which the reward was moved (earlier, $n = 15$ FOVs; later, $n = 7$ FOVs). We did not observe any significant differences. Thus, we pooled data for both directions of reward changes. For environment switch experiments, we began with the same environment used for reward location changes as above (env1), but then moved through the following sequence in a single session: env1–1.5 m, env1–2.3 m, env2, env3, env4 and then back to env1–1.5 m, with roughly 20 laps (5–10 min) in each environment (Extended Data Fig. 3c).

To assess the response in LEC to reward delivery and consumption outside of the context of virtual navigation, water-restricted naïve

mice ($n = 3$) were habituated to head fixation and provided randomly delivered rewards while imaging was performed (Extended Data Fig. 5). During these sessions, the display monitors were off. After these imaging sessions, one of the mice was trained on the VR navigation task as described above. Imaging was repeated once behavior reached the criteria detailed above.

Imaging

Widefield images were taken with a custom-built fluorescence microscope with a GFP filter cube through a 2× objective (TL2X-SAP, Thorlabs) and captured with a scientific CMOS camera (Prime BSI Express, Teledyne Photometrics).

Two-photon imaging was performed using a customized microscope with a resonant scanning module (Sutter Instruments) and a 20× objective (LUCPlanFL N, Olympus). Excitation was provided by a mode-locked Ti:Sapphire laser tuned to 920 nm (Chameleon Ultra II, Coherent) with ~100 mW of average power out of the objective. Emission light was split by a 560 longpass dichroic (FF560-Di01, Semrock) and filtered into red (FF01-620/52, Semrock) and green (FF01-510/84, Semrock) channels before being detected by a pair of GaAsP PMTs (H10770PA-40, Hamamatsu Photonics). Imaging was controlled by ScanImage software (Vidrio). A frame sync signal was sent to the data acquisition card (National Instruments) on the VR computer using custom-written code (MATLAB, Mathworks).

Timeseries videos ranging from 32,000 to 60,000 frames per imaging session were collected at 29.8 Hz. At full magnification, the field size was 700×700 µm with 512×512 pixels, with most imaging performed at magnifications of 1.0× to 1.5× (maximum of 2.2×). At the start and end of each imaging session, the depth of the imaging field from the surface (dura for entorhinal cortex imaging or the axonal layer for CA1 imaging) was estimated by focusing up using a calibrated micromanipulator (MP-285, Sutter Instrument). In LEC, we could distinguish layer II and layer III as a cell-free zone separates them, providing a convenient method to differentiate the layers⁷⁵ (Extended Data Fig. 1). Such a clear distinction between layers was not present in MEC; we used a threshold of 150 µm below dura to distinguish nominal layer II and nominal layer III. To block stray light from the VR monitors from contaminating the detected emission signal, a light-blocking cylinder formed from electrical tape was placed between the headbar and the objective.

Optical inhibition of LEC during behavior

Once implanted mice learned the task (as described under 'Behavior'), optical inhibition sessions were performed. A 633 nm fiber-pigtailed laser diode (LP633-SF50, Thorlabs) was split 50:50 using a fiber optic coupler (TW630R5F1, Thorlabs) and coupled to the implanted fiber cannulae using a pair of patch cables (M83L01, Thorlabs). The laser diode driver (LDC205C, Thorlabs) was used alongside an LD/TEC mount (LDM9LP, Thorlabs) driven by a temperature controller (TED200C, Thorlabs). Driver current was set to produce 6–8 mW of total power coming into each implanted fiber cannulae (typically 130 mA of total current from the laser driver). Power was switched on and off using TTL control of the driver.

A 'reward move' session was run as described under 'Behavior' but with the inclusion of inhibition laps, where the laser light turned on once the mouse reached 30 cm along the track and stayed on until a position of 270 cm. These positions were chosen so that they would encompass all possible reward positions (70, 150 and 230 cm). In a given session, the first 20–30 laps were run with no light with the reward in the familiar position. Then, at least ten inhibition laps were run (minimum, 10; maximum, 25) with the reward still in the familiar position. Next, ~20 more inhibition laps were run (minimum, 17; maximum, 23), but now with the reward in the new position. Finally, at least another 20 laps were run with no light and the reward in the new position.

Once mice learned the first reward position (230 cm), we performed an acclimation session with optical inhibition for ~20 laps but

with no reward move. The next day, the reward was moved from 230 cm to 150 cm with inhibition laps as described above. Then, we interleaved days with no reward move (and no inhibition) with days with reward moves (and inhibition), thus giving mice an opportunity to adjust to each reward location before the next move. The first session with a reward move was excluded from analysis so that mice had a chance to learn that a move could occur.

Image processing

To extract inferred firing of individual cells from two-photon movies, we performed the following steps. First, two-photon timeseries videos were motion-corrected using rigid registration⁷⁸. A target image was found from repeated rounds of registration of 4,000 frames. The full video was registered to that target image. Cells were then segmented and raw fluorescence traces extracted by passing the first 200 spatial components identified by singular value decomposition of the downsampled (20× in time) video into a customized implementation of Suite2p⁷⁹ in MATLAB (typical parameters: cell diameter, 10 μm; neuropil ratio, 12× or 120 μm). Cells were curated semimanually based on a number of properties, such as size, shape and brightness. A brightness-over-time signal was produced for each cell mask along with the corresponding neuropil signal surrounding it.

Next, to decompose the activity signal into interpretable units, we used an integrated iterative algorithm (available at github.com/DombeckLab/Issa2023) we developed to recover an estimate of r , the ratio of neuropil contamination into the cell signal; F_0 , the baseline fluorescence of the cell and S , the firing rate of the cell. F_0 is the linear summation of a set of basis functions, chosen to be slowly varying sinusoids and exponentials. S is estimated from deconvolution⁸⁰; thus, we assume $\Delta F/F_0$ is a convolution of S with a kernel. We chose the kernel to be a two-exponential function with 'on' and 'off' time constants taken from published data^{81,82} for GCaMP6s and GCaMP6f. S is not an exact measure but does correlate with $[Ca^{2+}]$ and thus the number of action potentials fired in a given time window⁸³. Thus, we scaled S by a number based on the transient dynamics to a number that is meant to approximate the number of action potentials (120× for GCaMP6s and 80× for GCaMP6f, chosen so that the mean value of S matches the measured number of action potentials that generate the same mean $\Delta F/F_0$ as observed for simultaneous imaging and cell-attached recordings of neurons in vivo^{81,82}). The units for S are meant for convenience since the true signals are nonlinear and the dynamics can vary from cell to cell, especially when the sensor is expressed virally⁸⁴. For analysis, S was then smoothed with a Gaussian filter (standard deviation of five bins or 170 ms). We use 'firing' throughout to refer to these neuropil-corrected, baseline-adjusted and deconvolved neural Ca^{2+} transients.

Analysis

Analysis was performed in four different reference frames, calculated lap-by-lap. Peak locations are defined by the peak in the lap-averages in a given reference frame:

- (1) Absolute position: calculated by taking frames during running (>10 cm s⁻¹) and excluding the first 2 s after reward delivery (if within 10 cm of reward location) and then binning inferred firing S by position into 1-cm bins.
- (2) Relative position: calculated similar to absolute position but for the 100 cm before and after the reward location, also in 1-cm bins.
- (3) Relative time: no frames excluded, for 10 s before and 10 s after the reward time, binned at 30 Hz. Although mice could run the 3.1-m track in under 10 s, they typically took ~20 s (across 2,888 laps in our LEC dataset, the mean lap time was 18.7 s with only one lap faster than 10 s). This discrepancy is because mice ran slower than their peak (typically 50 cm s⁻¹) along a substantial portion of the track and spent time stopped to consume the

reward (typically 2–4 s) and at the end of the track (4-s 'time-out' used to separate each lap as a distinct trial). We chose a window of 10 s to ensure we captured most of the dynamics in activity before and after reward delivery.

- (4) Epochs: formed by defining three events for each lap and linearly interpolating in time 60 bins for each epoch. The three events are start of running, deceleration or HMM transition time and reward time. These three events were found automatically for each lap from the recorded behavior. Start of running after reward was found by examining the position of the animal in the virtual world and finding when the animal first passed 15 cm beyond the reward position. Next, deceleration time (or HMM transition time for the prereward population) was found as defined below (under 'Deceleration criteria' and 'HMM transition criteria'; which measure was used is indicated as appropriate in the text). Finally, reward delivery time was recorded by the VR program and defined the third event. For each interevent period, we then linearly interpolated the timespan between events into 60 bins using the `interp1` function in MATLAB.

Criteria for categorizing cells are as follows.

Active cells. Mean $\Delta F/F_0 > 0.1$. The fluorescence signal was averaged over all laps and time points for the imaging session.

Spatial cells. Spatial information >0.3 bits per event and significance on 98% or more of shuffles. Spatial information³⁴ was calculated by taking frames during running (>10 cm s⁻¹) and excluding the first 2 s after reward delivery (if within 10 cm of reward location) and then binning inferred firing S by position into 5-cm bins. For the shuffled data, firing on individual laps was permuted randomly and the spatial information calculated. The spatial information was considered significant if at most 2 shuffles out of 100 returned a higher spatial information score.

Reward cells. Spatial cells with a peak within 40 cm of the reward location (prereward, 40 cm before reward and up to and inclusive of reward location; postreward, after reward and up to and inclusive of 40 cm after reward location).

RCA cells. Peaks between 0 and 1 s after reward (inclusive), 98% or above on shuffle test for first second after reward (considered significant if at most 2 shuffles out of 100 returned a higher mean firing rate in a 1-s bin than the actual mean firing rate between 0 and 1 s).

Other measures used in the manuscript are defined here.

Reward clustering ratio. For spatial cells, the number of cells with peak locations in the zone of interest (reward, prereward or postreward) was divided by the number of cells with peak locations anywhere along the track, normalized by the expected fraction if cells were distributed uniformly. For example, for a 40-cm prereward zone along a 310-cm track, if 20 cells out of 100 have peaks in the prereward zone, then the ratio is 20/100 (actual) divided by 40/310 (expected), which yields a prereward clustering ratio of 1.55.

Tuning width. Length of track over which lap-averaged cell firing is greater than 30% of the maximum.

Transient duration. The autocorrelation for $\Delta F/F_0$ was calculated after mean subtraction and smoothing with a nine-point rectangular filter. The half-height half-width was then found as a proxy measure of transient duration. This measure is influenced not just by the kinetics of the Ca^{2+} indicator itself but also by the concentration of the indicator in the cell⁸⁵, endogenous buffers and other properties of each cell, and the autocorrelation in time of the cell's actual action potential train.

Deceleration criteria. The 98th percentile of velocity (after smoothing with a 31-point rectangular filter, -350 ms) was used as the 'peak' velocity, v_{peak} . $v_{\text{th}} = v_{\text{peak}} \times 0.8 - 5$, so if peak velocity was 50 cm s^{-1} , we used 35 cm s^{-1} as the threshold. We then detected when the smoothed velocity (11-point rectangular filter, -120 ms) last was above this threshold in a time window from 10 s to 0.5 s before reward.

HMM transition criteria. For imaging sessions with at least five pre-reward cells, we calculated transitions from inactive to active in the 10 s period leading up to reward using an HMM. The sequence was the number of cells that passed a firing threshold in each time bin (>1.8 events per second). The model assumed only two states, inactive and active, with each lap beginning in the inactive state and transitioning to an absorbing active state. This transition matrix and the probability distributions for number of active cells in each state was estimated using the Baum-Welch algorithm (hmmtrain, MATLAB). Then, for each lap, this model was used to estimate the posterior state probabilities (hmmdecode, MATLAB) and hence the time of transition from inactive to active. To calculate variance explained, we modeled the 10-s pre-reward period for each lap as a step function using the HMM to set the transition point of the step. Then, we calculated 1 minus the variance of the difference between the data and the step function divided by the variance of the data itself: $1 - \text{var}(\text{data} - \text{step}) / \text{var}(\text{data})$.

Velocity HMM criteria. We also detected decelerations using an HMM. Velocity was rounded up to the nearest integer value in centimeters per second. Similar to the model used above for pre-reward cells, we used two states with a single transition to the absorbing state. In this case, the first state was a high velocity state and the second absorbing state was a lower velocity state. We compared the results of this method with those of the first method ('Deceleration criteria') and also re-ran our analysis comparing HMM-detected decelerations with HMM-detected changes in pre-reward and found both methods produced similar results (Extended Data Fig. 4c).

Lick selectivity index. LSI is the ratio of licking at the familiar (rew1) or new (rew2) reward locations. L1 is the licking in a zone around rew1, stretching from 30 cm before to 10 cm after the reward location, and excluding any stationary periods. L2 is the licking in a zone around rew2. The LSI is then calculated as $(L2 - L1) / (L2 + L1)$ for each lap. A value of -1 indicates licking at rew1 and not rew2 (and the opposite for a value of +1), while a value of 0 indicates equal amounts of licking at both locations. If no licking was detected at either reward location on a given lap, the index was undefined and that particular lap is excluded when calculating averages across sessions.

Bayesian decoding⁸⁶ was performed with respect to absolute position for datasets with reward located at 2.3 m (Extended Data Fig. 2c). Data binned every 10 cm and training and test datasets were formed from odd and even laps, respectively.

Statistics and reproducibility

No statistical methods were used to predetermine sample sizes, but sample sizes are similar to those reported in previous publications^{23,87}. Data distribution was assumed to be normal but this was not formally tested. For optogenetic inhibition experiments, the first session with a reward move was excluded from analysis so that mice had a chance to learn that a move could occur. For imaging experiments, we included all imaged mice, which are mice with good optical windows and that reached the predetermined behavioral criteria. Where appropriate, data were cross-validated by comparing results from odd and even trials. Further, data were grouped and compared either by session or by animal (across sessions) to ensure that data were representative across sessions and across animals.

The same experiments and measures were made for each region studied with imaging experiments. We did not attempt to randomize

these allocations as experiments targeting each region were performed in batches; however, in some cases we allocated littermates to MEC and LEC implants and trained/imaged them in parallel and at least qualitatively observed results consistent with our larger cohort. For optogenetic experiments, littermates were pseudorandomly assigned to either positive or control groups for virus injection; the remainder of the experiment (habituation, training and behavior manipulation) were performed identically in the two groups.

Data collection and analysis were not performed blind to the conditions of the experiments. This was not possible to do for imaging because the location of the imaging window (and all the procedures related to that experiment) is part of the experiment, so there was no practical way to blind the experimenter. To determine whether the differences in reward-related neural responses across LEC layers could be explained by factors other than the imaged region, we performed a multiway analysis of variance using imaging depth, age, sex and deceleration (a behavioral measure of task performance) as predictors of reward clustering and found that only depth provided a significant prediction (Extended Data Table 1).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Datasets generated in this study have been deposited online and are publicly available (<https://doi.org/10.5281/zenodo.10160991>). Source data are provided with this paper.

Code availability

Original code used for source extraction of two-photon imaging is available at GitHub (<https://github.com/DombekLab/Issa2023>) and has been deposited on Zenodo (<https://doi.org/10.5281/zenodo.10162356>).

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Author contributions

J.B.I., B.A.R. and D.A.D. conceptualized the study. J.B.I. and D.A.D. analyzed the data. J.B.I. performed the investigation, with CA1 cannulation by J.B.I. and F.X. D.A.D. supervised the study. J.B.I. and D.A.D. wrote the manuscript with input from all coauthors.

Competing interests

The authors declare no competing interests.

Additional information

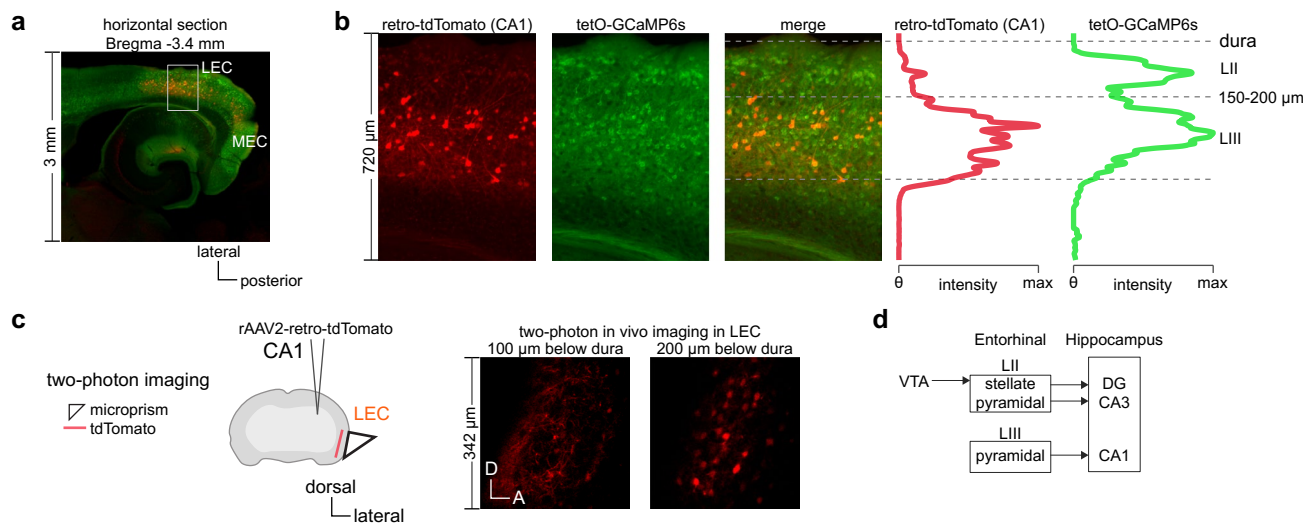
Extended data is available for this paper at <https://doi.org/10.1038/s41593-023-01557-4>.

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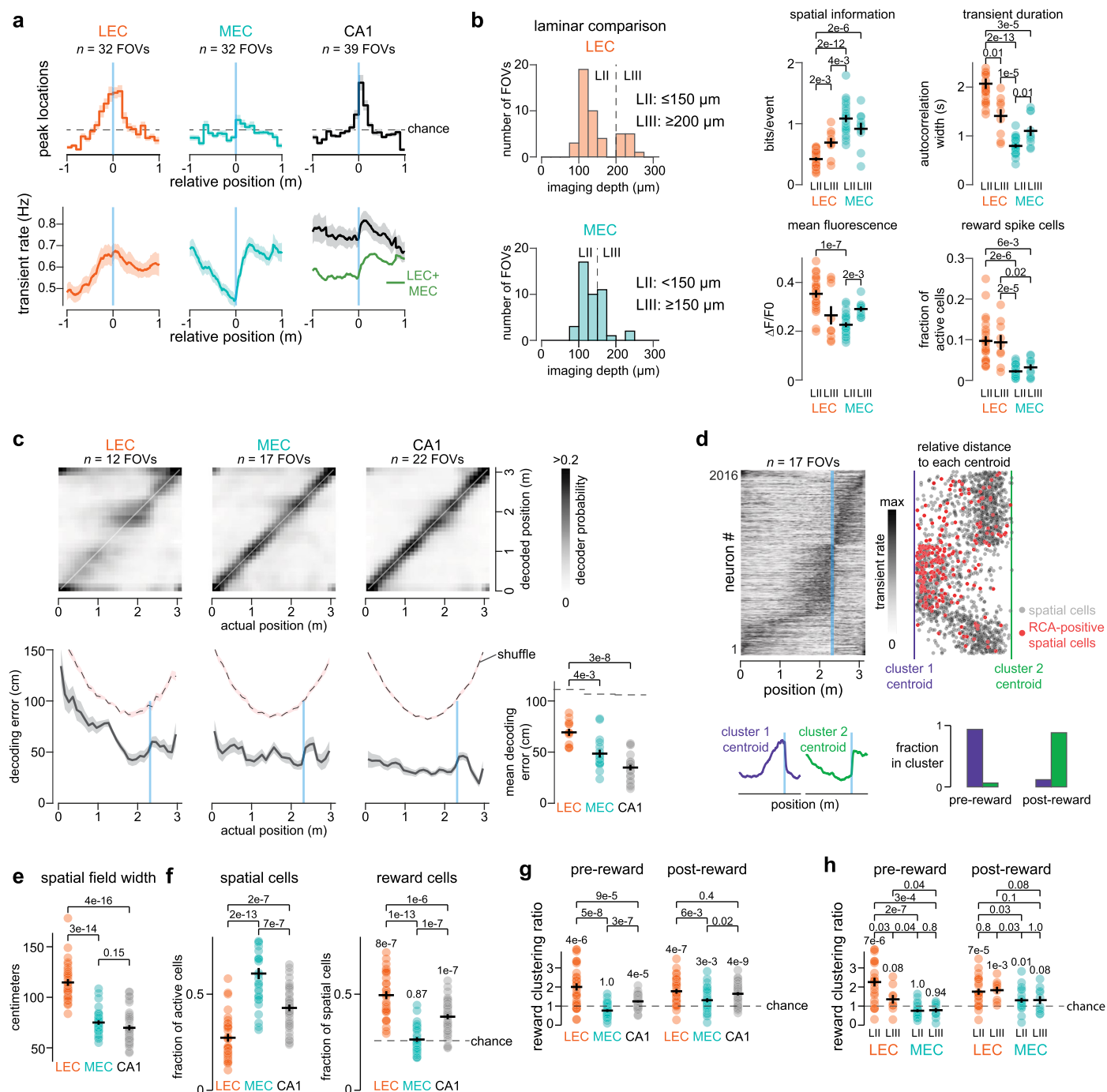
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Extended Data Fig. 1 | Histological confirmation of imaging window over LEC.

a) Histology of lateral entorhinal cortex (LEC). Injection of retrograde tracer in CA1 labels CA1-projecting LIII pyramidal cells of entorhinal cortex in a mouse with GCaMP6s expression. Horizontal section is taken after PFA fixation. Tracer injection and histology was performed on $n=3$ mice with similar results across mice; data here is shown for a representative exemplar. **b**) Zoomed in images of tdTomato and GCaMP6s labeling of the LEC in this horizontal slice. Histogram quantifies the amount of fluorescence from each of these channels as a function

of depth from the dural surface. The cell-free zone separating layers II and III is approximately 150 to 200 μm beneath dura. **c**) To validate the prism placement, CA1-projecting LIII cells in LEC were again labeled with a retrograde tracer and two-photon imaging was performed on an implanted mouse. Labeled cell bodies were only seen at depths of at least 200 μm. This validation experiment was performed on $n=1$ mice. **d**) Simplified diagram of connectivity between superficial layers of entorhinal cortex and regions of the hippocampus along with dopaminergic inputs from the ventral tegmental area (VTA).

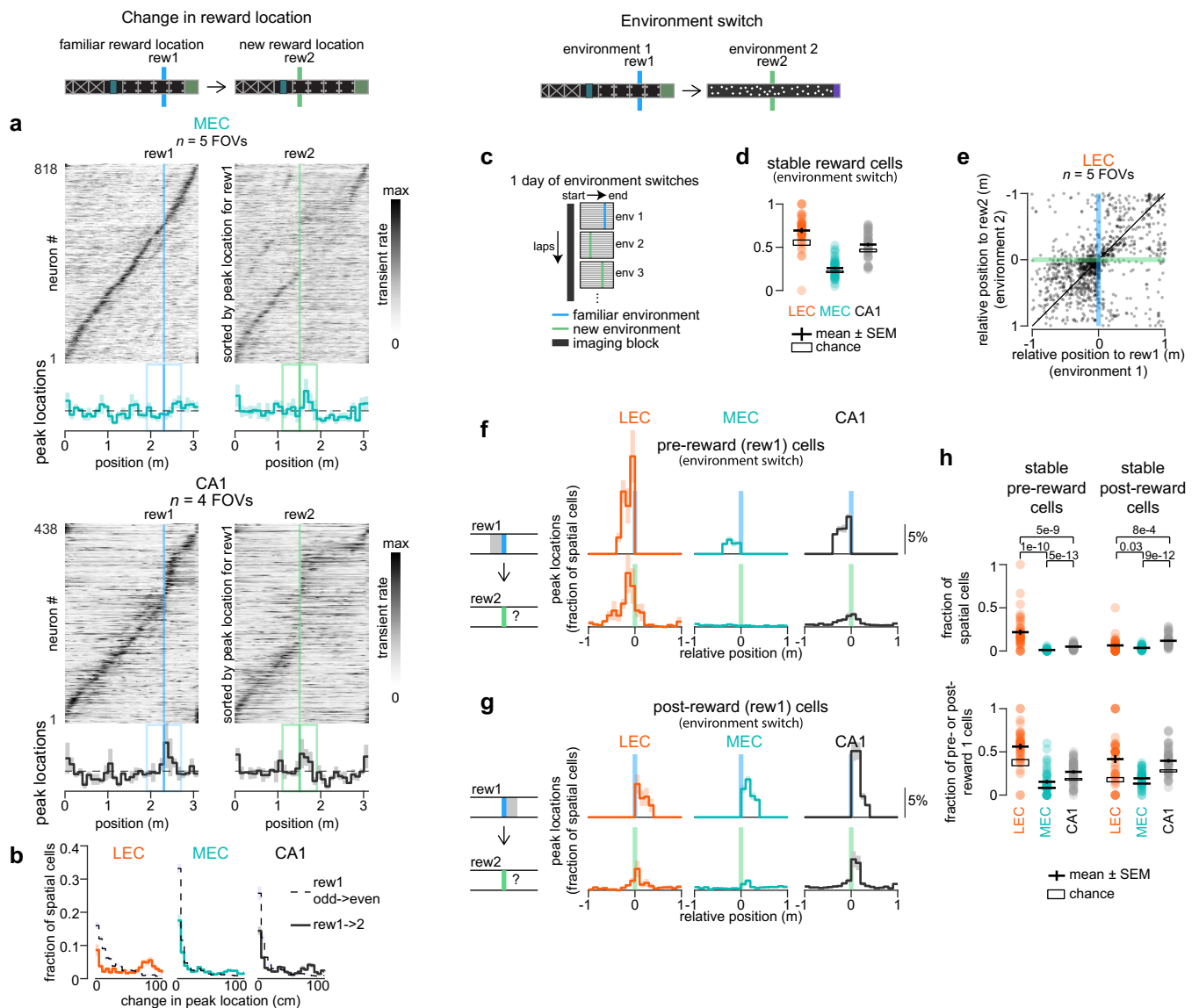


Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | LEC firing peaks around reward and differs by layer.

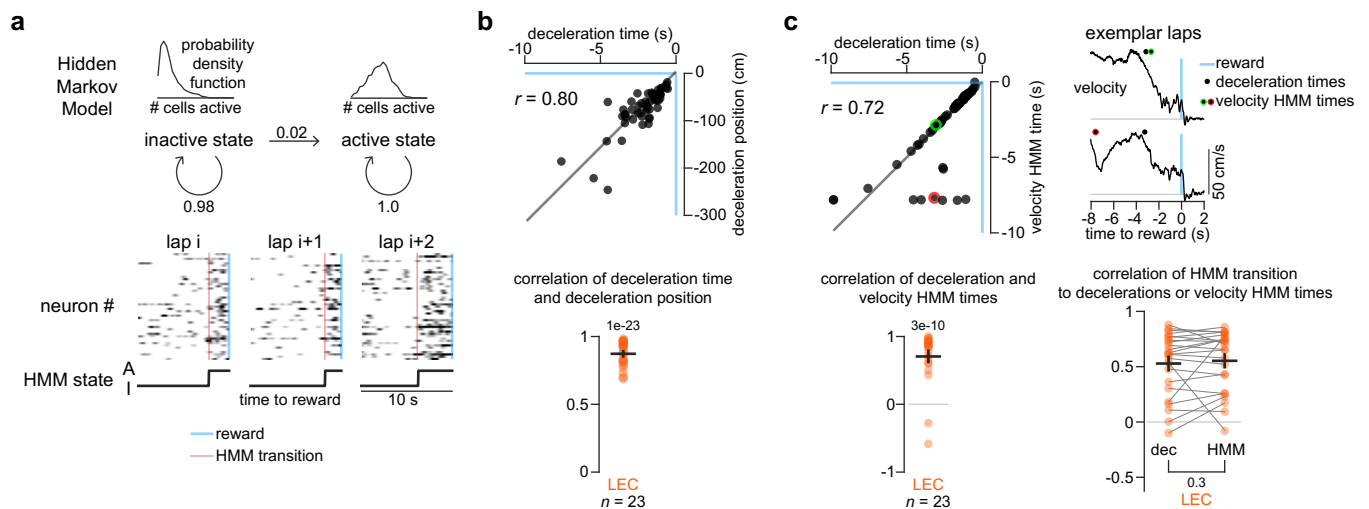
a) Histogram of spatial cell peaks and mean transient rates relative to reward location. Datasets were combined across days with reward at 2.3 m (as in Fig. 2a–d), 0.7 m, and 1.5 m. Histograms binned every 10 cm; transient rate sampled every 1 cm. Mean $\pm$ SEM shown as dark line with light shading. Green LEC+MEC trace is average of LEC and MEC traces. **b)** Comparison of Ca^{2+} transients and cell firing properties from LII ($n=23$ FOVs) and LIII ($n=9$ FOVs) of LEC and LII ($n=22$ FOVs) and LIII ($n=10$ FOVs) of MEC. Data quantified for each imaging session; black cross represents mean $\pm$ SEM across sessions. Statistical tests between pairs (2-sided 2-sample t-test, Bonferroni correction); significant p-values ($p < 0.05$) shown. Imaging depths for all LEC and MEC fields (47 and 44 total, respectively, which include some sessions with tasks not included here) shown on left. **c)** Bayesian decoding of spatial position for sessions with reward at 2.3 m. Decoder trained with data from odd laps for a random subset of 40 non-reward spatial cells for each FOV and tested on even laps. Only sessions with ≥ 40 non-reward spatial cells were used. Mean $\pm$ SEM shown as dark line with light shading. Decoded error taken as the mean absolute difference between most likely decoded position and the actual position in 10-cm bins. Data quantified for each imaging session; black cross represents mean $\pm$ SEM across sessions. p-value from 2-sided 2-sample t-test shown. Shuffle (shown as dashed lines) achieved by randomly permuting position data for test set (even laps) and performing the same decoding analysis. $n = 12, 17, 22$ FOVs for LEC, MEC, and CA1, respectively. **d)** Unsupervised k-means clustering of spatial firing patterns for LEC with reward location at 2.3 m (data

shown in Fig. 2c). Calinski-Harabasz criterion identified the optimal number of clusters as 2. Cluster centroids shown in purple and green, along with fraction of prereward and postreward cells that identify as each cluster. Dot raster: relative distance to each, taken as $(d_2 - d_1)/(d_2 + d_1)$, where d_i is the squared Euclidean distance to centroid i . **e)** Quantification of spatial field widths in each region, computed as distance of the track with firing rate $> 30\%$ of each cell's maximum. Each point represents mean of spatial cells from one imaging session. **f)** Fraction of active cells that are spatial cells and fraction of spatial cells that are reward cells in each region. Each point represents one imaging session. Chance was calculated assuming uniform distribution of spatial cell peaks along the track. Since the reward zone is 80 cm total and the track length is 310 cm, chance is $80/310$, or ~ 0.26 . **g)** Reward clustering ratio, calculated as number of cells that peak in each reward zone divided by expected number of cells if peaks were uniformly distributed along the track. Ratio greater than one (chance) indicates an increased number of cells in that reward zone. **h)** Reward clustering ratio stratified by imaging depth. For LEC: $\leq 150 \mu\text{m}$ below dura (layer II) and $\geq 200 \mu\text{m}$ below dura (layer III); for MEC: $\leq 150 \mu\text{m}$ below dura (nominal layer II) and $> 150 \mu\text{m}$ below dura (nominal layer III). **e–h)** One-sided 1-sample t-tests used for individual regions compared to chance and 2-sided 2-sample t-tests for comparisons between regions (indicated by horizontal line). p-values indicated on panels. $n = 32$ (23+9), 32 (22+10), 39 FOVs for LEC, MEC, CA1, respectively, with LII+LIII breakdown in parentheses; black cross represents mean $\pm$ SEM across sessions.



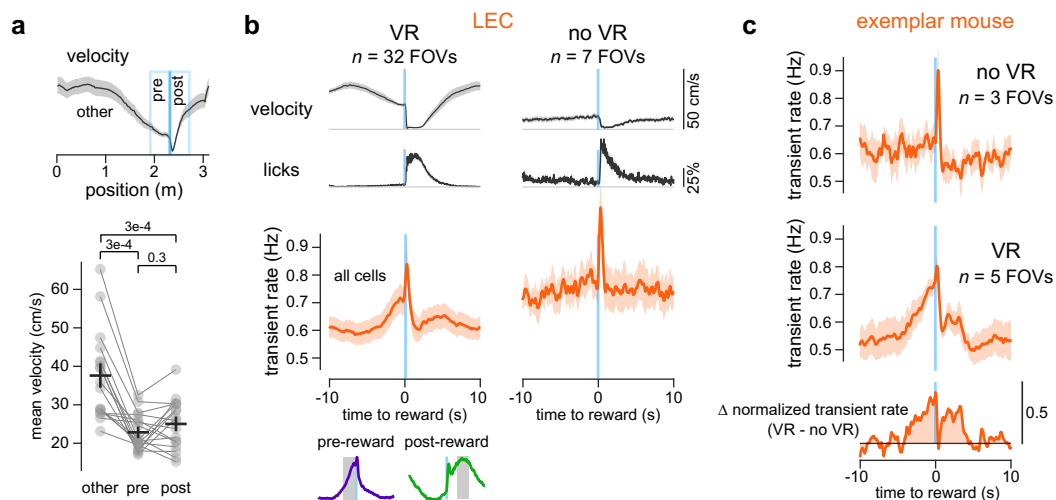
Extended Data Fig. 3 | Additional details for pre- and postreward populations across reward and environment switches. **a)** Spatial firing patterns along the track and histogram of firing peaks for spatial cells sorted by their firing peaks with the familiar reward location for MEC and CA1. Same format as Fig. 3c. **b)** Distribution of difference in peak location for MEC and CA1 spatial cells across the two conditions. Dashed line is the distribution for cross-validated data with the familiar reward location (even versus odd laps). **c)** Paradigm for environment switches. Four different environments were visited in the same session, with ~20 laps run in each environment before switching to the next one. **d)** Fraction of reward cells that remain reward cells after environment switch, quantified as fraction of reward cells that maintain their peak location within 40 cm of the new reward location. Data quantified for each environment switch in an imaging session. **e)** Peak locations shown relative to reward locations with first environment on the x-axis and second environment on the y-axis. Each point

represents one LEC neuron that was a spatial cell in both. **f)** Peak locations for second environment (rew2) for cells that are spatial in both conditions and prereward relative to first environment (rew1). Histograms calculated for each environment switch in an imaging session; mean ± SEM shown as dark line with light shading. **g)** Same as panel **f** but for postreward cells. **h)** Quantification of stable pre- or postreward cells for both environments, either as fraction of all spatial cells or of pre- or postreward cells for first environment. **d, h)** Two-sided 2-sample t-tests for comparisons between regions, indicated by horizontal line with p-values. $n = 5, 5, 6$ FOVs for LEC, MEC, CA1, respectively, each of which has 9 pairs of environments that are used for assessing stability of reward cells; each dot represents average for one pair of environment switches from one imaging session and black cross represents mean ± SEM (black rectangle represents chance).



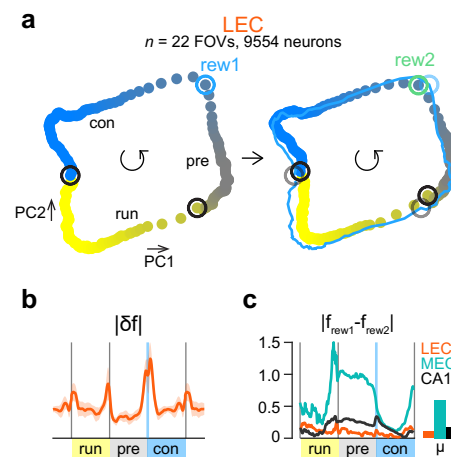
Extended Data Fig. 4 | Additional details on hidden Markov model for LEC prereward population firing. **a** A hidden Markov model (HMM) was used to detect transitions in the prereward population activity. The 10 seconds before reward delivery were considered. The system begins in an inactive state and can transition to an absorbing active state on each lap. 'Number of cells active' is the observed state. The HMM is trained to learn the emission probabilities for each state (number of cells active) and the transition probability matrix. **b** Deceleration times and position relative to reward are highly correlated, thus indicating that running velocity is fairly consistent during approach to reward. Analysis in main text uses time because it was more reliable in practice. **c** Transition from high running speed to lower running speed during reward approach was measured in two ways: deceleration time and velocity HMM time

(see Methods). For exemplar session (top left: points represent transition times for individual laps; top right: exemplar laps where velocity HMM time agreed or disagreed with the deceleration time, indicated by green or red highlight, respectively) and across sessions (bottom left: each point represents correlation between the two measures for one imaging session; black cross represents mean $\pm$ SEM across sessions), the two measures were highly correlated. Both measures were also similarly highly correlated to the HMM-detected state change in the prereward cell firing (bottom right: Pearson correlation coefficients for each session, calculated as in Fig. 4g; each point represents one imaging session; black cross represents mean $\pm$ SEM across sessions). **b–c** Statistical tests performed against zero (1-sided 1-sample t-test) and between each pair (2-sided Wilcoxon signed-rank test); p-values indicated on figure. $n = 23$ FOVs.



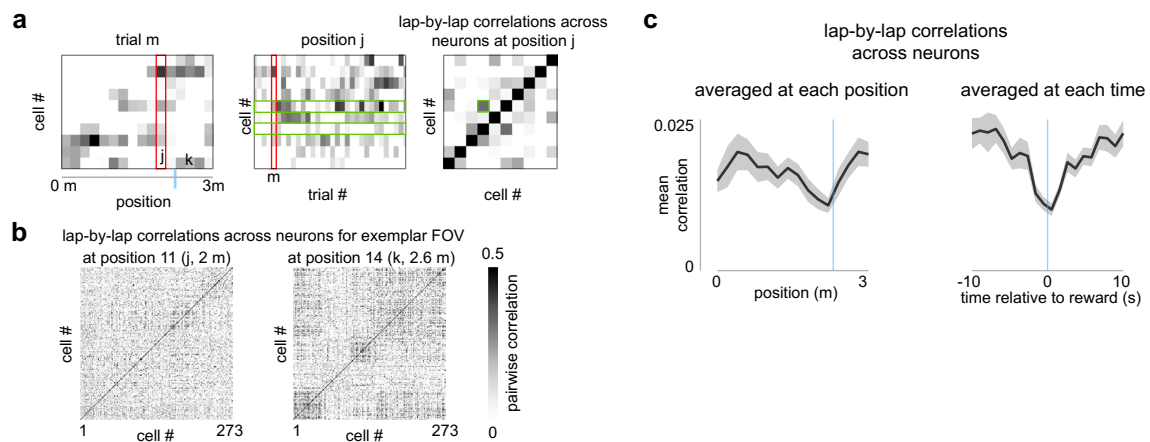
Extended Data Fig. 5 | Pre- and postreward firing in LEC is specific to a virtual navigation task. **a** Mean running velocity at track positions surrounding reward are similar (pre: 40 cm before reward, mean of 22.8 cm/s; post: 40 cm after reward, mean of 25.0 cm/s) but lower than running speed along the rest of the track (other: all track positions excluding 40 cm around reward, mean of 37.6 cm/s). Each point represents one imaging session in LEC for reward at 2.3 m; black cross represents mean $\pm$ SEM across sessions. Statistical tests performed between each group (2-sided Wilcoxon signed-rank test; p-values indicated on figure). $n = 17$ sessions. **b** VR (left column): mean velocity, lick rate, and transient rate across all active cells in LEC, as a function of time relative to reward. LEC transient rate plot is reproduced from Fig. 5a. Inset shows mean transient rate for prereward neurons and postreward neurons relative to reward time. Gray boxes highlight periods when prereward and postreward cells are highly active outside of the immediate reward consumption period. No VR (right column):

same measures but for naïve head-fixed mice (7 FOVs in 3 mice) receiving random (unpredictable) rewards. We only included rewards with immediate consumption (first lick within 0.5 s of reward delivery) and with some treadmill movement in the 2.5 s preceding reward. Mean $\pm$ SEM shown as dark line with light shading, respectively. **c** After imaging LEC during delivery of random rewards ('no VR' condition), the same exemplar mouse was trained in virtual reality over a few days and imaging was performed during a reward navigation task ('VR' condition). Transient rate averaged across all active neurons for each session. Mean $\pm$ SEM shown as dark line with light shading. After normalizing transient rate (by dividing by the mean transient rate in the period from -10 s to -5 s), difference in bulk firing between 'VR' and 'no VR' conditions calculated, which captured two periods of large difference: prereward and postreward, relieved by a brief moment of equality for 0.5-1 s immediately after reward delivery.



Extended Data Fig. 6 | Trajectory of LEC population firing using state space analysis. a) Population LEC firing with respect to behavioral epochs is reduced to two dimensions using principal component analysis. The first two components (PC1 and PC2) explained 20% and 10% of the variance, respectively. The mean trajectory is plotted, color-coded by the behavioral epoch as established in Fig. 4 (yellow: postreward/running, gray: reward approach, blue: reward consumption). Open circles highlight the points in the trajectory that correspond to the transition points between epochs. The same components are used to plot the population firing for familiar reward (rew1) and novel reward (rew2). The trajectory for familiar reward is reproduced on the right plot using

a thin blue line for comparison. **b)** Magnitude of difference between successive points (1-norm of the derivative) using first 10 principal components. Mean $\pm$ SEM (across the 10 components) are shown as dark line with light shading. **c)** Quantification of difference between trajectories for familiar and novel rewards using the first two principal component dimensions, taken as the 2-norm of the difference normalized by the 2-norm of the familiar reward trajectory. For reference, we compared this quantity to the same measure applied to MEC and CA1. Unlike LEC, the trajectories in MEC and CA1 differed more after the reward location was moved.



Extended Data Fig. 7 | Pairwise correlations between LEC neurons across trials decrease near rewards. a) Correlation matrices were formed for the activity patterns of a neural population in a given imaging session at a given position. We calculated the correlation between the activity of each pair of cells at a given position on the track across all the laps for that session. **b)** Example correlation matrices at two track positions are shown. **c)** Correlations as a function of track position or time relative to reward. At a given position

(or time), the correlation matrix was averaged across all cell-cell pairs (excluding the diagonal) and computed for each imaging session. Mean $\pm$ SEM shown as dark line with light shading, respectively. **a–c)** Cell-cell correlations were calculated at a given position (or time point relative to reward) across laps. For an imaging session, the mean population correlation was then taken as the average cell-cell correlation across all cell pairs (autocorrelations excluded). Positions were binned in 20 cm intervals and time in 1 s intervals.

Extended Data Table 1 | Number of imaging sessions and analysis of variance

	LEC	MEC	CA1
# mice (F/M)	7 (4/3)	6 (2/4)	8 (3/5)
# fields	32	32	39
Age (mean +/- std)	22.5 ± 9.6 weeks	22.5 ± 4.4 weeks	17.4 ± 1.8 weeks
ANOVA: F (p-value)			
Depth	6.80 (0.01)	0.05 (0.82)	0.52 (0.47)
Deceleration	3.53 (0.07)	2.42 (0.13)	2.68 (0.11)
Sex	0.29 (0.59)	1.30 (0.26)	3.66 (0.06)
Age	0.41 (0.53)	1.42 (0.24)	0.63 (0.43)

Multiway analysis of variance (ANOVA) was performed using the anovan function in MATLAB. The effects of the following factors were tested for their effect on reward clustering for each imaging session: 1) imaging depth, discretized into 50 μm bins; 2) degree of anticipatory deceleration, measured as mean velocity in 1 s before reward dropping to at least half of the maximum velocity; 3) sex, 4) age, in weeks.

Extended Data Table 2 | Number of imaging sessions and neurons for each reward location

Reward location	# FOVs	Total neurons	Active neurons	Spatial neurons
0.7 m	5	2106	2001	577
1.5 m	10	4812	4387	1363
2.3 m	17	8538	8101	2016
Total (familiar reward)	32	15456	14489	3956
Total (reward change)	22	10208	9554	2890, 2447

Number of LEC imaging fields and number of neurons for each of the three reward locations used. Final row shows data for sessions where the reward location was changed. For this data, 'Spatial neurons' shows number of spatial neurons for familiar and novel reward location, respectively.

Extended Data Table 3 | Number of sessions for optogenetic experiments

	control	Jaws
# mice (F/M)	4 (1/3)	5 (0/5)
# sessions	25	26
Age (mean +/- std)	25.3 ± 2.8 weeks	23.3 ± 1.7 weeks

Number of sessions for optogenetic inhibition experiments in LEC.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

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|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | A description of all covariates tested |
| ☐ | ☒ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection ScanImage 5, MATLAB R2013b/R2017b

Data analysis MATLAB R2022a; Custom code available at GitHub (<https://github.com/DombeckLab/Issa2023>) and deposited on Zenodo (<https://doi.org/10.5281/zenodo.10162357>).

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- Accession codes, unique identifiers, or web links for publicly available datasets
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Datasets generated in this study have been deposited online and are publicly available (<https://doi.org/10.5281/zenodo.10160991>).

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes, but sample sizes are similar to those reported in previous publications (Kaufman et al, Neuron 2020, DOI:10.1016/j.neuron.2019.12.029; Heys and Dombeck, Nat Neuro 2018, DOI: 10.1038/s41593-018-0252-8).
Data exclusions	For optogenetic inhibition experiments, the first session with a reward move was excluded from analysis so that mice had a chance to learn that a move could occur. For imaging experiments, we included all imaged mice, which are mice with good optical windows and that reached the predetermined behavioral criteria.
Replication	Where appropriate, data was cross-validated by comparing results from odd and even trials. Further, data was grouped and compared either by session or by animal (across sessions) to ensure that data was representative across sessions and across animals.
Randomization	The same experiments and measures were made for each region studied with imaging experiments. We did not attempt to randomize these allocations as experiments targeting each region were performed in batches; however, in some cases we allocated littermates to MEC and LEC implants and trained/imaged them in parallel and at least qualitatively observed results consistent with our larger cohort. For optogenetic experiments, littermates were pseudorandomly assigned to either positive or control groups for virus injection; the remainder of the experiment (habituation, training, and behavior manipulation) were performed identically in the two groups.
Blinding	Data collection and analysis were not performed blind to the conditions of the experiments. This was not possible to do for imaging because the location of the imaging window (and all the procedures related to that experiment) is part of the experiment, so there was no practical way to blind the experimenter.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Mouse strains - used as adults 2-6 months old:

1. tetO-GCaMP6s (JAX 024742)
2. Camk2a-tTA (JAX 007004)

Wild animals

No wild animals were used in this study.

Reporting on sex

Both males and females were used. Numbers of each are reported where appropriate. No significant differences between sexes were found.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All animals used in this study were maintained and cared following protocols approved by the Northwestern Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.