# Integrating time from experience in lateral entorhinal cortex

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**Abstract (150 words)**

**The encoding of time and its binding to events are critical components for episodic memory, but how these processes are carried out in hippocampal-entorhinal circuits is unclear. Here we show in freely foraging rats that temporal information is robustly encoded across time scales from seconds to hours within the overall population state in lateral entorhinal cortex (LEC). Similarly pronounced encoding of time was not present in medial entorhinal cortex (MEC) or hippocampal areas CA3-CA1. When animals' experiences were constrained by behavioral tasks to become similar across repeated trials, encoding of temporal flow across trials was reduced while encoding of time relative to the start of trials was improved. The findings suggest that populations of LEC neurons represent time inherently through the encoding of experience. This representation of episodic time may be integrated with spatial inputs from MEC in the hippocampus, allowing the hippocampus to store a unified representation of what/where/when.**

## Introduction

The representation of time is a critical component of episodic memory[1-3]. Although a significant body of work has now demonstrated that the hippocampus plays an essential role in generating a

representation of time[4-11], our understanding of how the brain represents time for episodic memory (episodic time) is still in a nascent stage. The primary function of episodic time is to record the order of events within experience, which does not require a precise representation of metric time, differentiating it from interval and circadian timing [12-14]. Rather than being able to keep precise metric time, the neural code for episodic time should have the following two fundamental properties: 1) the code should arise automatically without any behavioral training, to support one-shot formation of episodic memory, and 2) the code should be able to capture the different scales of time at which experience may occur. Recently, two types of representation of time have been observed in the hippocampus and MEC: 'time cells' which fire at specific points in time as an animal performs a task[15-19], and the decorrelation of place cell activity across hours to days[20-25]. However, neither of these representations of time has been shown to fully support one-shot formation of episodic memories in combination with variable timescales. Furthermore, how either of these representations of time arises is unknown. Here, we investigated temporal coding outside the place-cell system, in LEC. We focused on LEC because (i) this area is a major source of cortical input to the hippocampus, (ii) previous work has shown that responses in LEC to physical stimuli could be unstable across time[26,27], and (iii) a clear underlying function has not yet been defined for LEC. We found a representation of time in LEC which exhibited both of the expected signatures of episodic time, and thus could support episodic memory.

**Temporal coding in individual LEC cells**

To explore temporal coding in LEC, we recorded neural activity over more than an hour while rats ran in a box whose walls alternated semi-randomly between black and white on 12 trials (BW12 experiment, **Fig. 1a, Extended Data Fig. 1**). An extended number of trials was used to increase the likelihood that animals defined multiple temporal contexts across the experiment, while the interleaved design was chosen to avoid confounding changes in wall color with progression of time. Data was also recorded from CA3 and MEC for comparison. Examining LEC responses by eye, we noticed that some cells exhibited clear ramping activity (**Fig. 1a, b**), raising the possibility that individual LEC cells track the passage of time through their firing rates. Responses to specific environmental features such as walls and cue cards[26] were also observed, consistent with LEC's established role in encoding environmental context[28-30].

We quantified the influence of wall color and time on the activity of single cells using a generalized linear model (GLM) incorporating time, wall color, and position as variables for fitting the firing rates of individual neurons, which were binned temporally into blocks of 500 ms (**Extended Data Fig. 2a-d**). A significant number of LEC cells were selective specifically for time (20.4% of all recorded cells), while only 2.0% of CA3 cells and 4.5% of MEC cells were selective for time alone (number of cells significantly influenced by at least one variable for LEC: 186/451, 41.2%; CA3: 72/148, 48.6%; MEC: 49/133, 36.8%; **Fig. 1c**). The distributions of cell selectivities for LEC, CA3, and MEC were consistent across individual animals (**Fig. 1c**).

Because time was modeled as a linearly-increasing function in the GLM, all time-selective cells exhibited some form of ramping activity. Both increasing and decreasing ramps across a range of time constants were observed (**Extended Data Fig. 2c**), as would be expected if they performed a Laplace transform of the recent past[31]. Ramping cells were found in both deep and superficial layers, with no clear difference in the time constants (**see Supplemental text**). Ramping responses, particularly across the entire recording session, were not due to recording instability (**Extended Data Fig. 2e-g, see Methods**). We found little evidence in LEC for non-ramping time-specific activity similar to that of time cells (**Extended Data Fig. 3**).

**LEC population states encode temporal information**

We next focused on the overall population of LEC cells and asked whether its dynamics reflected time coding. We first visualized the overall population data from individual animals using linear discriminant analysis (LDA) to determine the dimensions which optimally discriminate the 24 experiment-defined states (12 trial, 12 intertrial), and plotting the 2D projection yielding the best discrimination (**Fig. 2, Extended Data Fig. 4a-c**). Population activity for the entire session separated into distinct modes corresponding to the environmental contexts of the experiment, as expected from previous work demonstrating LEC involvement in encoding context[28-30]. In addition, there was a prominent progression of states corresponding to the temporal order of the experiment. Thus, across the entire session, the three environmental contexts could be separated along one axis in state space, while the temporal epoch of each trial could be separated along another.

To quantify the temporal information present, we trained a linear multiclass support vector machine (SVM) to identify temporal epochs (e.g. trial 1, intertrial 1, trial 2, etc.) based on neural activity from individual animals, pooled across recording sessions. Population activity defined by the firing rates of cells was binned into 10 s bins, with each bin labeled by the temporal epoch it was in. The decoder was then trained to identify temporal epochs based on population activity, with 10-fold cross-validation (**Extended Data Fig. 4d**). Statistical significance was evaluated using a permutation method (**see Methods**). Very high decoding accuracy for temporal epoch identity across the whole session spanning both trial and intertrial epochs was observed for all LEC animals during the BW12 experiment, indicating significant temporal information was present in LEC population activity (88.0% mean accuracy, chance level 4.2%; **Fig. 3a**). The high decoding accuracy was not due to pooling cells across recording days, changes in behavior or noise-driven variability of population activity states (**Extended Data Fig. 4e, f, 5**). To further verify our observations, we trained decoders using data from additional LEC animals which ran a simplified 4-trial version of the BW12 experiment (BW4), and obtained similar results (93.1% mean accuracy, chance level 25%; **Extended Data Fig. 4g, h**). Thus, population activity in LEC clearly defined a unique temporal context for every epoch of experience on the timescale of minutes.

Visualization of data from CA3 and MEC revealed population activity patterns different from LEC (**Fig. 2**). Quantification of temporal information across the entire session, including both trial and intertrial periods, using decoders trained on CA3 and MEC data revealed decoding accuracies above chance, but lower than that found in LEC (23.0%, 34.4% mean accuracy for CA3, MEC respectively). In order to properly compare decoding accuracy across the three areas, we trained separate decoders for each area using populations with equal size to ensure that higher accuracy was not simply due to the data having higher dimensionality. LEC still contained significantly more temporal information than either CA3 or MEC, as decoding accuracy for temporal epoch was higher for LEC by a sizeable margin (45.5% vs. 20.4% vs. 26.2% mean accuracies for LEC, CA3, and MEC respectively; **Fig. 3b-c**). The difference in decoding accuracy was not simply due to temporal information being more distributed across the population in CA3 or MEC, as LEC required significantly fewer cells to reach high decoding accuracy (**Fig. 3d, Extended Data Fig. 4i**). In the simplified BW4 experiments, we also had data

from CA2 and CA1, allowing us to compare decoding accuracies for temporal epoch across entorhinal cortex and all CA subfields. We again found, in separate animals, that decoding accuracy was highest for LEC (**Extended Data Fig. 4h**). In total, these observations point to LEC as a possible source of temporal context information necessary for episodic memory formation in the hippocampus.

Is the robust encoding of time in LEC at the population level due primarily to cells with ramp-like activity? To test whether cells not classified as selective for time nonetheless also encoded temporal information, possibly in a nonlinear form[32], we trained decoders for temporal epoch using only cells which were not selective for time, and found that decoding accuracy remained very high. Comparing against size-matched populations with cells drawn randomly from the entire dataset, decoding accuracy for time using only non-selective cells was not significantly different (77.2% vs. 79.2% mean accuracy for trial identity using population with no time-selective cells, compared to a size-matched randomly-drawn population, **Fig. 3e**).

If the temporal information observed within LEC population activity is actually used as a temporal code, is it limited to representing just the macroscopic temporal context of experiences, on the timescale of minutes and longer, or is it also capable of representing the order of events within experiences, which may be on timescales shorter than minutes? We examined whether LEC could encode temporal epochs of shorter length than entire trial or intertrial periods by dividing trial-period data into shorter epochs and training decoders to identify these shortened epochs (**Fig. 3f**). Decoding accuracies for 20 s, 10 s, and 1 s epoch-lengths across the entire session were all significantly above chance (27.8%, 21.4%, 1.6% mean accuracy for 20 s, 10 s, and 1 s epochs respectively; chance levels 0.4%, 0.2%, 0.02% respectively; **Fig. 3f, g, Extended Data Fig. 6a-c, see Supplemental text**). Although decoding accuracy decreased with shorter epoch lengths, decoding errors predominantly predicted adjacent epochs, as opposed to temporally distant epochs (**Fig. 3g**). Decreased accuracy may have been due to the limited number of cells that we recorded from. Pooling together all recorded cells from every animal to generate a population of 451 cells, decoding accuracy for 20 s epochs was 86.0%, while decoding accuracy for 10 s and 1 s epochs were 83.0% and 30.5% respectively. Decoding accuracy for shortened epochs was not due to differences in population activity on the timescale

of trial or intertrial periods, as decoding accuracy for shortened epochs within single trial and intertrial periods was still above chance (**Extended Data Fig. 6d, e**). Thus, temporal information in LEC was flexible enough to support encoding of events happening across a wide range of timescales.

In a separate set of experiments, we tested whether changing the content of the animal's experience affected temporal information in LEC by introducing an object into the recording environment. We found a similar representation of time at both the single-cell and population level (**Extended Data Fig. 7**). Information about the current environmental context (B or W) as well as information about the immediately preceding context was also present in LEC (**Extended Data Fig. 4j-l, see Supplemental Text)**. Overall, these results suggest a large component of LEC population activity codes for time across multiple scales, expressed both through cells explicitly coding time as well as cells having mixed selectivity for time that requires population decoding to extract.

**Temporal information arises inherently**

What is the mechanism by which time is incorporated into the population representation in LEC? One possibility ("explicit mechanism") is that LEC actively, in a clock-like manner, generates timestamps for representations of experience. Another possibility ("inherent mechanism") is that temporal information in LEC arises simply due to the fact that the animal's moment-to-moment experience constantly changes, and time can be extracted from this changing flow of experience by integrating the amount of change[9,12,33] (**Extended Data Fig. 8, 9a**). We sought to distinguish these two possibilities by constraining experience through the use of a more structured task in which the animal's behavior was stereotyped. If the temporal information present within LEC arose from an explicit clock-like process, we would expect to see no change in the amount of temporal information present when compared to results from the free-foraging BW experiments. By contrast, if the temporal information within LEC arose inherently through its encoding of experience, we would expect to see a decrease in the amount of temporal information due to the repetitive nature of the task.

We examined the variability of temporal representation in LEC in a separate experiment where animals performed a learned continuous-alternation task (figure-eight task, **Fig. 4a**). Trials were aligned by the time-point at which the animal entered the central stem, and trials consisted of activity spanning from three seconds before to three seconds after entering the central stem. Visualizing the data, we observed the separation between trials was reduced in comparison to BW data (**Fig. 4b**). Decoding accuracy for trial identity across the entire session was above chance, but much lower than for matched BW data (figure-eight, 10.6% vs. BW, 21.5% mean accuracy, chance level 5%; **Fig. 4c, Extended Data Fig. 9b**). In a separate experiment where animals ran repeated laps on a circular track, reduced decoding accuracy for trial identity during repetitive experience was also observed (**Extended Data Fig. 9c-e**). Overall, these results are consistent with temporal information in LEC arising not in an explicit clock-like manner, but inherently from the dynamics underlying LEC's representation of ongoing experience.

**Temporal coding varies under changing behavioral conditions**

The hippocampus represents learned time intervals[15,16,19,34], and this representation of time may depend on the retrieval of stable temporal contexts[35]. Does the reduction in across-session temporal information during learned behavior reflect a similar instatement of a stable temporal context in LEC for the purpose of representing the relevant features of the task, which may include the progression of time within single trials? Analysis of single-cell responses using a GLM incorporating trial type (left/right turn), trial time (time within single trials), and session time showed that the ratio of cells encoding trial time versus session time was significantly altered, with more cells encoding trial time and fewer cells encoding session time in the figure-eight experiment (5.7% vs. 34.0% vs. 18.2% cells selective for session time or mixed session time [session time+trial type] for figure-eight, BW12, and object experiments respectively; 35.7% vs. 6.2% vs. 7.7% cells selective for trial or mixed trial time [trial time+trial type] for figure-eight, BW12, and object experiments respectively; **Fig. 5a, Extended Fig. 10b-e**). The proportion of cells selective for trial type also differed significantly (20.8% vs. 4.9% vs. 8.0% cells selective for context for figure-eight, BW12, and object experiments respectively; **Fig. 5a**).

Visualization of overall population activity using PCA to plot 2D projections of neural trajectories through population activity space showed that neural trajectories were relatively

constant across trials, suggesting that LEC was in fact in a different, more stable mode of activity compared to that observed during free foraging (**Fig. 5b, Extended Data Fig. 10f**). Consistent with this observation, decoding accuracy for time relative to the start of each trial was significantly above chance and much higher than for matched BW data (45.3% vs. 18.3% mean accuracy, chance level 16.6%, **Fig. 5c**). Although trial time was tightly correlated with position, the significant decoding of trial time did not appear to be due purely to LEC activity reflecting spatial location (10.0% cells selective for trial time exclusively, **Fig. 5d**). In addition to changes in the type of temporal information present in LEC population activity, the amount of task-related information also appeared to change, as the decoding accuracy for trial type was higher for figure-eight data than the decoding accuracy for wall color using matched BW data (81.3% vs. 72.1% mean accuracy, chance level 50%; **Fig. 5e).** Finally, the degree to which information was distributed across the entire population was decreased compared to BW experiments, as decoding accuracy for both trial type and trial time were significantly reduced compared to size-matched randomly-drawn controls when cells selective for trial type or trial time respectively were removed (56.1% vs. 76.4% mean accuracy for trial type, 32.2% vs. 41.9% mean accuracy for trial time, **Fig. 5f**). Overall, our results suggest that as animals engaged in a structured, learned task, the dynamics of LEC activity became significantly more stable compared to when animals were engaged in free behavior.

**Discussion**

Being able to recall the temporal details of past experiences is a fundamental element of episodic memory. Our recordings demonstrate a unique temporal signal in LEC that can encode time across multiple scales from seconds to hours and across different environmental contexts. Ordinarily this code for time marks the free-flowing progression of time, reflecting the structure of ongoing experience. However, when animals engage in a structured behavioral task in which experience is similar across repeated trials, time coding becomes relative, encoding time with respect to temporal landmarks. The adaptable nature of this code for time makes it particularly well-suited for defining the temporal component of episodic memory (′episodic time′) and differentiates it from previously-described interval-timing mechanisms[36-38].

What mechanism could produce spontaneous representations of episodic time in LEC? Our results support recent theoretical work demonstrating that encoding of time can arise inherently as a result of interactions between externally- and internally-driven states[31,33,39]. This form of time coding represents different points in time using different high-dimensional population states that can be easily differentiated by downstream readout neurons (**Extended Data Fig. 8**). In LEC, such high-dimensional states may be generated by a combination of the recurrent local connectivity of LEC[40] and the uniquely diverse set of inputs that the LEC receives[41]. Given LEC's anatomical position as a major gateway for information entering the hippocampus, it is possible that representations of time outside of the entorhinal/hippocampal circuit[42,43] are integrated in LEC to form a single representation of time for episodic memories.

Within the entorhinal/hippocampal circuit, two representations of time have been identified in previous work: 'time cells' which fire at specific points in time as an animal performs a task[15-19], and the decorrelation of place cell activity across hours to days[20-25]. Continuously-changing LEC activity may underlie both scales of temporal representation: drift of place cell activity in may be governed by a constantly-changing LEC input which is time-varying on the scale of minutes to hours, while sequential activity of time cells may be driven in part by LEC input which is time-varying relative to task events on the scale of seconds [31,44]. Thus, while an episodic memory may contain both a fine-grained representation of the sequence of events composing that memory as well as a coarser temporal context for the overall episode, both of these scales of temporal representation may originate from a single temporal signal within LEC. This signal may then reach the hippocampus to become part of a unified what-when-where representation of experience, space, and time[3], in which the representation of experience and time arising in LEC is integrated with the representation of space arising in MEC[45].

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## Competing interests statement

The authors declare that they have no competing financial interests.

## Keywords

entorhinal cortex, hippocampus, episodic memory, time, temporal coding.

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**Figure 1**

**Temporal information within LEC single-cell activity**

**a**, Experiment structure: animals ran twelve 250 s trials in a box with either black or white walls. Trials were separated by 140 s intertrial periods where the animal was placed in a holding pot. Total session length was 1.3 h.

**b**, Four example LEC cells. Left: Path plots showing animal's location in grey and cell spikes in red. Right: Firing rate plots for the same cells. Dark and light grey indicate trial periods with black and white walls respectively. Unshaded regions indicate intertrial periods. Cells 1-3 responded to features of the recording box with no obvious temporal component to their activity, while cell 4 exhibited ramping activity across each trial.

**c**, Left, example GLM fit results for four cells with selectivity for different features, with observed firing rate shown in grey, predicted firing rate in blue. Right, average tetrode waveforms for first (green) and last (black) quarter of the session.

**d**, Distribution of selectivity for wall color, position, time, and mixtures of variables for LEC (top, $n = 3$ animals with 90, 141, 220 cells), CA3 (middle, $n = 3$ animals with 78, 42, 28 cells), and MEC (bottom, $n = 2$ animals with 31, 102 cells). Circles indicate individual animals, solid lines indicate mean ± s.e.m., shade indicates individual animals.

**Figure 2**

**Visualizing LEC population activity**

2D projections of neural population responses. Axes correspond to the first two linear discriminants (LD1, LD2, arbitrary units). Left column shows LEC population responses, middle column shows CA3 population responses, right column shows MEC population responses, each from an example animal. Each trial's wall color indicated by a shade of green (black walls) or purple (white walls); intertrial periods shown in grey. Progression of shade from dark to light for trial and intertrial periods indicates progression of time. Note progression by time for LEC (see also **Extended Data Fig. 4a**). LDA projections were used for visualization purposes only.

**Figure 3**

**Temporal information within LEC population activity**

**a**, Decoding accuracies for trial-length temporal epochs from whole recording session for LEC ($n$ = 3 animals), CA3 ($n$ = 3 animals), and MEC ($n$ = 2 animals).

**b**, same as **a**, but for size-matched populations (unpaired $t$-test with Bonferroni correction, LEC vs. CA3: $t(4) = 5.77$, $P < 0.01$, LEC vs. MEC: $t(3) = 4.81$, $P < 0.05$, matched population size = 28 cells).

**c**, Confusion matrices from example animals. Each matrix shows entire session, with each entry corresponding to a single epoch (i.e., trial 1, intertrial 1, trial 2, etc.). Epoch type indicated along left and bottom (grey for intertrial periods, black for black wall trial periods, white for white wall trial periods).

**d,** Relation between population size and decoding accuracy. Lines indicates exponential curve fit to data (shown as points, pooled across $n$ = 3, 3, 2 animals for LEC, CA3, MEC respectively).

**e**, Decoding accuracy for temporal epoch using subpopulations with all time-selective cells removed, compared to randomly drawn size-matched controls (paired $t$-test, $t(2) = 1.00$, $P = 0.42$, $n$ = 3 animals with 150, 107, 62 cells not selective for time).

**f**, Decoding accuracies for different temporal epoch lengths for LEC. Left, method for constructing trial-length epochs. Right, decoding accuracies for different epoch lengths ($n$ = 3 animals, matched population size = 90 cells).

**g**, Confusion matrix for 20 s epochs from example animal. The matrix contains 228 epochs (each trial period truncated to 240 s and divided into twelve 20s epochs, each intertrial period of 140 s divided into seven 20s epochs, nineteen epochs per trial/intertrial pair, twelve total pairs across the session, giving 228 epochs). Left, confusion matrix for the entire session. Right, sum across trial/intertrial sections of the whole session matrix (outlined by dotted line in whole session matrix).

Circles indicate individual animals, solid lines indicate mean decoding accuracies ± s.e.m., dashed lines indicate chance.

**Figure 4**

**Temporal information arises inherently**

**a**, Continuous alternation task, where animals alternated between left- and right-turns when it reached the top of the central stem for 40 total trials. Black circle indicates base of central stem.
**b**, Left: 2D projection of LEC neural population response during figure-eight experiment from example animal. Right: 2D projection of LEC neural population response during matched periods from BW experiments.
**c**, Left, decoding accuracy for trial identity during figure-eight experiment compared to decoding accuracy for temporal epoch using matched data from BW experiments (unpaired *t*-test, $t(8) = 8.35$, $P < 0.0001$, matched population size = 31 cells, $n = 3, 7$ animals for figure-eight and BW respectively). Circles indicate individual animals, solid lines indicate mean decoding accuracies ± s.e.m, dashed lines indicate chance. Right, confusion matrix for figure-eight experiment from example animal.

**Figure 5**

**Temporal coding depends on behavioral context**

**a**, Proportion of cells exhibiting selectivity for session time, trial time, and trial type for figure-eight (light blue), BW12 (purple), and object experiments (magenta). Session/mixed session time: figure-eight vs. BW12, $t(4) = 4.35$, $P < 0.05$, figure-eight vs. object, $t(4) = 10.10$, $P < 0.01$; Trial/mixed trial time: figure-eight vs. BW12, $t(4) = 6.70$, $P < 0.01$, figure-eight vs. object, $t(4) = 7.28$, $P < 0.01$; Trial type: figure-eight vs. BW12, $t(4) = 5.56$, $P < 0.01$, figure-eight vs. object, $t(4) = 4.45$, $P < 0.05$; unpaired *t*-test with Bonferroni correction, $n = 3$ animals for all.
**b**, 2D projection of neural trajectories for LEC data during figure-eight experiment from example animal. Axes correspond to the first two principal components (PC1, PC2; arbitrary units).
**c**, Decoding accuracy for trial time compared to matched BW data (unpaired *t*-test, $t(8) = 38.98$, $P < 10^{-10}$**,** matched population size = 31 cells, $n = 3, 7$ animals for figure-eight, BW respectively).
**d**, Decoding accuracy for trial time using subpopulations with all spatially-selective cells removed, compared to randomly drawn size-matched controls (paired *t*-test, $t(2) = 2.17$, $P = 0.16$, $n = 3$ animals with 38, 44, 22 cells not selective for space).

**e**, Decoding accuracy for trial type compared to decoding accuracy for wall color using matched BW data (unpaired *t*-test, $t(8) = 2.74$, $P < 0.05$, matched population size = 31 cells, $n = 3, 7$ animals for figure-eight, BW respectively).

**f**, Decoding accuracy for trial type (left) or trial time (right), with cells selective for decoded variable removed, compared to randomly drawn size-matched controls (paired *t*-test, trial type: $t(2) = 4.83$, $P < 0.05$; trial type: $t(2) = 4.40$, $P < 0.05$; $n = 3$ animals with 29, 31, 15 cells not selective for trial type, 27, 29, 8 cells not selective for trial time).

Circles indicate individual animals, solid lines indicate mean ± s.e.m. of described measurement, dashed lines indicate chance.

## Methods summary

Methods, along with any additional Extended Data display items, are available in the online version of the paper; references unique to this section appear only in the online paper.

## Subjects

Experiments were carried out using twenty-one male Long Evans rats at NTNU and two male Long-Evans rats at Johns Hopkins University. Animals were housed individually in Plexiglas cages. Ten rats had tetrodes in LEC, two rats had tetrodes in LEC and CA3, two rats had tetrodes in MEC, and nine rats had tetrodes in either CA3, CA2, or CA1. Data from seven of the LEC animals and the nine animals with tetrodes solely in hippocampus have been published previously, but examined activity of individual cells rather than the overall population[46,47]. Animals were maintained on a 12-h light/12-h dark schedule, and kept at 85-90% of free-feeding body weight. Experiments were performed in accordance with the Norwegian Animal Welfare Act and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, or the Institutional Animal Care and Use Committee at Johns Hopkins University. The study contained no randomization to experimental treatments and no blinding. Sample size (number of animals) was set based on conventions in the field.

## Surgery and electrode preparation

Rats were anesthetized with isoflurane (air flow: 1.0 L/min, 0.5-3% isoflurane, adjusted according to physiological monitoring). For LEC recordings, either one microdrive with four tetrodes (10 rats) or one hyperdrive with 18 tetrodes was targeted to LEC (2 rats). Coordinates for microdrive LEC animals were: AP: 0-0.2 mm anterior to lambda, ML: 5.3-5.5 mm lateral to midline, DV: 4.3-5.0 mm below dura, 4-8 degree angle in the coronal plane, with electrode tips pointing away from midline. Coordinates for hyperdrive LEC animals were: AP: 7.5-7.6 mm posterior to bregma, ML: 3.0 mm lateral to midline, DV: at brain surface, 25-degree angle in the coronal plane, with electrode tips pointing away from midline. For MEC recordings, one microdrive was targeted to MEC with coordinates: AP: 0.2-0.4 mm anterior to the transverse sinus, ML: 4.6 mm lateral to midline, DV: 1.8 mm below dura, 20-degree angle in the sagittal plane, with electrode tips pointing towards bregma. For hippocampal recordings, either one microdrive was targeted to CA3, with coordinates: AP: 3.8 mm posterior to bregma, 3.0 mm

lateral to midline, DV: 1.5 mm below dura (3 rats), or one hyperdrive was placed over hippocampus with coordinates: AP: 2.5-4.3 mm posterior to bregma, ML: 2.2-3.8 mm lateral to midline (9 rats). Drives were fixed to the skull using jeweller's screws and dental cement. One screw served as a ground for each drive. Tetrodes were made from four twisted 17 μm polyimide-coated platinum-iridium (90%-10%) wires (California Fine Wire, CA). Electrode tips were plated with platinum to reduce electrode impedances to between 150-300 kΩ at 1 kHz.

**Recording procedures**

Microdrives were connected to a multi-channel unity gain headstage, which was connected via a cable to a Neuralynx recording system for BW12 and circular-track experiments, or an Axona recording system for object and figure-eight experiments (Axona Ltd., Hertfordshire, U.K.). Unit activity was amplified by a factor of 1000-10000 and band-pass filtered from 600 to 6000 Hz for Neuralynx recordings or from 800 to 6700 Hz for Axona recordings. Spike waveforms above a set threshold were time-stamped and digitized at 32 kHz for 1 ms for all recordings. Tetrodes were lowered in 50 μm steps while the rat rested on a towel in a flower pot on a pedestal. Turning stopped when well-separated units appeared. Data collection started when signal amplitudes exceeded approximately 5 times the noise level (root mean square 20-50 μV) and units were stable for >3 hours. Animal position was tracked at 25-30 Hz for Neuralynx recordings or 50 Hz for Axona recordings using an overhead video camera and either two (for microdrive animals) or multiple (for hyperdrive animals) LEDs attached to the headstage.

**Behavioral procedures**

For BW12, BW4, and object experiments, animals were trained to collect randomly scattered chocolate cereal crumbs in a square box. For BW12 experiments, animals ran in an 80 cm × 80 cm × 50 cm box with interchangeable walls, a single cue card along one wall, and curtains on two sides of the box. For BW4 experiments, the box was 100 cm × 100 cm. For object experiments, the box was 100 cm × 100 cm, the walls were black with a single cue card, and no curtains were present, such that there were many distal cues. Once animals were able to achieve good coverage of the recording environment, they began running standard sessions of 12 trials for BW12 experiments or 4 trials for BW4 experiments, during which wall color was changed across trials. For object experiments, animals ran 3 trials, during which an object consisting of a

6 cm × 6 cm × 37 cm tower made of Legos was placed in a fixed location during the second trial[59].

For circular track experiments, animals were trained to run back and forth between food wells for food pellets (BioServ, NJ) on a circular track (diameter 97 cm, width 10 cm). The food wells were separated by a 15 cm-tall black barrier with 0.4 cm sidewalls. For figure-eight experiments, animals were trained to run in a figure-eight pattern on a square figure-eight maze made of plexiglass with vinyl flooring which had runways 15 cm wide with 2 cm high side walls, a central stem that was 150 cm long, and total dimensions of 150 cm × 150 cm. Rewards were placed in dishes at the end of each goal arm. The maze was elevated 50 cm above the ground, surrounded by black curtains with a cue card 100 cm × 25 cm on the left side of the maze. Animals were trained in three stages[48]. In the first stage, wood-block barriers were placed at the bottom and top of the central stem so that rats could only run a fixed path. The position of the barriers was alternated on each trial once the rat reached the reward zone, and animals remained at this stage until they ran >20 trials within a 20-minute session. In the second stage, the barrier at the top of the central stem was phased out so that animals could enter either reward arm. Only correct alternating choices were rewarded, and animals were blocked from backtracking to explore the stem or other reward arm once a choice was made. In the third and final stage, the barrier at the bottom of the central stem was phased out, and animals had to continue to run in a figure-eight pattern, with backtracking blocked by a barrier. After reaching the third stage, animals ran 20-minute sessions, averaging ≥50 trials per session with >95% correct choices.

**Spike sorting and cell classification**

Spike sorting was performed offline using graphical cluster-cutting software to examine two-dimensional projections of the multidimensional parameter space (Tint [Neil Burgess] for Axona data; MClust [A.D. Redish] or custom-written spike-sorting software for Neuralynx data). Spike clusters were compared across successive days to ensure that the same cell was not counted twice.

Cell stability across BW12 experimental sessions was determined by checking for stationarity of spike waveforms. For each cell, the Euclidean distance between spike waveforms across the

recording session and the average waveform of the first ten spikes was measured. For each spike, the four spike waveforms were compressed into a single point in a 128-dimensional space (4 waveforms, each with 32 values), and Euclidean distance was measured in this 128-dimensional space. Subsequently, the stationarity of this distance was determined using three tests: augmented Dickey-Fuller test, the Kwiatkowski-Phillips-Schmidt-Shin test, and the Ljung-Box test, with stable cells being those which were categorized as stationary across all three tests. Following this stability test, stability was additionally assessed by calculating the Pearson correlation between the measured Euclidean distances across the recording session and firing rate across the recording session. Cells were excluded from further analysis if their correlation values exceeded chance levels. Chance levels were estimated individually for each cell by temporally shuffling the firing rate for a given cell and calculating the correlation between the cell's waveform distance and the shuffled firing rates, repeating this process 1000 times, and taking the 95$^{th}$ percentile as the threshold. Firing rate was estimated for 500 ms bins, with no smoothing or additional preprocessing.

**GLM fitting**

A Poisson generalized linear model was fit to each cell individually using the MATLAB stepwiseglm function. The variables used to fit the model for BW12 data were wall color, position, trial time, and session time. A single predictor was used for each variable. Session time was the total elapsed time, and took into account intertrial periods. Adjusted $R^2$ was used as the criterion for adding or removing terms (0.01 for adding, 0.005 for removing). Firing rate was estimated for 500 ms bins, with no smoothing or additional preprocessing. The same process was used for object and figure-eight data, excluding position as a variable.

**Estimating time constants**

For cells that were classified as selective for trial or session time, a single-term exponential model was fit to each cell's firing rate $f = ae^{bx}$ where $f$ is the firing rate of the cell, $a$ and $\underline{b}$ are constant coefficients, and $b$ is the cell's time constant. For cells selective for session time, the firing rate across all trial periods was used. For cells selective for trial time, the average firing rate across trials was used.

**Dimension-reduced visualization of data**

2D projections of population activity states were constructed by reducing the dimensionality of raw neural data using PCA, followed by applying LDA to the dimension-reduced data and taking the top two linear discriminants. Dimension reduction for visualization was carried out separately for 'Whole session' and 'Trial periods only' plots. For neural trajectories, PCA was applied to spiketrains which were first smoothed using a Gaussian kernel 500 ms wide. The top two PCs were taken for 2D projections. In both cases, neural data were from single animals but pooled across recording days. Bin size was 500 ms for both projections of population activity states and neural trajectories.

**Decoding analysis**

Decoding was done with linear SVM classifiers implemented using the LIBLINEAR package[49], specifically using L2-regularized L2-loss SVC, 10-fold cross-validation, and cost parameter C = 1. Data consisted of raw spike counts from individual animals, pooled across recording days, and binned in 10 s bins, except for results varying epoch length, circular track, and figure-eight experiments. For shortened epochs, bin size was 1 s for 20s-long epochs, 500 ms for 10s-long epochs, and 50 ms for 1s-long epochs. For circular track and figure-eight results, bin size was 500 ms. Multiclass decoding was done using the one-versus-all method. The cost parameter was varied between $10^{-3}$ and $10^{3}$ with no significant effect. 10-fold cross-validation was implemented by splitting the data into 10 subsamples, each randomly drawn from across the entire session. Decoders were then trained on 9 of the subsamples, and tested on the remaining subsample, with this process repeated using all 10 subsamples were used as test data once (**Extended Data Fig. 4d**). Bin-size was sufficiently shorter than epoch length to ensure that training data covered all temporal epochs. Decoding accuracy was taken as the average accuracy across all 10 trained decoders. Cross-validation was repeated 1000 times with overall decoding accuracy taken as the mean across the 1000 repetitions, except for when population subsampling occurred, in which case 1000 different subsamples were taken, each with 10 repetitions of cross-validation. For comparing decoding accuracies across areas, size-matched populations were used. Size-matched populations were generated by subsampling the total population of cells for a given animal without replacement. For each animal, subsampling was repeated 1000 times, and the mean was taken as the decoding accuracy for that animal. For decoding analyses in which GLM-classified

cells were removed, the size-matched populations for comparison were randomly drawn from the full population of cells recorded for each individual animal without replacement.

For figure-eight and circular track experiments, matched BW data consisted of 6s long epochs with 500 ms bins and an intertrial interval of 22 seconds. Because inclusion of a matched intertrial interval caused matched data to exceed the length of a single BW trial, matched data spanned across 3 trials of BW data. Wall color in matched BW data corresponded to trial type for figure-eight data (black walls/left trial, white walls/right trial), and running direction for circular track data (black walls/clockwise, white walls/counterclockwise). Twenty epochs were used for figure-eight data to match the twenty trials from the experiment, with population size set to 31 cells. Fifteen epochs were used for circular track data to with population size set to 47 cells. Figure-eight and circular track data were also compared against matched BW data which did not account for the intertrial period. In this case, matched data was generated by taking activity from the first trial only of each session, with matched bin size and population size (twenty epochs, each 6 s long, made up of twelve 500 ms bins, in total spanning the first 120 s of the first trial, with population size set to 31 cells for figure-eight data; fifteen epochs, each 6 s long, made up of twelve 500 ms bins, in total spanning the first 90 seconds of the first trial, with population size set to 47 cells for circular track data).

**Determining statistical significance for decoding accuracies**

Statistical significance for decoding accuracy was determined by comparing mean decoding accuracy from the original data against mean decoding accuracy from temporally shuffled data. Shuffled comparisons were generated by first temporally shuffling population activity such that the shuffled order was the same for each cell within the population. Decoding accuracy was then determined using the original labels for time bins and 10-fold cross-validation. This process was repeated 1000 times to generate shuffled comparisons for full-population decoding accuracies, and 1000 times for each subsampled population of cells to generate shuffled comparisons for size-matched decoding accuracies. All decoding accuracies were significantly above chance, with the least significant decoding accuracy having $P < 10^{-5}$.

**Estimates of distance between high-dimensional population states**

For within-trial or intertrial period distances, distance between the population state in the first time bin and population states in all subsequent time bins within a trial or intertrial period was measured. Population states were defined by the firing rates of cells from individual animals, pooled across recording days, for 10 s time bins. Firing rates were not smoothed. This process was repeated twelve times, once for each trial or intertrial period, and then the average was taken as the final result. For across-trial or intertrial period distances, population states were defined in the same way, but then the average population state for each trial or intertrial period was calculated. Distance was then measured from the average state of the first trial or intertrial to all the average states of all subsequent trials or intertrials. Because the data were high-dimensional, Manhattan distance was used instead of Euclidean distance[50]. To account for differences in dimensionality across animals and facilitate comparison, distance measures were z-scored.

**Classifying spatial cell types and measuring spatial tuning**

Spatial rate maps were generated by binning activity into 3 cm × 3 cm spatial bins, calculating firing rates for each spatial bin, and then applying a two-dimensional Gaussian kernel with standard deviation of 7 cm in both directions. Only spikes recorded during running speeds above 2.5 cm $s^{-1}$ were used. Place cells were classified by comparing spatial information scores against a shuffled distribution[51]. Grid cells were classified by comparing autocorrelogram-based gridness scores against a shuffled distribution[51]. Speed cells were classified by comparing Pearson correlation values against a shuffled distribution[52]. Velocity, acceleration, and head direction were calculated using methods published previously[52]. Spatial selectivity for LEC cells was determined by measuring spatial information[53] for each cell and comparing against a distribution generated by calculating spatial information for data shuffled temporally using wrap-around shuffling.

**Code and data availability**

Custom code used and datasets generated and/or analysed during the current study are available from the corresponding author upon request.

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**Extended Data Figure 1**

**Histology for LEC animals**

Nissl-stained coronal sections showing recording locations for LEC animals used in BW12 experiments (**a**), BW4 and figure-eight experiments (**b**), circular track experiments (**c**), and object experiments (**d**). Red arrows indicate tetrode locations. Activity was recorded from neurons in all layers of the lateral half of LEC, across all layers. Dashed lines indicate approximate anatomical borders of LEC.

**Extended Data Figure 2**

**Single-cell responses in LEC related to time**

**a,** Schematic of the GLM used for determining cell selectivity. Learned weights for the relevant predictors, as determined by the stepwise selection process, were put through an exponential nonlinearity that returned the mean rate of a Poisson process from which spikes were drawn (14.0% selective for session time, 3.8% selective for trial time, 2.7% selective for a mixture of trial and session time, percentages averaged across all animals).

**b**, Explained variance for all LEC cells fit by the GLM for BW12 experiment ($n$ = 186 cells). Average explained variance was 0.05.

**c**, Distribution of time constants for trial- or session-time selective cells ($n$ = 80 cells). Time constants were estimated for each cell classified as trial- or session-time selective by fitting a single-term exponential.

**d**, Left, five additional example cells exhibiting ramping activity across the session. Each cell's firing rate is shown in grey, with the model-predicted firing rate in blue. $R^2$ value is shown in upper right corner for each cell. Right, average waveforms across four recording channels for the first quarter of the session (green line) and the last quarter of the session (black line).

**e**, Pearson correlation between waveform distances (Euclidean distance between pairs of consecutive spike waveforms) and firing rate for all LEC cells used in the BW12 experiment ($n$ = 451 cells).

**f**, Comparison of the correlation between waveform distance and firing rate, as in **j**, for time-selective LEC cells ($n$ = 92 cells) and all other LEC cells used in the BW12 experiment ($n$ = 359 cells).

**g,** Six example cells demonstrating that significant fluctuations in firing rate can occur during stable recordings. For each cell, left top row shows activity during trial periods, left bottom row shows activity during intertrial periods, and right shows average waveforms as in **d**.

**Extended Data Figure 3**

**Time cell-like activity**

**a-h**, Temporal specificity examined as a function of peak firing times of individual cells.

**a**, **b**, Mean activity for trial (**a**) and intertrial (**b**) periods, ordered by time of peak firing rate from example animals. Rows show mean firing rates for individual cells. Actual data shown in left column and shuffled data shown in right column. Shuffling was performed by time-shifting each cell's firing rate by a random amount with the end of the trial wrapped around to the beginning. Cell identities were not maintained across actual and shuffled data.

**c**, Fraction of cells with temporally significant activity for trial (top) and intertrial (bottom) periods ($n$ = 3, 3, 2 animals for LEC, CA3, and MEC respectively).

**d**, Time of peak activity for cells with temporally-significant activity.

**e**, Same as **d**, but for intertrial periods.

**f-h**, Temporal specificity examined by calculating temporal information.

**f**, Fraction of cells with significant temporal information for trial (top) and intertrial (bottom) periods ($n$ = 3, 3, 2 animals for LEC, CA3, and MEC respectively).

**g**, Distribution of significant temporal information scores for trial periods.

**h**, Same as **g**, but for intertrial periods.

**i**, Predictors added for expanded GLM: symmetrical ramps and single-trial ramps. Both predictors would reveal cells with increased activity within a specific range of time, whereas the trial and session ramping variables of the original GLM would identify cells whose activity was continuously changing across the session. This type of activity would be more similar to time cells, which fire at specific points in time, albeit at a much shorter timescale. Both predictors were synchronized to the start of trials, while the symmetrical ramps spanned a range of slopes, dependent on which trial was being synched to. Colored lines highlight the predictors for the respective trial indicated on top, gray lines are the rest of the available predictors.

**j**, Explained variance for all LEC cells fit by expanded GLM for BW12 experiment ($n$ = 350 cells). Average explained variance was 0.03.

**k**, Distribution of selectivity for time, wall color, and position for single LEC cells, determined using the expanded GLM ($n$ = 3 animals). Shade indicates individual animals.
**l**, Examples of expanded GLM fit results for four cells with selectivity for different features. Each cell's firing rate is shown in grey, with the model-predicted firing rate in blue. $R^2$ value is shown in upper right corner for each cell. Average waveforms shown on right, as in **d**.
Circles indicate individual animals, solid lines indicate mean fraction of cells ± s.e.m.

**Extended Data Figure 4**

**Decoding of temporal epochs**

**a**, 2D projections of neural population responses during BW12 experiment for trial periods only. Axes correspond to the first two linear discriminants (LD1, LD2, arbitrary units). Left column shows LEC population responses, middle column shows CA3 population responses, right column shows MEC population responses, each from an example animal. Each trial's wall color is indicated by a shade of green (black walls) or purple (white walls), with progression of shade from dark to light indicating progression of trials.
**b,** Fraction of variance explained by the first 20 principal components for each area. Principal components were computed using PCA on raw data. Lines indicate variance explained for individual animals ($n$ = 3, 3, and 2 animals for LEC, CA3, and MEC respectively).
**c**, Regressing the first two principal components from PCA results for individual animals against time leads to significant fits for all areas, but substantially higher explained variance for LEC (unpaired $t$-test, LEC vs. CA3: $t(4) = 9.79$, $P < 0.001$, LEC vs. MEC: $t(3) = 6.13$, $P < 0.01$). Top row, example fits for individual animals are shown with black lines indicating time and colored lines indicate regression fit, and $R^2$ indicated for the example fit. Bottom two rows, first two PCs for example fits.
**d**, Illustration of cross-validation procedure: 5-fold cross-validation is shown for data containing four temporal epochs, with five time bins in each epoch. A different subset of time bins is used as test data for each iteration of the cross-validation procedure. Actual data consisted of 24 epochs (trial and intertrial periods) with 25 or 14 time bins in each epoch, and 10-fold cross-validation was used.
**e**, Z-scored decoding accuracy using cells recorded in a single day (left, one-sided binomial test, $P = 0.68$, $n$ = 46 days), pairs of consecutive days (middle, one-sided binomial test, $P = 0.29$, $n$ =

72 pairs), pairs of days separated by half the total number of recording days (right, one-sided binomial test, $P = 0.37$, $n = 44$ pairs).

**f**, Decoding accuracy for temporal epoch using behavior tracking data in place of neural activity ($n = 3$ animals). 'All' tracking data consisted of the animal's position, velocity, acceleration, and head direction.

**g**, Left, decoding accuracy for temporal epoch using BW4 data, compared to decoding accuracy using BW12 data (unpaired *t*-test, $t(5) = 1.09$, $P = 0.32$, matched population size = 47 cells, $n = 4, 3$ animals for BW4 and BW12 respectively). Right, decoding accuracy for wall color from the BW4 experiment, compared to decoding accuracy using matched data from BW12 experiment (unpaired *t*-test, $t(5) = 1.03$, $P = 0.35$, matched population size = 47 cells, $n = 4, 3$ animals for BW4 and BW12 respectively,).

**h**, Decoding accuracy for temporal epoch using BW4 data from LEC, MEC, CA3, CA2, and CA1 (one-way ANOVA, $F(4) = 20.78$, $P < 1\times10^{-5}$, post-hoc Bonferroni multiple comparisons test, $P < 0.005$ for all LEC comparisons, matched population size = 24 cells, $n = 7, 2, 7, 3, 3$ animals for LEC, MEC, CA3, CA2, and CA1 respectively).

**i,** Relation between population size and decoding accuracy for LEC, CA3, and MEC. Left, decoding accuracy for varying population sizes; each line indicates the curve fit to data (shown as points) from one animal, with colors indicating recording area ($n = 3, 3, 2$ animals for LEC, CA3, MEC respectively). Right, relation between population size and decoding accuracy for LEC and CA3, pooled across animals from BW12 and BW4 experiments (data pooled from $n = 7$ animals for both LEC and CA3).

**j,** Decoding accuracy for wall color from trial period activity alone for LEC, CA3, and MEC (unpaired *t*-test, LEC vs. CA3: $t(4) = 0.81$, $P = 0.47$, matched population size = 28 cells, $n = 3, 3, 2$ animals for LEC, CA3, and MEC respectively).

**k**, Decoding accuracy for wall color using data which was shuffled in time (two-tailed paired *t*-test, $t(2) = 0.38$, $P = 0.74$, $n = 3$ animals).

**l**, Decoding accuracy for wall color using a subpopulation with all time-selective cells removed, compared to size-matched populations which were randomly drawn from full population (paired *t*-test, $t(2) = 2.66$, $P = 0.12$, $n = 3$ animals with 77, 126, 195 cells not selective for wall-color).

For **f, g, j-l**, circles indicate individual animals, solid lines indicate mean decoding accuracy ± s.e.m., dashed lines indicate chance.

**Extended Data Figure 5**

**Evolution of LEC population dynamics**

Structure and evolution of LEC (purple, $n = 3$ animals), CA3 (blue, $n = 3$ animals), or MEC (gold, $n = 2$ animals) activity, quantified by applying several distance measures to population activity states, see **Supplementary Text**.

**a**, Manhattan distance between the population state for the middle time bin of a trial or intertrial period and all other time bins within that period.

**b**, Manhattan distance between the population state for the last time bin of the preceding trial/intertrial period and all time bins within the current intertrial/trial period, z-scored.

**c**, Left, Manhattan distance between the population state for the first time bin of a trial or intertrial period and all subsequent time bins within that period. Right, fitting distance using an autoregressive model. Red line indicates model fit.

**d**, Manhattan distance between consecutive pairs of population states across trial or intertrial periods.

**e**, Pairwise angles, measured across consecutive points in time along neural trajectories during trial or intertrial periods as the angle between two vectors, each defined as the difference of consecutive population states.

**f**, Manhattan distance between the overall mean population state for the first trial or intertrial period and the overall mean population states of all subsequent trial or intertrial periods.

Solid lines indicate mean distance across all animals, shaded area indicates s.e.m., distance measures were all z-scored.

**Extended Data Figure 6**

**Decoding shortened temporal epochs**

**a**, Decoding accuracies for different temporal epoch lengths across LEC, MEC, CA3, CA2, and CA1 using BW4 data (matched population size = 24 cells, $n = 7, 2, 7, 3, 3$ animals for LEC, MEC, CA3, CA2, and CA1 respectively). Decoding accuracy for 20s epochs using LEC data was significantly better than all hippocampal areas (one-way ANOVA, $F(17) = 20.69$, $P < 1\times10^{-5}$, post-hoc Bonferroni multiple comparisons test, LEC vs. MEC: $P < 0.05$; LEC vs. CA3/CA2/CA1: $P < 0.001$; matched population size = 24 cells). Decoding accuracy was higher for LEC and MEC than CA3 for 10s epochs (one-way ANOVA, $F(17) = 8.00$, $P < 0.001$; post-

hoc Bonferroni multiple comparisons test, LEC vs. CA3: $P < 0.001$, all other comparisons were not significant; matched population size = 24 cells). Decoding accuracy was similar across all areas for 1s epochs (one-way ANOVA, $F(17) = 2.95$, $P = 0.05$).

**b**, Confusion matrix from an example LEC animal for 10 s epochs. The matrix contains 468 epochs (each trial period of 250 s divided into twenty-five 10 s epochs, each intertrial period of 140 s divided into fourteen 10 s epochs, thirty-nine epochs per trial/intertrial pair, 12 total pairs across the session, giving 468 epochs. Left shows confusion matrix for the entire session, right shows sum across trial/intertrial sections of the whole session matrix (outlined by dotted line in whole session matrix).

**c**, Decoding accuracy for re-binned confusion matrices (circles and solid lines with black indicating mean and shade indicating individual animals, comparing across epoch-lengths: one-way ANOVA, $F(3) = 32.28$, $P < 1\times10^{-4}$, $P = 0.12$ for trial vs. 20s, $P < 0.001$ for trial vs. 10s and trial vs. 1s) compared to decoding accuracy for re-binned confusion matrices following shuffling along columns, with diagonals preserved (triangles and dashed lines, with black indicating mean and shade indicating individual animals, comparing decoding accuracies from shuffled and unshuffled confusion matrices: two sided paired *t*-tests, for 20s: $t(2) = 7.42$, $P < 0.05$; for 10s: $t(2) = 14.31$, $P < 0.01$; for 1s: $t(2) = 4.46$, $P < 0.05$;). Gray dashed line indicates chance at 4.2%, shade indicates individual animals.

**d**, Decoding accuracy for different temporal epoch lengths for LEC (matched population size = 90 cells, $n = 3$ animals), based only on activity within individual trial or intertrial periods (top and bottom, respectively). Decoding accuracy was measured for each trial or intertrial period individually, and then the average was taken across all trials or intertrial periods, respectively, for each animal. Temporal bin size was: 1 s for 20s-long epochs, 500 ms for 10s-long epochs, and 50 ms for 1s-long epochs. Chance levels for trial periods were 8.3%, 4.0%, and 0.4%, and 14.3%, 7.1%, and 0.7% for intertrial periods.

**e**, Confusion matrices from an example LEC animal for single-trial or intertrial periods as in **c**, using 20 s (top) and 10 s (bottom) epochs, with intertrial results on left and trial results on right. For **a**, **d**, circles indicate individual animals, solid lines indicate mean decoding accuracies ± s.e.m., dashed lines indicate chance.

**Extended Data Figure 7**

**Temporal coding within a fixed environmental context**

**a**, Experimental paradigm:10 minutes without object, 10 minutes with object, and 10 minutes without object. Right:

**b**, Predictors used for object GLM: object presence, trial time, and session time (24.0% selective for session time, 4.8% selective for trial time, 4.3% selective for a mixture of trial time and session time, percentages averaged across all animals).

**c**, Explained variance for all LEC cells fit by GLM for object experiments ($n$ = 150 cells). Average explained variance was 0.05.

**d**, Distribution of selectivity for time, object, and mixtures of time and object for single LEC cells, determined using a GLM ($n$ = 3 animals with 56, 57, 150 cells). Shade indicate individual animals

**e**, Examples of GLM fit results for twelve cells from the object experiment with selectivity for different features. Each cell's firing rate is shown in grey, with the model-predicted firing rate in blue. $R^2$ value is shown in upper right corner for each cell.

**f**, 2D projection of LEC neural population response during object experiment from example animal.

**g**, Decoding accuracy for trial identity or object presence ($n$ = 3 animals).

**h**, Decoding accuracies for temporal epochs of shortened length ($n$ = 3 animals, matched population size = 56 cells).

**i**, Decoding accuracy for trial identity (left) or object presence (right), with cells selective for decoded variable removed, compared to size-matched populations randomly drawn from full population (paired $t$-test, trial identity: $t(2) = 4.95$, $P < 0.05$, $n$ = 29, 26, 79 cells not selective for time; object presence: $t(2) = 7.17$, $P < 0.05$, $n$ = 39, 47, 110 cells not selective for object presence).

Circles indicate individual animals, solid lines indicate mean ± s.e.m. of described measurement, dashed lines indicate chance.

**Extended Data Figure 8**

**Explicit vs. inherent mechanisms for temporal coding**

**a**, Top: a series of experiences occurs, each containing different event content and spanning different amounts of time. Bottom: two different ways in which temporal information within this series of experiences may be encoded. For both cases, a population code is used, but this may just as easily be replaced by a rate code within single cells. An explicit mechanism (left) purposely represents the passage of time, such that each chunk of time is represented equally. Thus, two experiences with the same temporal length but differing numbers of events would correspond to the same change in activity. An inherent mechanism (right) encodes temporal information entirely by representing the events within each experience. Thus, two experiences with the same temporal length but differing numbers of events would correspond to differing changes in activity. In either case, the high dimensionality of the representations would allow temporal information to be read out easily by downstream readout neurons, e.g. cells in the hippocampus.

**b**, Same as in **a**, but in this example, instead of a series of different experiences, the same experience is repeated three times (analogous to performing a learned task three times). Here, an explicit mechanism for temporal coding would exhibit the same amount of change in activity as in **a**, whereas an inherent mechanism would exhibit significantly reduced differences in activity across the experiences.

**Extended Data Figure 9**

**Decoding trial identity with additional matched data**

**a**, Euclidean distance between mean LEC population states for pairs of adjacent trials with different or same wall color (unpaired *t*-test, $t(31) = 8.81$, $P < 10^{-10}$; $n = 12, 21$ same color and different color transitions respectively, pooled from three animals).

**b**, Decoding accuracy for trial identity during figure-eight experiment, compared to decoding accuracy for temporal epoch using matched data from BW experiment which did not account for intertrial intervals or trial type (unpaired *t*-test, $t(8) = 4.91$, $P < 0.01$, matched population size = 31 cells, $n = 3, 7$ animals for figure-eight and BW respectively).

**c**, Circular-track task, where animals alternated between clockwise and counter-clockwise runs for 15 consecutive back and forth laps. Black circle indicates midpoint of the track.

**d**, Left: 2D projection of LEC neural population response during circular track experiment from example animal. Right: 2D projection of LEC neural population response during matched periods from BW experiments.
**e**, Left, decoding accuracy for trial identity during circular track experiment compared to decoding accuracy for temporal epoch using matched data from BW experiments (unpaired *t*-test, $t(7) = 6.21$, $P < 0.0001$, matched population size = 47 cells, $n = 2, 7$ animals for circular track and BW respectively). Right, same as left, but using temporally consecutive data which did not account for intertrial intervals or running direction (unpaired *t*-test, $t(7) = 3.17$, $P < 0.05$, matched population size = 47 cells, $n = 2, 7$ animals for circular track and BW respectively).
**f**, Confusion matrix for decoding trial identity in the circular track experiment from example animal.
Circles indicate individual animals, solid lines indicate mean ± s.e.m. of described measurement, dashed lines indicate chance.

**Extended Data Figure 10**
**Additional characterization of LEC activity during figure-eight task**
**a**, Manhattan distance between consecutive pairs of population states (binned in 500 ms time bins) across single trials, averaged across all animals.
**b**, Predictors used for figure-eight GLM: trial type, trial time, and session time (1.3% selective for session time , 9.3% selective for trial time, 6.5% selective for a mixture of trial time and session time, percentages averaged across all animals).
**c**, Explained variance for all LEC cells fit by GLM for figure-eight experiment ($n = 149$ cells). Average explained variance was 0.10.
**d**, Distribution of selectivity for time, trial type, and a mixture of time and trial type for single LEC cells, determined using a GLM ($n = 3$ animals with 72, 76, 31 cells). Circles indicate individual animals, solid lines indicate mean fraction of cells ± s.e.m., shade indicates individual animals.
**e**, Examples of GLM fit results for six cells from the figure-eight experiment with selectivity for different features. Each cell's firing rate is shown in grey, with the model-predicted firing rate in blue. $R^2$ value is shown in upper right corner for each cell.

**f**, LEC activity for twelve example cells during the figure-eight task. Each plot shows mean firing rate (top, 95% percentile confidence interval shaded), and peristimulus time histograms for left- (middle) and right-turn trials (bottom), with time centered on the point at which the animal reaches the base of the central stem on each trial. Cells 7-12 exhibited similar firing patterns for both left- and right-turn trials, including during the first three seconds of the trial, in which the animal occupied a different spatial location for left- versus right-turn trials. Such activity may be used for temporal information. Cells 13-18 exhibited highly divergent firing patterns for left- and right-turn trials, which may reflect the animal's spatial location, the behavioral context of the trial, or a combination of the two variables. All example cells exhibited relatively stable firing across trials, a common feature observed during the figure-eight task.

**Supplemental materials and methods**

**1. Single cell activity in LEC related to time**

*Mixed selectivity incorporating time*

For the BW12 experiment, the GLM analysis showed that a fraction of cells in all three recorded brain areas exhibited selectivity for a mixture of time and non-time variables (8.4% for LEC, 7.3% for CA3, and 4.7% for MEC, percentages averaged across all animals; **Fig. 1c**). We employed a stepwise model-fitting procedure, where the first variable to be added to the model is the one explaining the most response variance. In LEC, 15/40 of time/non-time mixed selective cells were selective first for time, while 2/11 of CA3 time/non-time mixed selective cells were selective first for time and 0/5 of MEC time/non-time mixed selective cells were selective first for time. For CA3 cells exhibiting mixed selectivity, 9/11 satisfied criteria for place cells, while for MEC cells exhibiting mixed selectivity, 2/5 were tuned for a spatial feature (1 speed cell and 1 grid cell). Thus, cells exhibiting mixed selectivity in LEC, CA3, and MEC had differing primary selectivities, with almost half of LEC cells being primarily selective for time. In total, the selectivity of LEC single-cell responses reflected the significant influence of time on LEC activity, in contrast to CA3 and MEC.

*LEC single-cell time constants*

The distribution of time constants for ramping cells spanned a broad range of time, from a few minutes to hundreds of minutes, and although a greater number of cells had negative time constants, the distribution was not significantly biased (52/80 LEC cells had negative time constants; one-sample *t*-test, $P = 0.46$; **Extended Data Fig. 2c**). There was not any apparent anatomical organization to the distribution of time constants between deep and superficial layers of LEC, as the absolute value of time constants in deep layers was not different than for superficial layers (mean for superficial layers: 48.9 min, mean for deep layers: 41.3; unpaired *t*-test, $P = 0.80$) and the distribution of time constants between deep and superficial layers was not different for trial-selective (short time constant) versus session-selective (long time constant) cells (two-proportion z-test, $P = 0.46$). Up-ramping and down-ramping cells were observed simultaneously on seven out of the seventeen instances where more than one ramping cell was recorded in a single session. The presence of cells with positive time constants, over a wide

range of timescales, suggests that the ramping activity we observe is not simply an effect of habituation[54], and may be generated by intrinsic mechanisms[55,56].

*Ramping activity is not due to recording instability*

One possible concern, given that the vast majority of time-selective cells encoded time across the entire session rather than across single trials, is that this apparent temporal coding merely reflects unstable recordings. This is unlikely, as only cells which passed spike waveform stationarity tests checking for stability across the entire recording session were included in analyses (**Extended Data Fig. 2e, f, see Methods**). Additionally, although cells firing during a few trials in the middle of a session may have reflected legitimate time-influenced activity, only cells which fired spikes in both the first and last trial or intertrial periods were included for analysis. Finally, for some cells showing significant changes in firing rate across trial periods (intertrial), activity during intertrial (trial) periods was relatively consistent across the session (**Extended Data Fig. 2g**), demonstrating that significant changes in activity across an experimental session were not simply an artifact of poor recording stability.

*Time cell-like activity*

In the hippocampus, time cells fire in relation to events[15,16] (e.g. the start of a delay period). Do such cells exist also in LEC? In the context of the BW12 experiment, synchronizing events may be distributed across the entire session without appearing in any obvious way to an observer. If this is the case, because we have not recorded the same cells across days, we would not be able to distinguish time cell activity from activity due to random fluctuations of external stimuli. However, it is also possible that time cells in the BW12 experiment are synchronized to the structure of the task, e.g. the beginning of trial or intertrial periods. If this is the case, we would expect to see repeated activity at the same time relative to the start of a trial or intertrial period. We tested the latter possibility in our data.

We began by ordering cells from individual animals by the latency of their peak firing rate and looking for sequential activity. Although a ridge of peak activity was present, consistent with sequential time cell-like activity, a similar ridge of peak activity was present for both actual and shuffled data across all areas, suggesting that time cell-like activity may not have been present in

LEC during the BW12 experiment (**Extended Data Fig. 3a, b**). . The low ratio of peak-to-background activity, in contrast to that of time cells which are mostly silent outside of their time field[15,16], also suggested a lack of time cell-like activity. We quantified these observations by calculating a peak-to-background ratio. The peak was defined as the mean rate within a 10 s window centered on the time point of the cell's peak firing rate, and the background was defined as the mean rate across the rest of the session. Significance was determined by comparing to a distribution generated by calculating peak-to-background ratios for temporally shuffled data (1000 shuffles per cell, shuffling was done as in **a**) and using the 95$^{th}$ percentile as a significance threshold. The number of cells in LEC with significant temporal information during trial periods was below that expected by chance (number of cells with significant temporal information during trial periods for LEC: 3.5%, 16/451; CA3: 2.0%, 3/148; MEC: 7.5%, 10/133; mean peak time during trial periods for LEC: 67.7 s; CA3: 3 s; MEC: 25.1 s; number of cells with significant temporal information during intertrial periods for LEC: 3.8%, 17/451; CA3: 2.7%, 4/148; MEC: 7.5%, 10/133; mean peak time during intertrial periods for LEC: 58.9 s; CA3: 73.3 s; MEC: 108.9 s, **Extended Data Fig. 3c-e**). A number of cells did have significant peak activity at the beginning of trial periods, which may reflect actual time cell-like coding, given that time cells have been observed to have more accurate timing closer to the start of their sequences[16]. However, we are unable to distinguish between this and the possibility that these cells are merely responding to the change in context from holding pot to recording box.

In a separate quantification, we calculated a temporal information score for each cell, analogous to the commonly-used spatial information score[53]. For a given cell, temporal information was calculated as $\sum P_i \left(\frac{R_i}{R}\right) log_2(\frac{R_i}{R})$, where $i$ is the time bin number, $P_i$ is the probability of occupancy for time bin $i$ (5s time bins were used), $R_i$ is the mean firing rate for bin $i$, and $R$ is the overall mean firing rate, analogous to the commonly used spatial information score. Significance was determined by comparing to a distribution generated by calculating temporal information for temporally shuffled data (1000 shuffles per cell, data was first binned into 1s time bins, wrap-around shuffling was performed individually on activity from each trial or intertrial prior to taking trial or intertrial averages, and temporal information were then calculated by binning the trial/intertrial-averaged shuffled data into 5s time bins). The 95$^{th}$ percentile of the shuffled distribution was taken as a significance threshold. The number of cells in LEC with significant

temporal information during trial periods was barely above that expected by chance (number of cells with significant temporal information during trial periods for LEC: 5.5%, 25/451; CA3: 4.7%, 7/148; MEC: 9.8%, 13/133; mean temporal information during trial periods for LEC: 1.11 bits/s; CA3: 3.36 bits/s; MEC: 0.53 bits/s; number of cells with significant temporal information during intertrial periods for LEC: 7.3%, 33/451; CA3: 6.8%, 10/148; MEC: 11.3%, 15/133; mean temporal information during intertrial periods for LEC: 0.67 bits/s; CA3: 1.61 bits/s; MEC: 0.47 bits/s, **Extended Data Fig. 3f-h**).

In addition to looking for time cell-like activity specifically, we also ran an expanded GLM which included single-trial ramps and symmetrical ramps, which have been suggested as inputs to time cells[31] (**Extended Data Fig. 3i**). Many cells were classified as exhibiting single-trial ramps, raising the possibility that ramping cells in LEC provide input to time cells (**Extended Data Fig. 3i-l**). Overall, the small number of temporally-selective cells detected by either method suggests a limited presence of time cell-like activity, with the exception of a few cells with activity locked to the start of trials. However, time cell-related activity may not necessarily be synchronized to the start of trials, and may instead by distributed across the entire experimental session, expressing time relative to any number of unaccounted-for events (e.g. random noises).

**2. Population activity in LEC related to time**

*Regressing for time using dimension-reduced data*

Given that a progression of population states across time could be seen in the dimension-reduced visualization of LEC data, was it possible to regress for time using the first few principle components from this dimension-reduced data? Regression was carried out using the linear model $y = \beta_1 * PC1 + \beta_2 * PC2$, with $y$ being time, $PC1$ and $PC2$ the first two principal components, and $\beta_1$, $\beta_2$ the fitted coefficients. Regressing the first two principal components from PCA results for individual animals against time lead to significant fits for all areas, but substantially higher explained variance for LEC (70.9% vs. 10.5% vs. 12.9% mean explained variance for LEC, CA3, and MEC respectively; **Extended Data Fig. 4b, c**). The improved fit for all LEC animals is due to one of the top two PCs exhibiting the session-length exponential decay seen in PC1 of the example LEC fit, and suggests a significant impact of time on LEC activity

during unconstrained behavior. For the specific example shown, the poorer fit towards the end of the session for LEC reflects the flattening of the first PC, while the oscillating nature of the fit for CA3 reflects rate remapping due to wall color being picked up by both PCs, and the predicted time for both CA3 and MEC being centered on the mean overall time reflects the minimization of error by the regression.

*Decoding controls for across-day pooling and behavior*

Given that decoding accuracy was determined using cell populations which were pooled across recording sessions, the concern arises that pooling cells across days would reduce the covariance amongst neurons and drive an artificial increase in decoding accuracy for temporal epochs. We first computed decoding accuracy for temporal epoch using groups of simultaneously-recorded cells, and found that all single-day decoding accuracies were significantly above chance (16.9% median decoding accuracy compared to 4.2% chance level, n = 46 single days). We then compared single-day decoding accuracy against a distribution of decoding accuracies formed using subpopulations with the same number of cells, but randomly drawn from the overall population pooled across days. This was done by calculating a z-score for the single-day decoding accuracy using the mean and standard deviation of the randomly-drawn distribution. Red line indicates 95$^{th}$ percentile boundary for null hypothesis that single-day decoding accuracy was not significantly lower than across-day decoding accuracy. Only 2 out of 46 single-day decoding accuracies were significantly lower than randomly-drawn decoding accuracies (mean z-score -0.39, **Extended Data Fig. 4d**). Comparison of randomly drawn data to data taken from consecutive pairs of days (middle, n = 72 days), or data taken from pairs of days which were separated by half of the overall number of recording days (right, n = 44 days), gave similar results (-0.24, -0.19 mean z-score for consecutive day-pairs and across-experiment day-pairs respectively, **Extended Data Fig. 4e**).

If decoding of temporal epoch were in part due to changes in animals' behavior across time, it would be possible to decode temporal epoch with accuracy greater than chance using just behavior tracking data. We trained separate decoders using behavior tracking data and compared against decoders trained using neural activity. Both tracking data and neural activity were binned into 500 ms bins. Decoding accuracy using just behavior tracking data did not exceed chance,

indicating that the high decoding accuracy for temporal epoch observed using LEC population activity was not due to behavioral changes across experimental sessions (**Extended Data Fig. 4f**).

*Decoding errors for CA3 and MEC reflect non-temporal coding*

In addition to lower decoding accuracy for temporal epoch, both CA3 and MEC also exhibited significantly more erroneous predictions for trials with the same wall color than trials with different colors (14.9% vs. 36.1% vs. 23.9% mean percentage of same-colored errors for LEC, CA3, and MEC respectively; unpaired t-test, LEC vs. MEC: $P < 0.05$, LEC vs. CA3: $P < 0.01$; **Fig. 3c**), consistent with a role for CA3 and MEC in representing aspects of the environment that are constant over time.

*Temporal information in CA3 and MEC is limited*

Although decoding accuracy for temporal epoch was significantly higher for LEC than CA3 or MEC, decoding accuracies for CA3 and MEC were nonetheless greater than chance. Was this simply due to a more widely distributed temporal code in each of these structures, such that with a sufficient number of observed cells, both MEC and the CA areas would reach similar decoding accuracies as LEC? We examined this possibility in the BW12 experiment by quantifying decoding accuracy for temporal epochs as a function of population size, and found that CA3 and MEC began to plateau before reaching accuracies close to what LEC achieved (**Fig. 3d**). Extending population sizes by pooling cells across animals gave similar results (**Extended Data Fig. 4i**). Thus, although the decoding accuracies for time in CA3 and MEC were above chance, temporal information was greater in LEC, requiring significantly fewer cells to reach high decoding accuracy.

*Additional evidence for temporal coding in LEC using BW4 data*

Existing BW4 data offered us a chance to compare temporal coding properties across CA2 and CA1, which have been shown to exhibit more significant place cell decorrelation than CA3[22,24]. Decoding accuracies for both trial identity and wall color using matched BW12 data restricted to the first four trials were comparable, suggesting that there were no conspicuous differences between the two data sets besides the reduced number of trials (trial identity: 88.0% vs. 83.1%

mean decoding accuracy for BW4 vs. BW12; wall color: 91.2% vs. 86.8% for BW4 vs. BW12; **Extended Data Fig. 4g**).

Subsequently, we compared decoding accuracy across all areas at several epoch lengths. Decoding accuracy for trial-length epochs using LEC data was significantly better than all hippocampal areas (mean accuracy for LEC: 75.1%; MEC: 39.8%: CA3: 44.4%; CA2: 51.6%; CA1: 51.1%; chance level 25%; **Extended Data Fig. 4h**). Decoding accuracy for 20s epochs using LEC data was significantly better than all hippocampal areas (mean accuracy for LEC: 16.4%; MEC: 12.2%: CA3: 8.5%; CA2: 8.3%; CA1: 9.2%; chance level 0.4%; **Extended Data Fig. 6a**). Decoding accuracy was higher for LEC and MEC than CA3 for 10s epochs (mean accuracy for LEC: 12.4%; MEC: 11.4%: CA3: 6.5%; CA2: 7.4%; CA1: 8.1%; chance level 0.2%; **Extended Data Fig. 6a**). Decoding accuracy was similar across all areas for 1s epochs (mean accuracy for LEC: 0.54%; MEC: 0.99%: CA3: 0.76%; CA2: 0.65%; CA1: 0.82%; chance level 0.02%; **Extended Data Fig. 6a**). The parity between areas at shorter epoch lengths is potentially due to similar dynamics across all areas at these short timescales but may also reflect the fact that time cells in MEC and hippocampus are capable of coding for time at these short timescales.

*Reduced temporal precision in LEC at shortest timescales*

The differences in decoding accuracy across epoch-lengths suggest a basic limitation in temporal precision within LEC (**Extended Data Fig. 6c**). We examined whether temporal information was in fact reduced at shorter timescales compared to longer, trial-length timescales. First, we re-binned confusion matrices for shorter-epoch decoders to match the size of confusion matrices for trial/intertrial-length epoch decoders (e.g. for 10s decoders, prediction counts within 25×25 bin areas and 14×14 bin areas were summed to match trial- and intertrial-epochs of the trial/intertrial-length epoch decoder, so that the 39×12 classes of the 10s decoder were converted into 24 classes, matching the trial/intertrial-length epoch decoder). We then calculated the decoding accuracy for this re-binned confusion matrix from its diagonal, and found that decoding accuracy was in fact decreased when epoch length was shortened, suggesting that decoders using shorter temporal epochs were comparatively worse than decoders using longer temporal epochs (mean accuracy for trial: 88.6%; 20s: 76.6%: 10s: 56.5%; 1s: 24.9%; chance level 4%; **Extended**

**Data Fig. 6c**). Even though decoding accuracy for shorter temporal epochs was worse, decoding errors may nonetheless have been close to the correct epoch. In order to examine whether errors were close to the correct epoch, we shuffled the confusion matrix entries for each row, but kept the diagonal original. We then applied the same re-binning procedure and compared decoding accuracies to those found using unshuffled data. If errors were close to the correct epoch, decoding accuracies should decrease for shuffled data compared to the decoding accuracies found using unshuffled data, which is in fact what we observed (**Extended Data Fig. 6c**).

*Distributed population coding of environmental context in LEC*

In addition to temporal information, it was clear from 2D visualizations of LEC population activity during the BW12 experiment that environmental context was also encoded within LEC population states (**Fig. 2**). The amount of information present about the current environmental context (B or W) could be measured using a linear binary classifier. Decoding accuracy for wall color was very high for LEC population activity (91.9% mean accuracy). Similar decoding accuracies were observed for CA3, suggesting that environmental context is represented robustly at the population level in both regions (78.6% vs. 83.1% mean accuracies for LEC and CA3, matched population sizes; **Extended Data Fig. 4j**).

High decoding accuracy for wall color was not simply an artifact of the separation of population states across temporal epochs, as decoding accuracy using data which was shuffled temporally while maintaining the original wall color condition was similarly high, suggesting that wall color defines two regions in population state space, within which different temporal epochs are encoded (91.8 vs. 91.9% mean accuracy for original and temporally shuffled data, **Extended Data Fig. 4k**). Information about wall color in LEC was also distributed across the population, as wall color could be decoded with high accuracy (77.9% vs. 91.4%, mean accuracy for wall color using population with no wall color-selective cells, compared to a size-matched randomly-drawn population; **Extended Data Fig. 4l**).

Information about the immediately preceding context was also present in LEC, as decoders trained on intertrial period activity could infer the preceding wall color with high accuracy when the data were labeled by the wall color of the previous trial (74.1% mean accuracy), in agreement

with previous work[30,47]. Thus, while temporal information was encoded through a continuously-changing population state, other streams of information such as environmental context and recent experience had a significant impact on this changing population state, constraining it to a limited range for any given period of time.

**3. LEC population dynamics**

High decoding accuracy for temporal epochs could be caused by purely random fluctuations in LEC activity, raising the possibility that temporal information emerges from a stationary noise-related process (e.g. white noise) that may not have any actual connection to temporal coding. The presence of a fair number of ramping cells argues against this possibility. Nonetheless, we further examined the structure of neural trajectories by measuring the distance between population states across trial and intertrial periods to population states at specific points in time.

First, we measured the distance between population states at all time bins within a trial period or intertrial period to the middle time bin of the same trial or intertrial period (**Extended Data Fig. 5a**), the last time bin of the preceding intertrial or trial period (**Extended Data Fig. 5b**), or the first time bin of the same trial or intertrial period (**Extended Data Fig. 5c**). If LEC activity was generating temporal information purely through stationary noise-related processes, one would expect no difference in population dynamics at the start versus the end of a trial. If LEC activity was instead representing time as it related to experience, one might expect differential dynamics at prominent temporal landmarks such as the start and the end of a trial or intertrial periods, given the significant change in external environment at the start of a trial. Across all three measures, changes in distance were indeed larger at the start of trials than the end, suggesting that the temporal information did not arise purely from trivial noise sources.

For LEC and MEC, the distance between the population state for the first time bin and all subsequent time bins in the same trial or intertrial period could be fit by an autoregressive model (**Extended Data Fig. 5c**). A fifth-order autoregressive model was used, with the order determined by looking at the autocorrelations of the distance measures for all three areas. The LEC model incorporated significant terms for lags of 1, 4, and 5, suggesting that activity at time points of 1, 4, and 5 time bins prior to the current time bin influence current time. The MEC

model incorporated a significant term for lag 1. Applying the autoregressive model to CA3 data did not lead to any significant terms. This influence of preceding activity may be due to decay of externally-driven activity that takes longer than the time bin length, with more lag terms implying that longer periods of preceding time are influencing current activity.

We also measured pairwise distances between consecutive population states, both directly (**Extended Data Fig. 5d**) and in terms of the angle formed between vectors defined by the consecutive population states (**Extended Data Fig. 5e**). For distance measured directly using the population states themselves, we found significantly longer distances at the start of epochs, which may reflect significantly more variability in activity when the animal is first placed in the recording box, as we observed a number of cells with higher firing rates for the first 5-10 seconds of each trial compared to the rest of the trial (data not shown). The short period of initial variability was followed by settling into a stable state, as indicated by the roughly uniform pairwise distances across the rest of the trial or intertrial periods.

For measuring pairwise angles across consecutive points in time, population state vectors were defined as the difference between consecutive population states, and the angle between them was calculated (**Extended Data Fig. 5e**). If trajectories evolved according to a stationary process (e.g. white noise), one would expect the distribution of angles across an epoch to be uniform. For LEC, we observed clustering around angles which were greater than 90 degrees although not quite reaching 180 degrees, indicating that LEC activity did not evolve as a stationary process. Both CA3 and MEC exhibited a wider range of angles, including instances which were less than 90 degrees (corresponding to a reversal in direction), suggesting that they were less structured.

Finally, we measured the distance between the overall mean population state for the first trial or intertrial period and the overall mean population states of all subsequent trial or intertrial periods for LEC (**Extended Data Fig. 5f**). A non-stationary progression was observed across trials. As a whole, these results indicate that population activity in LEC evolved in a non-stationary manner, as expected for a system expressing the evolution of time.

**4. Temporal coding within a fixed environmental context**

A robust temporal code for episodic experience should function across a range of external conditions. To what extent do the results observed during the BW12 experiment generalize to other contexts? We looked at LEC activity in a separate experiment where the environment was kept constant, and an object was present on a subset of trials (**Extended Data Fig. 7a**). First, we examined the activity of single cells using the same GLM method that we used for the BW12 experiment, incorporating the presence/absence of the object, trial time, and session time as variables. More than half of the cells we recorded were found to be selective for at least one of the variables used in the model (**Extended Data Fig. 7b-e**), with 31.6% of cells exhibiting selectivity for time and 8.0% exhibiting selectivity for the presence of the object (number of cells significantly influenced by at least one variable: 150/263, 57.0%; **Extended Data Fig. 7d**). For cells exhibiting mixed selectivity, 34/46 were primarily selective for time.

Next, we visualized LEC population activity and observed once again a separation of the three trials across the session, suggestive of temporal coding at the population level (**Extended Data Fig. 7f**). Decoders trained to determine trial identity using LEC population activity achieved near-perfect accuracy (98.7% mean accuracy, chance level 33.3%; **Extended Data Fig. 7g**). Object presence could also be decoded with near-perfect accuracy (98.4% mean accuracy, chance level 50%; **Extended Data Fig. 7g**). In addition to decoding trial-length epochs, it was also possible to decode shorter epoch-lengths within trials with accuracies well-above chance (14.7% mean accuracy for 20 s epochs, chance level 1.1%; 11.1% mean accuracy for 10 s epochs, chance level 0.6%; 1.1% mean accuracy for 1 s epochs, chance level 0.05%; **Extended Data Fig. 7h**).

Finally, we examined whether temporal information was distributed across the entire population, as observed in the BW12 experiment. We trained decoders for trial identity using only cells which were not characterized by the GLM to be selective for time. Decoding accuracy was reduced compared to decoding accuracy for size-matched populations which were randomly drawn from the full population, but still well above chance (84.2% vs. 93.8% for data with time-selective cells removed vs. randomly drawn control; **Extended Data Fig. 7i**). Similarly, decoding accuracy for object presence using cells not selective for object presence was reduced but still well above chance (81.5% vs. 96.9% for data with object-selective cells removed vs.

randomly drawn control; **Extended Data Fig. 7i**). Consequently, the encoding of information within LEC was again distributed across the general population. Importantly, the GLM and decoding results together suggest that under a different set of external conditions than the BW12 experiment, time nonetheless significantly influences LEC activity and both the population and single-cell level.

**5. Additional evidence that temporal information arises inherently in LEC**

Within the BW12 experiment, repetition of experience was potentially present in the pairs of trials for which wall color remained constant. We examined whether the population response to trials with repeated wall color differed from trials with changing wall color by measuring the Euclidean distance between mean LEC population states for pairs of adjacent trials with different or same wall color. Mean population states were calculated by taking the population activity vector (dimensionality equal to number of recorded cells pooled from all sessions) for each 10s bin, and averaging across all 25 of these vectors for each trial (trials 250s long). Distances were z-scored to allow comparison between animals. Population states for pairs of consecutive trials with the same wall color in the BW12 experiment were in fact more similar than population states for pairs of trials in which wall color changed, consistent with temporal information arising inherently in LEC through changing experience (**Extended Data Fig. 9a**).

Along this line, we also measured the distance between the population state for the first time bin and all subsequent time bins in each trial (**Extended Data Fig. 10a**). We observed fluctuations in distance which may have reflected significant changes in activity corresponding to when the animal was encountering features within the task (e.g., time bin 5 corresponded to when animal was at base of central stem, time bin 10 corresponded to when animal was turning onto the goal arm). The consistency of these fluctuations was again consistent with a change in dynamics compared to BW12 data: whereas activity evolved in a roughly uniform manner during free foraging in the BW12 experiment tracking the general flow of time, during performance of a stereotyped, learned behavior in the figure-eight task, activity instead became aligned to time within individual trials.

Finally, we examined the variability of temporal representation in LEC in a second experiment in which animals were trained to run back and forth on a circular track, alternating between clockwise and counter-clockwise laps for reward at a turnaround point (**Extended Data Fig. 9c**). As with the figure-eight experiment, animal behavior was much more structured than during free foraging in an open field, and was likely goal-oriented towards the reward location. Trials were aligned temporally by the time-point at which the animal reached the middle of the track halfway to the turnaround point, and trials consisted of activity spanning from 3s before to 3s after reaching the midpoint. Looking at 2D projections of LEC population states for each trial, there was a clear decrease in the separation between trials when compared to matched BW data (**Extended Data Fig. 9d**). Consistent with this, although decoding accuracy for trial identity across the entire session was above chance, it was far lower than for matched BW data (17.6% vs. 30.5% mean accuracy, chance level 6.6%; **Extended Data Fig. 9e**). In conjunction with the figure-eight experiment, these results suggest that constraining behavior to be more stereotyped reduced the amount of separation between population states encoding different epochs of time.

**6. Interpretation of discrepancies in past studies of LEC responses**

Overall, our results provide an explanation for discrepancies in previous recordings from LEC. During free exploration, LEC cells often display inconsistent responses to objects and other local cues[26,27,57,58]. In contrast, reliable selectivity for task-related features including objects has been observed during experiments where animals performed a structured behavioral task[29,44]. Since the representation of physical stimuli encountered during free foraging (e.g. objects or walls) is mixed with a representation of time, inconsistent responses would be expected across repeated exposures to these stimuli. During structured behavior, the influence of time decreases as mixed selectivity is reduced, leading to more consistent representations of task-related features. The mixing of time with other variables may be present in other brain areas as well, given that time is not important solely for episodic memory.


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