## TITLE

# Medial entorhinal cortex selectively supports temporal coding by hippocampal neurons


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**SUMMARY**

Recent studies have shown that hippocampal “time cells” code for sequential moments in temporally organized experiences. However, it is currently unknown whether these temporal firing patterns critically rely on upstream cortical input. Here we employ an optogenetic approach to explore the effect of large-scale inactivation of the medial entorhinal cortex on temporal as well as spatial and object coding by hippocampal CA1 neurons. Medial entorhinal inactivation produced a specific deficit in temporal coding in CA1, and resulted in significant impairment in memory across a temporal delay. In striking contrast, spatial and object coding remained intact. Further, we extended the scope of hippocampal phase precession to include object information relevant to memory and behavior. Overall, our work demonstrates that medial entorhinal activity plays an especially important role for CA1 in temporal coding and memory across time.

## INTRODUCTION

The hippocampus is crucial for episodic memory (Zola-Morgan et al., 1986, Vargha-Khadem et al., 1997), which is characterized by the temporal as well as the spatial organization of experience (Tulving, 1984, Eichenbaum, 2013). Correspondingly, recent studies have shown that the firing of hippocampal principal neurons, known as "time cells", fire at sequential moments in temporally structured experiences (Pastolkava et al., 2008, MacDonald et al., 2011, Kraus et al., 2013, Wang et al., 2015), adding temporal coding properties to the coding of specific events (Wood et al., 1999, Igarashi et al., 2014, Quiroga et al. 2005) and locations in space (O'Keefe and Dostrovsky, 1971, Ekstrom et al., 2003) by hippocampal neurons. Thus, hippocampal networks may organize events that compose an experience within a sequential framework of cell assemblies in support of episodic memory (Eichenbaum and Cohen, 2014, Dragoi and Buzsaki, 2006, Gelbard-Sagiv et al., 2008, Foster and Wilson, 2007, Feng et al., 2015, Silva et al., 2015, Girardeau et al., 2009, Pfeiffer and Foster, 2013). Characterizing how external inputs guide internal dynamics in the hippocampus to enable the spatial and temporal organization of memories is crucial to understanding hippocampal function.

The entorhinal cortex provides the major cortical input to the hippocampus and can be divided into medial (MEC) and lateral (LEC) subdivisions, which are thought to govern largely parallel processing pathways for spatial and object information respectively (Eichenbaum et al., 2012, Hafting et al., 2005, Jacobs et al., 2013, Sargolini et al., 2006, Kropff et al., 2015, Deshmukh and Knierim, 2011, Neunuebel et al., 2013,). Previous work has shown that MEC activity is also crucial for successful memory across a delay (Sauvage et al., 2010, Suh et al., 2011, Kitamura et al., 2014), and reported temporal firing fields in MEC grid cells (Kraus et al., 2015). MEC lesions disrupt the precise spike timing of CA1 neurons relative to the phase of the

theta rhythm (Schlesiger et al., 2015), suggesting that MEC may also support the temporal organization of hippocampal time cell sequences. Here we examined the effects of transient, large-scale medial entorhinal inactivation on memory, and on temporal, spatial, and object coding by CA1 neurons. In animals performing a behavioral task which required them to remember an object over a delay period, we find that MEC is essential for CA1 temporal coding and memory across time, but observe that spatial and object coding remains unaffected by the disruption. We also report novel theta rhythmic dynamics among highly object-selective CA1 populations. These findings expand our understanding of hippocampal-entorhinal functional interactions, and the integration of event information within hippocampal network operations.

## RESULTS

### MEC is critical for memory across a delay

To transiently disrupt MEC activity, an adeno-associated viral vector engineered to express the GFP-conjugated, red-shifted inhibitory opsin Jaws (Chuong et al, 2014) was bilaterally targeted to MEC in 9 Long Evans rats (7 Jaws animals, 2 GFP-only control animals), under the control of the synapsin (hSyn) promoter for pan-neuronal expression (Figure 1A). Anesthetized acute recordings demonstrated effective silencing of MEC neurons under this protocol (Figure S1). For behavioral experiments, we implanted bilateral optic fiber arrays that delivered light spanning the dorsoventral axis of the MEC, while simultaneously recording from the right dorsal CA1 region of the hippocampus utilizing a tetrode hyperdrive (Figure 1B). We characterized the effects of MEC inactivation on temporal, spatial, and object coding by hippocampal neurons in animals performing an object-delay-response association task (Figure 1C). On each trial the animal approached and sampled one of two objects for 3 sec (Object phase). Next, the object was removed, and the animal entered a treadmill and ran in place for 7 sec (Treadmill phase). Upon exiting the treadmill, the animal then either dug in a nearby ceramic pot or travelled to an alternative location to receive a reward, depending upon the object previously sampled. Following the response, animals then traversed the return arm of the maze (Figure 1C) to begin the next trial (Maze phase).

We first examined the effects of MEC inactivation during the Treadmill phase. Behavioral performance was high during Baseline trials (Fraction correct, 0.77 ± 0.026; mean ± s.e.m., 17 treadmill inactivation sessions from 7 Jaws animals, 0.81 ± 0.01, 8 sessions from 2 GFP animals, Figure 1D). Following the Baseline trial block, the laser was triggered during the Treadmill phase (Light-on Trials) perturbing MEC activity during the 2-4 sec window, which

resulted in a large behavioral impairment compared to Baseline in Jaws animals (Kruskal-Wallis (KW) test, $\chi^2$=15.78, p<0.0005, mean performance 0.62 ± 0.024, Tukey's *post hoc* test p<0.005, GFP controls remained stable, $\chi^2$=0.17, KW test p=0.92). Behavioral performance during pseudorandomly intermixed Light-off trials was also quantitatively reduced from Baseline (mean performance 0.68±0.02), although this effect did not reach significance (Tukey's *post hoc* test, p=0.08). However, analysis of the latency between the end of the treadmill delay and the time of the animal's decision revealed a pronounced increase in the variance of decision latency for both Light-on and Light-off trials, particularly during errors (Figure S1). Behavioral sessions in Jaws animals after implantation but without light delivery showed no deterioration in performance across the duration of the session (10 sessions, the first third of trials assigned as "Baseline" and the remaining pseudorandomly assigned into two additional groups, KW test, $\chi^2$=0.47, p=0.79), demonstrating that Jaws expression alone was insufficient to elicit the performance deficit. In sum, these data indicate that MEC activity is critical for remembering an object or response across a delay, while spatial location is fixed.

**MEC inactivation destabilizes CA1 firing sequences**

We next determined how MEC inactivation during the Treadmill period affected the temporal firing patterns of hippocampal neurons. 772 CA1 neurons were isolated in Jaws animals during Treadmill phase inactivation sessions, of which 206 (26.7%) were putative pyramidal cells that exhibited temporal coding during the delay in Baseline trials. This proportion was not significantly different than that observed in GFP-only controls (141 of 464 (30.4%) CA1 cells, Pearson's $\chi^2$ test of independence, $\chi^2$=1.96, p= 0.16). As reported previously, the firing patterns of CA1 "time cells" compose a sequence of temporally defined firing fields

that span the length of the mnemonic delay (Pastolkava et al., 2008, MacDonald et al., 2011, Kraus et al., 2013, Wang et al., 2015, Figure 2A). Transient inactivation of MEC during a window spanning 2-4 sec destabilized the sequences of time cells during both Light-on and Light-off trials. To quantify this effect, we used a template matching population vector decoder to examine how well we could predict the time of the animal in the delay from the spiking activity in each decoding window (see methods). Using the Baseline temporal tuning curves as a template, we analyzed mean spiking activity in non-overlapping 100ms windows across the delay during Baseline, Light-on, and Light-off trials to compute reconstructed temporal trajectories and their error compared to real time. Reconstruction error from Baseline spikes was low, demonstrating that the population activity at Baseline forms a sequence of unique activity profiles sufficient to discriminate each time point in the delay (Figures 2B and 2C). However, reconstruction using Light-on and Light-off spikes resulted in highly erratic trajectories (reconstruction error, 70 bins, signed rank test vs Baseline, Light-on: $Z$=-6.90, p<7.3e-12, Light-off: $Z$=-6.74, p<1.5e-12, Figure 2C), whose mean errors were not significantly different than expected using a temporally uninformative template (p-value from 10,000 shuffled templates, Baseline: p<0.0001, Light-on: p=0.46, Light-off: p=0.18, Figure 2D). Thus, the Baseline template was no longer predictive of spiking activity during Light-on and Light-off trials. This level of temporal reconstruction error compared to GFP controls was not present when the laser was triggered during the Maze or Object phases (Δ error from Baseline compared across inactivation paradigms, KW test, Light-on: $\chi^2$=25.42, p<1.2e-05, Light-off: $\chi^2$=20.54, p<0.0005, Figures 2E and S3). Only Jaws Treadmill inactivation sessions showed greater change in error than expected by chance (shuffle test with randomized trial type identities, p<0.0001 for Light-on and Light-off, Figure 2E), indicating the CA1 temporal firing field sequence during the delay

was selectively perturbed in Jaws animals when the MEC inactivation was triggered during the memory delay, corroborating the specificity of our behavioral result. We saw no change from Baseline in LFP theta power (17 sessions, signed rank test, Light-on: $Z$=0.40, p=0.69, Light-off: $Z$=0.21, p=0.83) or frequency (17 sessions, signed rank test, Light-on: $Z$=0.07, p=0.94, Light-off: $Z$=0.02, p=0.98) during the inactivation window (Figures 2F, 2G and S2). We note though that due tetrode placement primarily in the pyramidal layer, there may be unobserved oscillatory effects in stratum laconosum moleculare at the site of the direct entorhinal synaptic input.

**Time-locked impairment of CA1 temporal coding**

We observed a diverse set of changes in the temporal firing patterns of individual hippocampal neurons during MEC inactivation (Figure 3A). Cells with Baseline fields in the 2-4 sec inactivation window exhibited degradation of temporal coding and stability (36 cells, 2-4 sec temporal information, signed rank test, Baseline vs. Light-on: $Z$=2.45, p<0.02, Baseline vs Light-off: $Z$=2.51, p<0.02; field stability, Baseline vs. Light-on: $Z$=2.48, p<0.02, Baseline vs Light-off: $Z$=2.09, p<0.04, Figure 3B). Although cells with peak activity before the inactivation (133 cells, 0-2 sec group) appeared to largely maintain their field sequence (Figure 2A), we also detected decreases in temporal information (signed rank test, $Z$=3.57, p<3.6e-4, Figure 3B) and field stability (signed rank test, $Z$=2.32, p<0.02 Figure 3B) from Baseline during Light-on trials among this population, suggesting an increase in out-of-field spiking later in the delay. We did not find a consistent difference in these metrics for fields after the inactivation window (37 cells, signed rank test, 4-7 sec group, Figure 3B), nor did the distribution of mean firing rates for any group differ between trial types (Kolmogorov-Smirnov (KS) test, Figure 3B).

Analysis of time-resolved firing rate variance across trials however revealed a marked increase in variance from Baseline that was time-locked to the onset of the laser, remained elevated after the laser had turned off, and persisted in subsequent Light-off trials (206 cells, bootstrap test, $p<0.05$; Figure 3C). Only Jaws Treadmill inactivation sessions exhibited a variance elevation from Baseline significantly different from chance level (10,000 shuffles of trial-type identity, Light-on: $p<0.01$, Light-off: $p<0.01$) or GFP-only control sessions ($\Delta$ firing rate variance from Baseline compared across inactivation paradigms, KW test, Light-on: $\chi^2=69.7$, $p<4.9e-15$, Light-off: $\chi^2=73.1$, $p<9.5e-16$; Figure 3D). We observed a similar effect of elevated firing rate variance among putative interneurons specific to Jaws Treadmill inactivation sessions (Figure S2), though these increases were less strongly coupled to the timing of the laser. While many effects of the inactivation persisted in subsequent Light-off trials (Figures 2, 3, and S2), we found that the Baseline level of temporal information and proportion of temporally-tuned cells remained stable across sessions (Figure S4), indicating that the network recovered to a comparable level by the time of the next recording. Additionally, all measures of temporal coding remained stable in GFP group Treadmill inactivation sessions and in Jaws sessions where the laser was triggered during the Object or Maze phases (Figure S3), and temporal coding deficits in Jaws Treadmill inactivation sessions were not exclusively driven by reductions in mean firing rate (Figure S5) . These data suggest that the aberrant temporal activity was specific to MEC inactivation during the delay period, resulting in a destabilization of CA1 temporal firing fields in the inactivation window and a decreased signal-to-noise ratio among cells with fields outside this window.

**Reduction in population temporal decorrelation and specificity**

Correspondingly, the ensemble code no longer provided a reliable sequential progression of activity patterns across time. To quantify this effect, we examined the population vector cross correlation matrix using the temporal firing rate curves for each trial type (Figure 4A, see methods). To capture the rate of population activity change in each session, we computed the mean correlation value between population vectors spaced apart across a range of lags, and determined the lag at which the resulting temporal decorrelation curve falls below 0.5. This interval expands considerably during Light-on (17 sessions, signed rank test, $Z$=-2.91, $p<0.005$, Figure 4B) and Light-off (signed rank test, $Z$=-2.58, $p<0.01$, Figure 4B) trials, indicating that the specificity of the temporal code is diminished and the population activity is no longer sequentially progressing across the delay. In line with our reconstruction results, significant expansion compared to GFP Treadmill controls was only observed in Jaws Treadmill inactivation sessions ($\Delta$ interval value from Baseline compared across inactivation paradigms, KW test, Light-on: $\chi^2$=8.94, $p<0.05$, Light-off: $\chi^2$=9.16, $p<0.05$, Figure 4C), although Jaws Object inactivation sessions exhibited some expansion that was greater than expected by chance (10,000 shuffles of trial-type identities, Light-on: $p<0.05$, Light-off: $p<0.01$, Figure 4C), which may be due to the relative temporal proximity of the object sampling and treadmill delay task epochs during each trial. Combined, these findings demonstrate that MEC activity is necessary for orchestrating stable sequences of temporal activity in CA1 and for memory across time.

**CA1 place code is unaffected by MEC inactivation**

During separate sessions, we triggered the laser at a fixed spatial location during the Maze phase. During Maze inactivation sessions, performance of Jaws animals remained stable through Baseline (13 sessions, Fraction correct, 0.83 ± 0.013), Light-on (0.82±0.24), and Light-

off (0.82±0.024) trial blocks (KW test, $\chi^2$=0.12, p=0.94, GFP-only controls: 8 sessions, $\chi^2$=0.1, p=0.95, Figure 1D). As described earlier, temporally organized activity during the memory delay remained intact during maze inactivation sessions (Figures 2, 3, 4, and S3), corroborating the specificity of the behavioral and electrophysiological results from Treadmill phase inactivation. We further investigated whether MEC inactivation perturbed the CA1 place code along the return arm of the maze. We isolated and linearized this segment of the maze and identified cells with spatial firing fields during Baseline trials (Figure 5A). 551 CA1 cells were recorded from 13 Jaws maze return arm inactivation sessions, of which 144 (26.13%) were putative pyramidal neurons that passed our criteria for spatially tuned firing at Baseline (see methods). Similar numbers were obtained from GFP control sessions (553 cells recorded from 8 sessions, of which 159 (28.75%) passed criteria, Pearson's $\chi^2$ of independence, $\chi^2$=0.95, p=0.33). The sequence of place cells identified at Baseline remained highly stable across Light-on and Light-off trials (Figure 5A). Though spatial reconstruction error marginally increased from Baseline (75 bins, signed rank test, Baseline vs Light-on: p<1.73e-7, Baseline vs Light off: p<8.47e-8, Figure 5B), the mean errors from reconstructed trajectories for all trial types were significantly less than expected from decoding using spatially uninformative templates (10,000 shuffled templates, Figure 5C). Additionally, the difference in error from Baseline to Light-on/off trials was comparable to that observed in sessions when the laser was triggered during the Treadmill or Object phases, and GFP Maze phase control sessions (Δ spatial error from Baseline compared across inactivation paradigms, KW test, Light-on: $\chi^2$=24.91, p=0.16, Light-off: $\chi^2$=8.09, p<0.05, though no groups significantly different from GFP, Figures 5D and S6), and no inactivation paradigm produced error increases greater than expected by chance (10,000 shuffles of trial-type identity, Figure 5D).

Single unit measures of spatial tuning (144 cells, signed rank test, Figures 6A and 6B) and mean firing rate (144 cells, KS test, Figures 6A and 6B) also remained stable during Jaws Maze inactivation sessions and during all control session types (Figure S6). Similar to the spatial decoding result, we observed a minor increase in the spatial correlation decay interval (13 sessions, signed rank test, Baseline vs Light-on: $p<0.01$, Baseline vs Light off: $p<0.004$, Figures 6C and 6D), but the magnitude of this change from Baseline levels was not significantly different from GFP Maze control sessions, or Jaws Treadmill or Object phase inactivation sessions (KW test, Light-on: $\chi^2=0.37$, $p=0.95$, Light-off: $\chi^2=0.66$, $p=0.88$, Figure 6E) and no greater than expected by chance (10,000 shuffles of trial-type identity, Figure 6E). In brief, transient inactivation of MEC is insufficient to perturb the CA1 place code during this complex behavioral task in a rich and familiar environment.

**MEC disruption does not perturb CA1 object selectivity**

Separate sessions were also recorded in which the laser was triggered during the Object phase to assess the importance of MEC activity for hippocampal object selectivity. During Jaws object inactivation sessions, performance remained stable throughout all trial blocks (15 sessions, Fraction correct, Baseline: 0.78 ± 0.015, Light-on: 0.82 ± 0.022, Light-off: 0.81 ± 0.023, KW test, $\chi^2 = 1.43$, $p=0.49$; GFP-only controls remain stable: 6 sessions, $\chi^2 = 0.83$, $p=0.66$, Figure 1D). As described previously, temporal firing patterns on the Treadmill, as well as spatial firing patterns on the Maze, were not significantly impacted throughout sessions where the laser was triggered during the Object phase (Figures 2, 3, 4, 5, S3 and S6). To assess the integrity of CA1 object-coding activity during MEC inactivation, we identified neurons with mean firing rates > 2 Hz in the 3 second object sampling window during Baseline trials. We

isolated 496 CA1 neurons during 15 Jaws Object phase inactivation sessions, of which 89 (17.94%) were active during the object sampling period; this proportion was not significantly different than that observed in GFP-only control animals (66 of 449 total recorded neurons (14.70%), Pearson's $\chi^2$ of independence, $\chi^2 = 1.81$, p=0.12).

Within the object sampling period, a substantial fraction of the active cell population exhibited object-selective firing patterns (Figures 7A and 7B). To assess the integrity of CA1 object selectivity before, during, and after MEC inactivation, we computed selectivity indices (SI) for each neuron during Baseline, Light on, and Light off trial blocks and corresponding p-values (5000 shuffles of trial object identity, see methods). 'Selectivity' was defined for each cell as the difference in its firing rate for each object divided by the sum of these rates. We found that the proportion of significantly object-selective cells did not differ across trial types (Fraction of active cells with SI p<0.05, Baseline: 51.69%, Light-on: 50.56%, Light-off: 42.70%, Pearson's $\chi^2$ of independence, $\chi^2 = 1.71$, p=0.43, Figure 7C). Additionally, the SI values for neurons with significant selectivity during Baseline (n = 46) did not change during Light-on (signed rank test, $Z = 0.02$, p=0.99) or Light-off trials (signed rank test, $Z = -0.10$, p=0.92, Figure 7D). Lastly, we compared the absolute change in SI value from Baseline for cells with significant Baseline selectivity and found no significant differences among Jaws inactivation locations or GFP-only controls during Light-on (KW test, $\chi^2 = 5.03$, p=0.17, Figures 7E and S7) or Light-off trials (KW test, $\chi^2 = 6.59$, p=0.09, Figures 7E and S7). Thus, transient MEC inactivation did not produce an appreciable effect on CA1 object coding.

**CA1 object code exhibits theta phase precession**

We also examined spike timing relative to the phase of the local theta rhythm during CA1 object coding. The theta phase precession phenomenon has been well documented during hippocampal spatial (O'Keefe and Recce, 1993) and temporal (Pastalkova et al., 2008) coding, where a neuron's spikes systematically shift to earlier phases of the hippocampal theta oscillation as the animal traverses its receptive field. The resulting temporal compression of spike sequences among overlapping fields within single theta cycles has important mechanistic implications for neural sequence formation during memory and navigation (Jensen and Lisman, 2005, Koene et al. 2003, Feng et al., 2015). Here we report that object-specific firing patterns in CA1 also exhibit theta phase precession (Figures 8A and 8B). This phenomenon was observed in both Jaws animals in all inactivation location session types and GFP control animals, and was unaffected by MEC inactivation (Figure S7). For this reason, we pooled all cells active during the object sampling period among all animals and session types (n = 409 cells) and examined the prevalence of significant phase precession and significant object selectivity. Intriguingly, we found that among cells with significant phase precession during trials for at least one object (n = 110 cells, $p<0.05$ circular-linear correlation and negative spike time-phase slope, see methods), 70.91% exhibited significant object-selective firing rates (SI $p<0.05$), which was significantly greater than the proportion of non-precessing cells demonstrating firing rate object selectivity (55.52% of 299 cells, Pearson's $\chi^2$ of independence, $\chi^2 = 9.27$, $p<0.004$, Figure 8C). These data indicate that neurons engaged in theta phase precession during the object-sampling period preferentially encoded object information, compared to the remaining active population.

For neurons that exhibited phase precession, we further examined the SI values for the subgroups that exhibited significant firing rate object selectivity (SI $p<0.05$) and those that did not (SI $p>=0.05$). Precessing cells with significant firing rate selectivity exhibited a wide

distribution of SI values (Figure 8D), indicating that phase precessing object cells were not solely characterized by exclusive firing for only one object. However, both firing rate selective and non-selective populations largely exhibited significant phase precession for only one object (Pearson's $\chi^2$ of independence, firing rate selective: $\chi^2 = 35.67$, $p<2.3e-9$, non-selective: $\chi^2 = 12.6$, $p<3.9e-4$, Figure 8D), and object-selective phase precession occurs in equal proportion between cell populations with object selective and non-selective firing rates (Pearson's $\chi^2$ of independence, firing rate selective: $\chi^2 = 0.56$, $p=0.45$). Therefore, cells active during the object sampling period are capable of exhibiting object selectivity both through modulation of their firing rate and by the absence or presence of theta phase precession, and these two selectivity mechanisms can occur independently.

## DISCUSSION

Through optogenetic interrogation of the hippocampal-entorhinal network, our work provides further insight into afferent influences on hippocampal information processing. We analyzed hippocampal activity during epochs of experience dominated by three different modalities – temporal, spatial, and object coding – and found that disrupting medial entorhinal activity produces a specific, time-locked deficit in temporal coding during a mnemonic delay, which was associated with a large memory impairment. Previous work has suggested that neural sequences during a mnemonic delay are critical for guiding behavior, potentially through the maintenance of trial information across the delay or by triggering accurate retrieval of relevant information at the subsequent decision point (Harvey et al, 2012, MacDonald et al., 2013). The destabilization of these firing patterns in CA1 and the resulting behavioral impairment during MEC inactivation support this functional link.

Our work complements previous findings demonstrating the critical role of the hippocampus in temporal association memory (Farovik et al. 2010, Kesner et al. 2002, Kesner et al. 2005, McEchron et al. 1998, Wang et al. 2015). The impact that we observe on CA1 temporal coding persists into subsequent trials, which mirrors the behavioral impacts during the task in both Light-on (Figure 1) and Light-off trials (Figure S1). While it is possible that other extra-hippocampal targets of MEC efferents may contribute to the observed behavior deficits, our combined impact on CA1 activity and behavior strongly suggests a functional role for hippocampal temporal coding, which in turn is dependent on reliable MEC input. These data also indicate that MEC plays an important role in orchestrating hippocampal temporal coding in the absence of changing salient environmental information, which may normally guide activity during spatial navigation.

Additionally, our results complement a recent finding that memory and CA1 temporal firing patterns across the extent of a delay are disrupted following pharmacological inactivation of the medial septum (Wang et al., 2015) , a manipulation known to impair MEC grid cell firing fields (Brandon et al., 2011, Koenig et al., 2011). However, septal inactivation also results in large reductions in hippocampal firing rate and theta power, which may interfere with intrinsic hippocampal dynamics (Wang et al. 2015) . We did not observe these changes (Figures 2, 3, and S2), and thus our study highlights the particular importance of extrinsic entorhinal processing. Additionally, our temporally precise optogenetic intervention specifically disrupted temporal coding during the period of laser exposure. This effect persisted across subsequent intermixed Light-off trials which suggests that the intervention induced a destabilizing plasticity, in a manner largely localized to ensemble activity within the inactivation window.

The source and persistence of this effect could arise from temporary decoupling of coordinated input timing between CA3 and EC inputs, which is critical for recruiting the nonlinear input integration favorable for burst spiking, plasticity, and receptive field stability in CA1 (Takahashi and Magee. 2009, Basu et al. 2014, Bittner et al. 2015). Desynchronization of these input pathways during the inactivation could then lead to a temporary disruption of CA1 activity. MEC input may continue to be unreliable during subsequent Light-off trials due to impacts on local MEC microcircuits, resulting in a persistent downstream effect. However, the temporal specificity of the effect we observe in CA1 suggests the predominant effect is due to EC-HPC or intrahippocampal synaptic modifications that were induced at the time of MEC inactivation, potentially at CA1 perforant path synapses or in other hippocampal regions. As hippocampal temporal dynamics evolve on subsequent trials without MEC inactivation and reach the usual onset time of the laser, they may be affected by reduced input efficacy from MEC or

other impacts of plasticity at these synapses from past inactivation trials, resulting in a persistent impairment of receptive fields and the population temporal code. Indeed, previous work has implicated temporally-specific plasticity in shaping network time coding (Goel and Buonomano 2016). Temporal activity at the CA1 node is likely influenced by both direct entorhinal input and intrahippocampal activity from CA3 and CA2, which also receive input from MEC. Due to the extensive entorhinal innervation throughout the hippocampus and the lack of input pathway specificity in our optogenetic design, future work will be required to weigh the precise contributions of each network element to the observed effects at the level of synapses, receptive fields, and network activity states.

While we show that brief interruption of MEC input is sufficient to produce an intermediate persistent effect on CA1 coding that persists for several trials when MEC activity is later intact (Rueckemann et al, 2016), this effect was transient, as the network operated at a similar level of temporal selectivity at Baseline across consecutive sessions. Thus MEC supplies critical input that supports the organization of hippocampal cell assemblies over time or in the absence of changing external cues, and whose transient perturbation is sufficient to produce a marked impairment in temporal tuning and behavior. We hypothesize this input could be provided by MEC grid cell populations (Hasselmo, 2009), which we speculate may adaptively guide hippocampal assembly dynamics across cognitive dimensions beyond space (Kraus et al, 2015).

In agreement with other reports, the capacity of hippocampal neurons to represent space remained intact despite MEC inactivation (Hales et al. 2014, Miao et al., 2015, Rueckemann et al. 2016). Prior experiments have demonstrated cumulative or instantaneous "remapping" of hippocampal place fields during MEC inactivation, though perturbations generally exerted only

modest effects on the quality of spatial tuning (Hales et al. 2014, Miao et al., 2015, Rueckemann et al. 2016, Zhao et al. 2016) and featured comparatively simple behavioral tasks in larger environments. We did not observe any large-scale remapping or reductions in spatial information from transient MEC inactivation, in agreement with reports that the stability of hippocampal spatial maps can become independent of MEC or grid cell activity in familiar environments or those with rich proximal cues (Wang et al., 2015, Hales et al., 2014, Brandon et al. 2014). Further, a recent report employing a pharmacogenetic approach showed that depolarization, but not hyperpolarization, of MEC layer II stellate cells induced remapping in CA1 and impaired spatial memory (Kanter et al., 2017). Hippocampal spatial maps may be critically dependent on MEC input during learning or in less familiar or sensory-impoverished environments, where positional coding may be reliant on internal computations which could require MEC to effectively bridge disparate salient cues. However, we hypothesize that this dependency is transitory, and that with familiarity or rich environmental and cognitive features, the place code can be maintained or retrieved by other afferent or intrahippocampal activity. In line with other accumulating evidence, exclusive control of hippocampal spatial tuning by MEC inputs seems increasingly unlikely (Hales et al., 2014, Rueckemann et al., 2016, Wang et al., 2015, Brandon et al. 2014).

We observed robust object coding among CA1 ensembles, consistent with previous reports of object-selective coding and activity during object-sampling behaviors (Komorowski et al., 2009, Ranck, 1973). The stability of object coding in CA1 during MEC inactivation supports the theory that the medial and lateral subdivisions of the entorhinal cortex process distinct experiential modalities in parallel, where object information is predominantly supplied by LEC input (Igarashi et al. 2014, Eichenbaum et al., 2012, Deshmukh and Knierim, 2011). We

additionally report that these object responses exhibit theta phase precession, similar to canonical "place" cells (O'Keefe and Reece, 1993), "time" cells (Pastalkova et al., 2008), and other event-related discharges (Lenck-Santini et al., 2008), and that the population of phase precessing cells disproportionately encoded task-relevant information compared to the remaining active population. Further, cells active during the object sampling period demonstrated object selectivity both through modulation of firing rate and by the absence or presence of phase precession, and could exhibit these features independently. Future work will be required to explore how these object responses integrate with hippocampal assemblies during theta and sharp-wave ripple sequences. However, we suggest that phase precession and hippocampal sequencing may act as a more general mechanism for indexing event-related information within a sequential framework relevant for encoding, recall, and behavioral planning. Thus, the hippocampus may associate the elements of an episode across space and time by the generation, stabilization, and retrieval of neural sequences.

Overall, our work extends previous findings that have demonstrated temporal coding over a mnemonic delay in MEC grid cells (Kraus et al., 2015), as well as work that has implicated MEC activity in enabling memory over time (Sauvage et al., 2010, Suh et al., 2011, Kitamura et al., 2014). Here we offer direct evidence for a medial entorhinal role in the coherent orchestration of hippocampal temporal firing patterns important for memory. Though these results call into question whether spatial processing is the primary function supported by the MEC, we believe that our findings lay the groundwork for reinterpreting the contributions of the entorhinal cortex to hippocampal network operations.

## AUTHOR CONTRIBUTIONS

Conceptualization, N.T.M.R. and H.B.E.; Methodology, N.T.M.R.; Investigation, N.T.M.R., J.B.P., A.D.G., V.A.S., and F.A.M.; Software, J.B.P., N.T.M.R., and J.W.R.; Formal Analysis, J.B.P. and N.T.M.R.; Writing – Original Draft, N.T.M.R., J.B.P., and H.B.E.; Writing – Review & Editing, N.T.M.R., J.B.P., J.W.R., and H.B.E.; Funding Acquisition, H.B.E.

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**REFERENCES**

Basu, J., Srinivas, K.V., Cheung, S.K., Taniguchi, H., Haung, Z.J., Siegelbaum, S.A. (2014). A cortico-hippocampal learning rule shapes inhibitory microcircuit activity to enhance hippocampal information flow. Neuron *79(6)*, 1208-1221.

Bittner, K.C., Grienberger, C., Vaidya, S.P., Milstein, A.D., Macklin, J.J., Suh, J., Tonegawa, S., Magee, J.C. (2015). Conjunctive input processing drives feature selectivity in hippocampal CA1 neurons. Nature Neuroscience *18*, 1133-1142.

Brandon, M.P., Bogaard, A.R., Libby, C.P., Connerney, M.A., Gupta, K., and Hasselmo, M.E. (2011). Reduction of theta rhythm dissociates grid cell spatial periodicity from directional tuning. Science *332(6029)*, 595-599.

Brandon, M.P., Koenig, J., Leutgeb, J.K., and Leutgeb, S. (2014). New and distinct hippocampal place codes are generated in a new environment during septal inactivation. Neuron *82*, 789–796.

Chuong, A.S., Miri, M.L., Busskamp, V., Matthews, G.A., Acker, L.C., Sorensen, A.T., Young, A., Klapoetke, N.C., Henninger, M.A., Kodandaramaiah, S.B., Ogawa, M., Ramanial, S.B., Bandler, R.C., Allen, B.D., Forest, C.R., Chow, B.Y., Han, X., Lin, Y., Tye, K.M., Roska, B., Cardin, J.A., and Boyden, E.S. (2014). Noninvasive optical inhibition with a red-shifted microbial rhodopsin. Nature Neuroscience *17*, 1123-1129.

Deshmukh, S.S., and Knierim, J.J. (2011). Representation of non-spatial and spatial information in the lateral entorhinal cortex. Front. Behav. Neurosci. *5*, 69.

Dragoi, G., and Buzsáki, G. (2006). Temporal encoding of place sequences by hippocampal cell assemblies. Neuron *50*, 145–157.

Eichenbaum, H. (2013). Memory on time. Trends in Cognitive Science *17(2)*, 81-88.

Eichenbaum, H., and Cohen, N.J. (2014). Can We Reconcile the Declarative Memory and Spatial Navigation Views on Hippocampal Function? Neuron *83*, 764–770.

Eichenbaum, H., Sauvage, M., Fortin, N., Komorowski, R., and Lipton, P. (2012). Towards a functional organization of episodic memory in the medial temporal lobe. Neurosci. Biobehav. Rev. *36*, 1597–1608.

Ekstrom, A.D., Kahana, M.J., Caplan, J.B., Fields, T.A., Isham, E.A., Newman, E.L., and Fried, I. (2003). Cellular networks underlying human spatial navigation. Nature *425*, 184–188.

Farovik, A., Dupont, L.M., and Eichenbaum, H. (2010). Distinct roles for dorsal CA3 and CA1 in memory for sequential nonspatial events. Learning and Memory *17*, 12-17.

Feng, T., Silva, D., and Foster, D.J. (2015). Dissociation between the experience-dependent development of hippocampal theta sequences and single-trial phase precession. The Journal of Neuroscience, *35*, 4890–4902.

Foster, D.J., and Wilson, M.A. (2007). Hippocampal theta sequences. Hippocampus *17*, 1093-1099.

Gelbard-Sagiv, H., Mukamel, R., Harel, M., Malach, R., and Fried, I. (2008). Internally Generated Reactivation of Single Neurons in Human Hippocampus During Free Recall. Science *322*, 96–101.

Girardeau, G., Benchenane, K., Wiener, S.I., Buzsáki, G., and Zugaro, M.B. (2009). Selective suppression of hippocampal ripples impairs spatial memory. Nature Neuroscience *12*, 1222–1223.

Goel, A., and Buonomano, D.V. (2016). Temporal interval learning in cortical cultures is encoded in intrinsic network dynamics. Neuron *91*, 320-327.

Hafting, T., Fyhn, M., Molden, S., Moser, M.-B., and Moser, E.I. (2005). Microstructure of a spatial map in the entorhinal cortex. Nature *436*, 801–806.

Hales, J.B., Schlesiger, M.I., Leutgeb, J.K., Squire, L.R., Leutgeb, S., and Clark, R.E. (2014). Medial entorhinal lesions only partially disrupt hippocampal place cells and hippocampus-dependent place memory. Cell Reports *9*, 893-901.

Harvey, C.D., Cohen, P., and Tank, D. (2012). Choice-specific sequences in parietal cortex during a virtual-navigation decision task. Nature *484*, 62-68.

Hasselmo M.E. (2009). A model of episodic memory: mental time travel along encoded trajectories using grid cells. Neurobiology of Learning and Memory *92*, 559-573.

Igarashi, K.M., Lu, L., Colgin, L.L., Moser, M.-B., and Moser, E.I. (2014). Coordination of entorhinal-hippocampal ensemble activity during associative learning. Nature *510*, 143–147.

Jacobs, J., Weidemann, C.T., Miller, J.F., Solway, A., Burke,J.F., Wie, X., Suthana, N. Sperling, M.R., Sharan, A.D., Fried, I., and Kahana, M.J. (2013). Direct recordings of grid-like neuronal activity in human spatial navigation. Nature Neuroscience *16*, 1188-1190.

Jensen, O., and Lisman, J.E. (2005). Hippocampal sequence-encoding driven by a cortical multi-item working memory buffer. Trends in Neuroscience *28(2)*, 67-72.

Kanter, B.R., Lykken, C.M., Avesar, D., Weible, A., Dickinson, J., Dunn, B., Borgesius, N.Z., Roudi, Y., Kentros, C.G. (2017). A novel mechanism for grid-to-place cell transformation

revealed by transgenic depolarization of medial entorhinal cortex layer II. Neuron *93*, 1480-1492.

Kempter, R., Leibold, C., Buzsaki, G., Diba, K., and Schmidt, R. (2012). Quantifying circular-linear associations: Hippocampal phase precession. Journal of Neuroscience Methods *207*, 113-124.

Kesner, R.P., Gilbert, P.E., Barua, L.A. (2002). The role of the hippocampus in memory for the temporal order of a sequence of odors. Behavioral Neuroscience *116*, 286-290.

Kesner, R.P., Hunsaker, M.R., Gilbert, P.E. (2005). The role of CA1 in the acquisition of an object-trace-odor paired associate task. Behavioral Neuroscience, *119(3)*, 781-786.

Kitamura, T., Pignatelli, M., Suh, J., Kohara, K., Yoshiki, A., Abe, K., and Tonegawa, S. (2014). Island cells control temporal association memory. Science *343*, 896-901.

Koene, R.A., Gorchetchnikov, A., Cannon, R.C., and Hasselmo, M.E. (2003). Modelling goal-directed spatial navigation in the rat based on physiological data from the hippocampal formation. Neural Networks *15(5-6)*, 577-584.

Koenig, J., Linder, A.N., Leutgeb, J.K., and Leutgeb, S. (2011). The spatial periodicity of grid cells is not sustained during reduced theta oscillations. Science *332*, 592-595.

Komorowski, R.W., Manns, J.R., and Eichenbaum, H.B. (2009). Robust conjunctive item-place coding by hippocampal neurons parallels learning what happens where. The Journal of Neuroscience *29(31)* 9918-9929.

Kraus, B.J., Robinson, R.J.2nd, White, J. A., Eichenbaum, H., and Hasselmo, M.E. (2013). Hippocampal 'time cells': time versus path integration. Neuron *78,* 1090–1101.

Kraus, B.J., Brandon, M.P., Robinson, R.J.2nd, Connerney, M.A., Hasselmo, M.E., and Eichenbaum, H. (2015). During running in place, grid cells integrate elapsed time and distance run. Neuron *88,* 578-589.

Kropff, E., Carmichael, J.E., Moser, M.-B., and Moser, E.I. (2015). Speed cells in the medial entorhinal cortex. Nature *523*, 419–424.

Lenck-Santini, P., Fenton, A.A., Muller, R.U. (2008). Discharge properties of hippocampal neurons during performance of a jump avoidance task. The Journal of Neuroscience *28(27),* 6773-6786.

MacDonald, C.J., Lepage, K.Q., Eden, U.T., and Eichenbaum, H. (2011). Hippocampal "time cells" bridge the gap in memory for discontiguous events. Neuron *71*, 737–749.

MacDonald, C.J., Carrow, S., Place, R., and Eichenbaum, H. (2013). Distinct hippocampal time cell sequences represent odor memories in immobilized rats. The Journal of Neuroscience *33(36)*, 14607-14616.

McEchron, M.D., Bouwmeester, H., Tseng, W., Weiss, C., Disterhoft, J.F. (1998). Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. Hippocampus *8(6)*, 638-46.

Miao, C., Cao, Q., Ito, H.T., Yamahachi, H., Witter, M.P., Moser, M.B., and Moser, E.I. (2015). Hippocampal remapping after partial inactivation of the medial entorhinal cortex. Neuron *88*, 590-603.

Mitra, P., Bokil, H. (2008). Observed Brain Dynamics. Oxford University Press, New York.

O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Research *34*, 171–175.

O'Keefe, J., and Recce, M.L. (1993). Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus *3*, 317–330.

Neunuebel, J.P., Yoganarasimha, D., Rao, G., and Knierim, J.J. (2013). Conflicts between local and global spatial frameworks dissociate neural representations of the lateral and medial entorhinal cortex. The Journal of Neuroscience *33*, 9246–9258.

Pastalkova, E., Itskov, V., Amarasingham, A., and Buzsaki, G. (2008). Internally Generated Cell Assembly Sequences in the Rat Hippocampus. Science *321*, 1322-1327.

Pfeiffer, B.E., and Foster, D.J. (2013). Hippocampal place-cell sequences depict future paths to remembered goals. Nature *497*, 74–79.

Quiroga, R.Q., Reddy, L., Kreiman, G., Koch, C., and Fried, I. (2005). Invariant visual representation by single neurons in the human brain. Nature *435*, 1102–1107.

Ranck, J.B. (1973). Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. Part I. Behavioral correlates and firing repertoires. Experimental Neurology *41(2)*, 461-531.

Rueckemann, J.W., DiMauro, A.J., Rangel, L.M., Han, X., Boyden, E.S., and Eichenbaum, H. (2016). Transient optogenetic inactivation of the medial entorhinal cortex biases the active population of hippocampal neurons. Hippocampus *26*, 246-260.

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B., Witter, M.P., Moser, M.B., and Moser, E.I. (2006). Conjunctive representation of position, direction, and velocity in entorhinal cortex. Science *312*, 758–762.

Sauvage, M.M., Beer, Z., Ekovich, M., Ho, L., and Eichenbaum, H. (2010). The caudal medial entorhinal cortex: a selective role in recollection-based recognition memory. The Journal of Neuroscience *30*, 15695-15699.

Schlesiger M.I., Cannova C.C., Boublil B.L., Hales J.B., Mankin E.A., Brandon M.P., Leutgeb J.K., Leibold C, and Leutgeb S. (2015). The medial entorhinal cortex is necessary for temporal organization of hippocampal neuronal activity. Nature Neuroscience *18*,1123-1132.

Silva, D., Feng, T., and Foster, D.J. (2015). Trajectory events across hippocampal place cells require previous experience. Nature Neuroscience *18,* 1772-1779.

Skaggs, W.E., McNaughton, B.L., and Gothard, K.M. (1992). An information-theoretic approach to deciphering the hippocampal rate code. NIPS *12,* 1030-1037.

Suh, J., Rivest, A.J., Nakashiba, T., Tominaga, T., and Tonegawa, S. (2011). Entorhinal cortex layer III input to the hippocampus is crucial for temporal association memory. Science *334*, 1415–1412.

Takahashi, H., and Magee, J.C. (2009). Pathway interactions and synaptic plasticity in the dendritic tuft regions of CA1 pyramidal neurons. Neuron *62(1),* 102-111.

Tulving, E. (1984). Précis of Elements of Episodic Memory. Behav. Brain Sci. *7*, 223–268.

Vargha-Khadem, F, Gadian, D.G., Watkins, K.E., Connelly, A., Van Paesschen, W., and Mishkin M. (1997). Differential effects of early hippocampal pathology on episodic and semantic memory. Science *277*, 376-380.

Wang, Y., Romani, S., Lustig, B., Leonardo, A., and Pastalkova, E. (2015). Theta sequences are essential for internally generated hippocampal firing fields. Nature Neuroscience *18*, 282–288.

Wood, E.R., Dudchenko, P.A., and Eichenbaum, H. (1999). The global record of memory in hippocampal neuronal activity. Nature *397*, 613–616.

Zhao, R. Grunke, S.D., Keralapurath, M.M., Yetman, M.J., Lam, A., Lee, T.C., Sousounis, K., Jiang, Y., Swing, D.A., Tessarollo, L., Ji, D., and Jankowsky, J.L. (2016). Impaired recall of positional memory following chemogenetic disruption of place field stability. Cell Reports *16*, 793-804.

Zola-Morgan, S., Squire, L.R., and Amaral, D.G. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. The Journal of Neuroscience *6*, 2950-2967.

## FIGURE LEGENDS

**Figure 1. Transient optogenetic inactivation of MEC during a mnemonic delay selectively impairs memory performance**

**(A)** Top: DAPI-counterstained sagittal sections through MEC. White lines: optic fiber tracks. Green fluorescence shows expression of GFP-conjugated Jaws in MEC. Bottom: coronal section through dorsal hippocampus in the same animal. GFP signal is detectable in stratum lacunosum moleculare of proximal CA1 and the middle molecular layer of the dentate gyrus, indicative of viral expression localized among hippocampal-projecting axonal fibers from MEC. SP: stratum pyramidale, SR: stratum radiatum, SLM: stratum lacunosum moleculare. mML: middle molecular layer, GCL: granule cell layer, DG: dentate gyrus. Scale bar is 2000μm.

**(B)** Schematic for simultaneous hippocampal tetrode recordings and MEC optogenetic disruption.

**(C)** Diagram of maze used for behavioral task. During separate sessions, the laser was triggered on the treadmill mnemonic delay (Treadmill Phase), during the return arm traversal (Maze Phase), and during object sampling (Object Phase) to assess effects of MEC inactivation at each epoch.

**(D)** Behavioral performance during each inactivation paradigm. Performance is impaired only during sessions where the laser was triggered during the Treadmill phase (Kruskal-Wallis ANOVA (KW), asterisks indicate significant Tukey's *post hoc* test).

Data are represented mean ± SEM, * $p< 0.05$, ** $p<0.01$, *** $p<0.001$. See also Figure S1.

**Figure 2. CA1 firing patterns during the delay are destabilized during and following MEC inactivation**

**(A)** Top: diagram of behavioral epoch (Treadmill Phase). The animal runs for 7 seconds on a treadmill during the mnemonic delay. Bottom: sequence of firing fields during the treadmill delay on trials before (Baseline, blue), during (Light on, red), and after (Light off, green) the laser exposure. On Light on trials, the laser is triggered from 2-4 sec (red dashed lines). After the first Light on trial, Light on and Light off trials are pseudorandomly intermixed for the remainder of the session. Neurons are sorted according to the latency of their peak firing rate during Baseline trials.

**(B)** Template matching population vector decoder (see methods). The mean spiking activity at successive windows across the delay is used to estimate a reconstructed time in each trial type based on the Baseline sequence template. Hotter colors indicate stronger matches. White-dashed line: idealized perfect decoding (perfect match between real and reconstructed time at each bin). Cyan line: observed decoding results, defined as the peak bin (x axis) in each decoding window (y axis). Deviation from the diagonal reflects the error in temporal reconstruction.

**(C)** Mean±s.e.m decoding error in **B** (absolute difference between the white-dashed and cyan lines, signed rank test).

**(D)** Comparison of mean error in **C** (colored vertical lines) to distribution of simulated mean errors (grey), where the template vectors were randomly shuffled to be temporally uninformative (see methods). Only Baseline is less erroneous than chance (p-value from 10,000 shuffles).

**(E)** Summary of change in temporal reconstruction error from Baseline, for different inactivation paradigms. Legend specifies animal viral type (Jaws or GFP-only) and laser trigger location (Treadmill, Maze, or Object phase). Increased error compared to GFP-only controls is observed

in Jaws-expressing animals only when the laser is triggered during the Treadmill phase (KW test, black asterisks indicate significant Tukey's *post hoc* tests compared to the GFP-only control; gold asterisks indicate the median change in error from Baseline is significantly different from zero, p-value from 10,000 shuffles of trial type identities; gold bar indicates 95th percentile of the null distribution).

**(F)** Baseline-normalized LFP theta power during the 2-4 sec inactivation window (signed-rank test).

**(G)** Peak LFP theta frequency during the 2-4 sec inactivation window (signed-rank test).

Data are represented mean ± SEM, box and whiskers are IQR and 1.5 x IQR respectively, * $p < 0.05$, ** $p<0.01$, *** $p<0.001$. See also Figure S2, S3, .

**Figure 3. Time-locked perturbation of temporal firing fields**

**(A)** Activity of four simultaneously recorded CA1 pyramidal cells on the treadmill during Baseline (blue), Light on (red), and Light off (green) trials.

**(B)** Temporal information rate and field stability (signed rank test), and mean firing rate (Kolmogorov-Smirnov (KS) test) for cells in each time block of the delay (Before the laser window/0-2 sec, during the laser window/2-4 sec, and after the laser window/4-7 sec).

**(C)** Percent change in firing rate variance across trials from Baseline as a function of time during the delay, for Light-on and Light-off trials (mean and 95% bootstrap confidence interval). Black asterisks indicate time periods where change in variance is significantly different than zero ($p<0.05$, bootstrap test).

**(D)** Summary of firing rate variance changes from Baseline in different inactivation paradigms, for Light-on and Light-off trials. Only Jaws Treadmill phase inactivation sessions showed significant increases in firing rate variance compared to GFP controls (KW test, black asterisks indicate significant Tukey's *post hoc* test compared to GFP-only controls; gold asterisks indicate the median change in variance is significantly different from zero, p-value from 10,000 shuffles of trial-type identities; gold bar indicates 95th percentile of the null distribution).

Data are represented mean ± SEM, box and whiskers are IQR and 1.5 x IQR respectively, * $p< 0.05$, ** $p<0.01$, *** $p<0.001$. See also Figures S2, S3, S4, and S5.

**Figure 4. Speed of population temporal decorrelation is diminished**

**(A)** Population vector cross-correlation matrices (see methods) for each trial type, example session. The width of the diagonal band of heat reflects how quickly the neuronal population transitions through time. Lower right: mean correlation coefficient at each population vector lag for example session. Note where the curves cross the 0.5 correlation threshold.

**(B)** Summary of the interval measuring the time it takes for the correlation curve in **c** to fall below 0.5 for each trial type, across sessions (signed rank test).

**(C)** Summary of temporal correlation decay results for different inactivation paradigms, reported as the % change from the Baseline decay interval for Light on and Light off trials. Only Jaws Treadmill inactivation sessions showed significant decay interval expansion compared to GFP-only controls, although Object inactivation sessions show a slight expansion that is greater than expected by chance (KW test, black asterisks indicated significant Tukey's *post hoc* tests compared to GFP-only controls; gold asterisks indicates the mean change in interval expansion is significantly different from zero, p-value from 10,000 shuffles of trial-type identities; gold bar indicates 95th percentile of the null distribution).

Data are represented mean ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Figure 5. Spatial firing patterns are resilient to transient MEC inactivation**

**(A)** Top: diagram of behavioral epoch (Maze phase). The animal runs for 1.5 m along the return arm of the maze to begin the next trial. In separate sessions from the Treadmill phase inactivation, the laser is triggered on the maze. Bottom: sequence of spatial firing fields across the return arm on trials before (Baseline, blue), during (Light on, red), and after (Light off, green) the laser exposure. On Light on trials, the laser is triggered for 2 seconds at a fixed point in space on the return arm (red-dashed line). Light on and Light off trials are blocked.

**(B-D)** Template matching population vector decoder, as in Figures 2C, 2D, and 2E but across the spatial extent of the return arm.

**(B)** Summary of spatial decoding for each trial type. Error is minimal though increases marginally from Baseline in later trial types (signed rank test).

**(C)** Comparison of mean error in **B** (colored vertical lines) to shuffled distributions (grey). The mean errors for all trial types are significantly lower than expected from spatially uninformative templates (p-value from 10,000 shuffled templates).

**(D)** Summary of spatial reconstruction error for different inactivation paradigms. Error increases are minimal, and no groups exhibit significant differences from GFP-only controls (KW test; gold bars indicate 95th percentile of shuffle distribution where trial type identities were randomized and change in error recomputed 10,000 times).

Data are represented mean ± SEM, box and whiskers are IQR and 1.5 x IQR respectively, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. See also Figure S6..

**Figure 6. Place fields remain intact**

**(A)** Activity of two simultaneously recorded CA1 pyramidal cells on the maze during Baseline (blue), Light on (red), and Light off (green) trials.

**(B)** Spatial information and field stability (signed rank test), and mean firing rate (KS test) of neurons with spatial firing fields during Jaws maze inactivation sessions.

**(C-E)** Population vector cross-correlation and decay interval analysis, as in Figure 4 but across the spatial extent of the return arm.

**(C)** Example session cross-correlation matrices and spatial correlation decay curve (lower right).

**(D)** Mean spatial decay interval for Jaws Maze phase inactivation sessions (signed rank test). Light-on and Light-off trials show a small increase in mean decay interval compared to Baseline.

**(E)** Summary of spatial correlation decay results for different inactivation paradigms. The % change in spatial decay interval does not differ between inactivation paradigms for Light on or Light off trials (KW test), suggesting the change in **D** is no more than expected from normal population drift across recording session (gold asterisks indicate mean change is significantly different from zero, p-value from 10,000 shuffles of trial type identities; gold bar indicates 95th percentile of the null distribution).

Data are represented mean ± SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. See also Figure S6.

**Figure 7. MEC inactivation does not affect CA1 object selectivity**

**(A)** Selectivity responses for all cells active (>2 Hz mean firing rate) during the –3 to 0 second object sampling period, sorted by preferred object. Each row shows a neuron's color-coded object selectivity profile (see methods) around the time of object sampling (purple indicates selectivity for object A, orange for object B. Substantial fractions of cells exhibit selective firing for one object or the other during the object sampling period.

**(B)** Activity of two highly object-selective neurons during object sampling, displayed separately for object A (left) and object B (right) trials, and for Baseline (blue), Light on (red), and Light off (green) trials.

**(C)** Left: cumulative distribution of p-values for object selectivity. The black dashed line indicates the threshold ($p<0.05$) for significance. Right: the percentage of cells with significant selectivity indices (SI) in each trial type (Pearson's $\chi^2$ of independence).

**(D)** SI values in each trial type for cells with significant baseline selectivity. These values do not change significantly (signed rank test).

**(E)** Summary of absolute change in SI values during Light on and Light off trials for cells with significant baseline selectivity, shown for different inactivation paradigms. These values are not significantly different across inactivation paradigms (KW test).

Data are represented mean ± SEM, box and whiskers are IQR and 1.5 x IQR respectively, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. See also Figure S7.

**Figure 8. Theta phase precession in CA1 object responses**

**(A)** Left: unfiltered (light grey) and filtered (4-12 Hz) traces of LFP from a single trial object sampling period, with spike phase advancement (purple dots) from a highly object-selective neuron. Right: scatter plot of spike phases shown at left against their timestamp within the object sampling window (-3 to 0 sec), with circular-linear regression lines (red), correlation coefficient, and significance overlaid (see methods).

**(B)** Two example highly-object selective cells, shown separately for object A (purple) and object B (orange) trials, with each spike plotted as a function of theta phase and time, and smoothed firing rate curve, regression line, correlation coefficient, and significance overlaid. Data is plotted across two theta cycles for clarity.

**(C)** Fraction of cells that exhibit significant firing rate changes according to object identity (determined by SI p-value), calculated separately for the population of cells with significant phase precession for at least one object and those without significant precession (determined by circular-linear correlation coefficient p-value and regression slope sign for spiking during trials for the preferred object). Neurons with object-selective firing rates are comparatively overrepresented in the phase precessing group (Pearson's $\chi^2$ test of independence).

**(D)** Distribution of absolute object SI values for cells with significant phase precession for at least one object, shown separately for cells with significant firing rate changes according to object identity (red) and for cells that do not (grey). Precessing cells exhibit a wide distribution of firing rate differences between objects (1 would indicate all spikes were fired for only one object).

**(E)** Fraction of cells that exhibit phase precession for both objects (open bars) or a single object (shaded bars), shown separately for cells that show significant firing rate selectivity for objects

(red) and those that do not (grey). Most cells exhibit phase precession for only one object, and this fraction does change according to whether there is a significant firing rate change between objects (Pearson's $\chi^2$ test of independence).

See also Figure S7.

## STAR★Methods

### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for reagents and resources may be direct to the lead contact, Dr. Howard Eichenbaum (hbe@bu.edu).

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

Eleven male Long Evans rats (400-600g, 5-8 months old at surgery) were kept on a 12hr light/12hr dark schedule and housed individually. After behavioral training, 9 rats were implanted with 24 individually movable tetrodes targeted at CA1 of the hippocampus and two bilateral 3 fiber optic arrays targeting MEC. Prior to implant, a series of 6 adeno-associated virus (rAAV) infusions (3 each hemisphere) were performed into MEC. 2 additional rats were used in pilot inactivation recordings. During periods of behavioral performance rats were food deprived to maintain 85-90% of free feeding weight. All behavioral training and recording sessions were performed during the light phase of the cycle. All procedures were approved by the Boston University Institutional Animal Care and Use Committee.

### METHOD DETAILS

#### AAV Viral Constructs

7 experimental animals (Jaws) and 2 pilot inactivation animals were injected with rAAV5-hSyn-JAWS-KGC-GFP-ER2 (a gift from Dr. Ed Boyden) at a concentration of $3.3x10^{12}$ viral genomic particles per ml to enable Jaws expression in MEC under the neural specific promoter synapsin. 2 control animals (GFP) were injected with rAAV5-hSyn-EGFP (UNC Vector Core) to control for viral infusion, expression and light delivery.

**Stereotaxic Viral Injection and Implants**

Animals were anaesthetized with isoflurane at 5% (vol/vol) for induction with an air flow of 1.0 $lmin^{-1}$. Buprenorphine (0.05mg/kg) and Carprofen (1mg/kg) were injected subcutaneously for analgesia. The rat was fixed into a stereotaxic frame (Kopf) and isoflurane levels were reduced to 1-2.5% (vol/vol) and adjusted subsequently depending on physiological markers. The areas for tetrode drive implantation, viral infusion and fiber optic array implant were measured using a stereotaxic arm (Kopf). Craniotomies were made over MEC on both hemispheres and a durotomy was performed to allow clean needle penetration. Viral infusions were made using a 1 µL Neuros Model syringe with a 32 gauge needle (Hamilton) and an Ultra Micro Pump (World Precision Instruments Inc.) attached to a stereotaxic arm. 3 injections were made into each hemisphere at a flow rate of 50nl/min (1µL at AP: -9.1, ML: +/- 4.9, DV: - 3.2; 0.64 µL at AP: -8.4, ML: +/- 4.8, DV: -4.1; 0.36 µL at AP: -7.7, ML: +/- 4.7, DV: - 4.7, DV relative to cortical surface). Prior to infusion the needle was allowed to settle for 5 minutes, and after infusion was left for 10 minutes to allow viral diffusion before being slowly retracted. Custom designed triple fiber optic arrays with three 200µm core fibers (Doric Lenses Inc.) were implanted stereotaxically, one into each hemisphere, so as to position fiber tips 0.5mm above each injection point. Seven skull screws were inserted to fix the implants in place and 2 ground screws over the cerebellum to ground the recording drive. Hyperdrives consisted of 24 individually drivable tetrodes each consisting of four entwined 12.7µm Nickle-Chrome wires (Kanthal), gold plated to reduce impedance to between 180-225kΩ. Drives were implanted unilaterally over the right hippocampus with a craniotomy centered at AP: -4.1 and ML: -3.1. The drive and fiber optic arrays were secured to the skull using dental acrylic. During surgery the tetrodes were lowered

into the brain to a depth of ~0.8mm. Post-surgery the animals were given subcutaneous buprenorphine (0.05mg/kg) and Rimydl (1mg/kg) and intramuscular Cefazolin (50/mg/kg) for 4 days with water and food *ad libitum* for ten days to aid recovery.

**Data Acquisition**

All electrophysiological recordings were made using a 96-channel Multichannel Acquisition Processor (MAP) system (Plexon). Each channel was amplified (3,000-10,000×), band pass filtered in the 0.3Hz-6kHz range and digitized at 32kHz. Tetrodes were connected to the system via an electronic interface board (Plexon Inc.). Spike channels were referenced to a quiet electrode to remove noise. Events were captured using thresholding and digitized at 40kHz. The animals' position was tracked using two light emitting diodes fixed to the hyperdrive implant. After animal recovery, tetrodes were gradually lowered into the CA1 cell layer over 2-4 weeks, guided by LFP theta and sharp-wave ripple amplitude. Tetrodes were left to stabilize a minimum of 4 hours before a recording session. To minimize cell re-sampling, tetrodes were moved between recording sessions of the same session type (every 3-4 days). Some recurrence of cells across sessions is unavoidable though, and we have recomputed main analyses limited to a single cell from each unique wire (Figure S4).

**Light Delivery**

A 635nm 300mW laser (Optoengine) was coupled to a 1×2 equal intensity division fiber optic rotary joint (Doric Lenses Inc.) with a 1m 200μm core fiber and FC/PC connector. Two 2m 600μm fibers were used to carry light from the rotary joint to the fiber optic arrays and secured using a zirconia sleeve. Light power was adjusted to achieve an intensity of ~8mW/mm$^2$ per

array fiber tip. The laser power was controlled through TTL embedded electronic modulation build into the laser driver circuit, which precluded the need for mechanical shutters. The laser power was directly turned on/off through delivery of a 0-5 VDC square wave TTL pulse generated by an Arduino Uno (Arduino), which was controlled with custom written software in MATLAB (MathWorks). The laser was activated at specific locations via the animal position tracking or at a set time point into the treadmill run, and our electronic control scheme allowed modulation of the laser power between complete extinction to full power without any audible cues.

**Trial Block Design**

During recording sessions and post-operative re-training, the treadmill was activated automatically as the animal entered the treadmill area via animal position tracking data. A typical recording session consisted of 20 each of Baseline, Light on, and Light off in that order, where the object identity on each trial was pseudorandomized. During treadmill inactivation sessions, Light on and Light off trials were pseudorandomly intermixed, while during spatial and object inactivation sessions, they occurred in subsequent blocks. The difference in trial block design between session types is due to earlier attempts to maximize the chance of impacting spatial or object coding via repeated stimulation, whereas the intermixed design proved sufficient to perturb temporal coding and permitted us to examine the presence or absence of short-term network recovery. We do not believe the difference appreciably impacts the interpretation of our results, as we still did not see an impairment in spatial or object coding using the blocked design. MEC inactivation was performed during one consistent task epoch (i.e. during either the

Treadmill, Maze or Object phase) and the manipulated task phase was rotated each recording day.

**Spike Sorting and Data Processing**

Spikes were assigned to individual neurons by offline manual clustering of waveform characteristics (peak-valley, valley, energy, principal components) across tetrode wires using Offline Sorter (Plexon). Unit integrity was verified by the existence of a clean 1ms refractory period and by contrasting the spike autocorrelograms against cross-correlograms with other units. Recording stability was confirmed by comparing cluster characteristics across the duration of the session. Cluster quality and isolation were measured by computing $L_{ratio}$ values for each cluster (Pfeiffer and Foster, 2013; Schmitzer-Torbert et al, 2005), using an 8-dimensional feature space according to the energy and 1$^{st}$ principal component coefficient of the waveform on each wire

**Behavioral Procedures**

Animals were trained on a 335cm long 7.5cm wide approximately triangular track with an integrated custom treadmill made using commercially available parts (Colombus Instruments). The track was elevated 95cm above the floor and surrounded by salient nearby cues on all sides. After 2 days of initial exposure to the environment animals were trained to run unidirectional laps before learning to run on the treadmill, initially at 30cm/s before being incrementally increased until the animal was comfortably running at 50cm/s for 7s. This acclimation took approximately 1-2 weeks. The treadmill had walls funneling into a small exit to ensure minimal lateral head movement while running. Next animals were trained to dig in a small ceramic pot

containing sand after each treadmill run to receive food reward. Finally animals were taught to sample of one of two objects during a 3 sec sampling period prior to treadmill runs, which were then associated with either a dig or withhold-dig response to obtain reward after the treadmill. The objects were initially presented in blocks to aid response learning before being pseudo randomized. Once an animal performed >75% for three consecutive days they were surgically implanted. Typically, this training schedule took between 6-8 weeks prior to surgery for a completely naive animal to reach training criteria (1-2 weeks of treadmill training, and 4-6 weeks to learn the task), and 2-3 weeks post surgery for retraining.

**Treadmill Firing Fields**

For firing fields on the treadmill, each trial's spike train during the 7 second treadmill delay was aligned to the onset of the run, downsampled to 1250 Hz, and convolved with a Gaussian kernel (s.d.=200ms). The smoothed trial spike trains were averaged within trial type (Baseline, Light-on, Light-off) to obtain the firing rate tuning curves. To assess significance of Baseline temporal tuning for each cell, we created null tuning curves by circularly shifting each trial's spike train by a random amount (up to a maximum 7 second shift), smoothing with the 200ms Gaussian kernel, then averaging across trials to obtain the null tuning curve. This procedure was repeated 5000 times for each cell to produce a null distribution of firing rates at each bin in the tuning curve. We detected candidate segments of the Baseline tuning curve where it exceeded the $99^{th}$ percentile of the shuffled distributions for a consecutive period between 0.1 and 4 seconds in length. A cell was determined to have a temporal firing field if it exhibited a candidate segment that contained a peak firing rate >2 Hz, if this peak firing rate was at least twice the cell's mean firing rate, and if the cell's mean firing rate was < 8 Hz.

## Maze Firing Fields

The return arm of the track was linearized and then binned into non-overlapping 1 cm bins. Instantaneous firing rate over space was calculated for each trial by calculating spike counts and occupancy at each bin, smoothing each with a Gaussian kernel (5 cm s.d.), and dividing the smoothed spike counts by the smoothed occupancy to obtain rates. Only spikes recorded while the animal was running >5cm/s were included in this analysis. To determine cells with significant Baseline spatial tuning, we computed null firing rate distributions at each spatial bin by random circular shifts of trial spike trains, as described above for temporal firing fields. Trial null spike bins were then smoothed with the Gaussian kernel and then divided by the smoothed occupancy bins. Candidate segments between 5 and 75 cm in length in the Baseline tuning curve were identified against the $99^{th}$ percentile of the null distribution and vetted by firing rate criteria as described above for time.

## Neuronal Sequences

Firing fields on the treadmill and in the maze were ordered according to the latency of their peak firing bin from the start of the treadmill or maze run during Baseline, and then normalized by their Baseline peak firing rate.

## LFP Power and Frequency Analysis

Local field potential power spectra were estimated using the multi-taper spectral method included in the Chronux toolbox (mtspectrumc, NW = 2, with k = 3 tapers, Mitra and Bokil 2008). For treadmill theta power comparisons, LFP segments during the laser exposure period

(2-4 seconds) were extracted for each trial and concatenated by trial type. For each session, the spectral content for these periods was then estimated for Baseline, Light on, and Light off using the concatenated vectors. We detected the peak power and frequency in the theta range (6-10 Hz) and normalized each session's power estimates by the baseline value for comparison. We limited this analysis to one wire per session, using the wire with the highest Baseline theta power. For LFP spectrograms, we computed time-resolved Morlet wavelet spectrograms using 150 linearly spaced frequencies from 1 to 90 Hz. We computed instantaneous power in theta and high gamma bands by bandpass filtering the LFP with a 4th order Butterworth filter and taking the absolute value of the Hilbert-derived analytic signal.

**Template-matching Population Vector Decoder**

We evaluated temporal and spatial position coding via reconstruction using a template-matching population vector decoder. The smoothed Baseline tuning curves were individually z-scored, and the ensemble was binned into 100 ms population vectors (for treadmill activity) or 2.5 cm vectors (for maze activity). Thus at every position **x** for an ensemble of **N** neurons, the activity of the ensemble was described by a vector **f** containing the z-scored firing rate $\boldsymbol{f}_i(x)$ for $i$ = 1,2…N. These baseline vectors served as the template. For reconstruction, we calculated the unsmoothed mean spike count $\mathbf{n}_i$ for each 100 ms or 2.5 cm window, for $i$ = 1,2…N. For each decoding window, the reconstructed position was determined by the position template vector that maximized the cosine of the angle between the mean spike vector and the template vector:

$$\hat{x}_{template} = \underset{x}{\operatorname{argmax}} \frac{\sum_i^N n_i f_i(x)}{\sqrt{\sum_i^N n_i^2}\sqrt{\sum_i^N f_i(x)^2}}$$

We moved the decoding window across non-overlapping segments of the treadmill delay or maze return arm to compute reconstructed trajectories from the ensemble spiking activity during Baseline, Light on, and Light off trials. Reconstruction error was calculated as the absolute difference between the real and constructed position at each decoder window. We assessed the significance of the mean error for a reconstructed trajectory via a null distribution of shuffled templates to destroy their temporal information. We generated 10,000 sets of null template vectors by randomly shuffling the order of the population vector templates. We then asked whether the observed error in each trial type was significantly different than the distribution of null errors obtained when the spiking activity was decoded using the shuffled templates, and thus whether the template was significantly predictive of the observed spiking. We derived p-values for the mean error as the percentage of null errors less than the observed error (Figure 2D). We further compared the change in decoding error from Baseline, across inactivation paradigms (Figure 2E). To estimate the likelihood of obtaining the observed change in decoding error by chance, we compared these values to a null distribution where trial-type identities were shuffled between Baseline and Light-on, or Baseline and Light-off, before constructing Baseline templates and computing decoding error. We calculated p-values for the observed change in decoding error by comparing the median of the observed change in error to the distribution of shuffled median error calculations; the 95th percentile of the null median distribution is plotted in 2E.

**Information Rate**

Information rate (bits/sec) was calculated for time or space as $I = \sum \lambda(x) * \log_2(\lambda(x)/\lambda) * p(x)$, where $\lambda(x)$ is the firing rate of the neuron at spatial or temporal bin x, $\lambda$ is the mean firing rate of

the neuron's tuning curve, and p(x) is the probability of the animal being in bin (x) (Skaggs et al, 1993).

**Field Stability**

For each neuron, we computed the correlation between the smoothed average tuning curve of a 5 trial window and the smoothed average tuning curve of the remaining trials. We slid the 5 trial window in 1 trial increments, and averaged the resulting sequence of correlation values to obtain a measure of field stability. This measure was computed separately for Baseline, Light on, and Light off trials.

**Firing Rate Variance**

For each neuron, we computed the firing rate in 200ms bins during the Treadmill period on each trial. The variance in firing rate across trials was then computed for each time bin, separately for Baseline, Light-on, and Light-off trials. We averaged the resulting variance curves across neurons to obtain a time-resolved estimate of the population firing rate variance for each trial type, and computed the % change from Baseline in the variance curves for Light-on and Light-off. To estimate the likelihood of obtaining the observed change in variance by chance, we compared these curves to a null distribution where trial type identities were randomized between Baseline and Light-on, or Baseline and Light-off, before computing each cell's variance curve. This procedure was repeated 10,000 times to create null $\Delta$ variance curves. We calculated p-values for the observed $\Delta$ variance by comparing the median of the observed curve to the distribution of null curve medians; the 95th percentile of the null median distribution is plotted in 3D.

**Population Vector Cross-Correlation**

We formed population vectors from activity in non-overlapping 100 ms (time) or 2 cm (space) bins across the treadmill delay or return arm. We then computed population vector cross-correlation matrices for each trial type (Figures 4 and 6). Adjacent vectors exhibit high correlations (hot colors) while more distant vectors are decorrelated (cool colors). Thus, the width of the central band of heat along the diagonal autocorrelation describes the rate at which the population activity changes over time or space. To quantify this, we calculated the mean correlation value for pairs of vectors separated by a range of lags, which forms a curve with a correlation value of 1 at the zero lag (autocorrelation) and decays at further lags. To index this rate of decay (and thus the rate of population activity change), we calculated for each session the spatial or temporal lag at which the correlation decay curve fell below 0.5 and compared this value across Baseline, Light on, and Light off trials. We further compared the % change of Light-on and Light-off lags from Baseline, across inactivation paradigms. To estimate the likelihood of obtaining the observed change in lag by chance, we compared these values to a null distribution with shuffled trial-type identities as explained previously.

**Spike-subsampling**

To more rigorously control for differences in activity level across trial types, we recomputed the main analyses on simulated datasets where the spikes were subsampled to equalize the firing rate across Baseline, Light-on, and Light-off trial types. We identified the trial type with the lowest mean firing rate, and then calculated the number of spikes to remove from the other two trial types in order to equalize the mean rate across trial types. 1000 simulated data sets were created

where we randomly removed these spike counts; new firing rate curves, information scores, and field stability correlation coefficients were computed for each simulation. We repeated unit and population vector analyses using the average simulated values for these measures. We further compared the change in unit statistics between Light-on and Baseline (observed and simulated) to the change in observed firing rate via Pearson correlation. We assessed whether there was a significant difference, between the observed and simulated data sets, in how well changes in firing rate predicted changes in unit statistics by comparing the coefficient of variation of the model fits. To estimate the likelihood of obtaining the observed difference in $R^2$ values by chance, we compared this difference with a null distribution where cell identity was randomized between the observed and simulated groups, and the magnitude of the difference in $R^2$ values for each group were recomputed. This procedure was repeated 10,000 times to build the null distribution.

**Object Population Responses**

For each cell active during the object sampling period (>2 Hz mean firing within the object period for at least one object during Baseline), we calculated separate tuning curves based on firing during Object A and Object B sampling, smoothed with a Gaussian kernel (200ms). Using these vectors **A** and **B**, we then calculated a selectivity curve defined as **S** = (A – B) / (max(A) + max(B)), which produces selectivity values bounded [-1 1], where more negative values indicate selectivity for Object B, and more positive values indicate selectivity for Object A. Here, the denominator is calculated using the maximum rates for each object rather than calculated by bin in order to minimize the visual impact of spuriously selective, low firing rate selectivity in isolated bins. To visualize the ensemble selectivity dynamics over time around the object

sampling period, we sorted all neurons by their Baseline maximum selectivity during object sampling and plotted the population as a heatmap, where Object A selectivity appeared purple and Object B selectivity appeared orange, with values near zero fading to black (Figure 7A).

**Selectivity Index**

To quantitatively compare cell selectivity across Baseline, Light on, and Light off trials, we calculated a selectivity index (SI) for each cell during each trial type, defined as SI = (A - B) / (A + B), where A and B are scalar firing rates for activity during Object A sampling and Object B sampling respectively. This produces an index bounded [-1 1], where -1 indicates complete selectivity for (i.e., all spikes during) Object B, while +1 indicates complete selectivity for Object A. To assess the significance of the SI values obtained, we assigned p-values by generating a null distribution of shuffled SIs, where for each cell and trial type, trial object identities were randomized while holding the original number of trials per object constant. This procedure was repeated 5000 times, and a null distribution of SIs was calculated from the resulting null object firing rates. A cell was considered to have significant object tuning if less than 5% of the null SIs had an absolute value greater than the absolute value of the true SI ($p < 0.05$).

**Theta Phase Estimation**

LFPs were filtered in the theta band (4-12 Hz) via a $4^{th}$ order Butterworth filter and phase derived via Hilbert transform. Instantaneous spike-phase was then estimated by linear interpolation using the LFP phases from the same wire, and the spike and LFP timestamps.

**Circular-Linear Regression and Correlation**

For a series of *n* spikes, we quantified the circular-linear relationship between spike theta phase $\phi_j$ and time $x_j$ for j = 1,2…n, using a linear model of the form $\bar{\phi}_j = 2\pi a x_j + \phi_0$, where *a* is the slope (in cycles per unit time) and $\phi_0$ is the phase-offset of the regression line (Kempter et al 2012). The slope parameter *a* can be found by maximizing the mean resultant length *R* of the circular errors between $\phi_j$ (measured phase) and $\bar{\phi}_j$ (model-predicted phase):

$$R = \sqrt{\left[\frac{1}{n}\sum_{j=1}^{n}\cos(\phi_j - 2\pi a x_j)\right]^2 + \left[\frac{1}{n}\sum_{j=1}^{n}\sin(\phi_j - 2\pi a x_j)\right]^2}$$

Where $\hat{a} = \underset{a}{\operatorname{argmax}} R$ is the estimated slope. We find $\hat{a}$ numerically via the MATLAB routine *fminbnd()*; since $\hat{a} = \underset{a}{\operatorname{argmax}} R$ is not unique on a cylinder, we constrain *a* within [-2 2] cycles per second for the numerical optimization. The phase offset is calculated by plugging in $\hat{a}$ to the following equation:

$$\hat{\phi}_0 = atan2\frac{\sum_{j=1}^{n}\sin(\phi_j - 2\pi\hat{a}x_j)}{\sum_{j=1}^{n}\cos(\phi_j - 2\pi\hat{a}x_j)}$$

The circular-linear correlation is then obtained by:

$$\rho = \frac{\sum_{j=1}^{n}\sin\left(\phi_j - \bar{\phi}\right)\sin(\theta_j - \bar{\theta})}{\sum_{j=1}^{n}\left[\sin\left(\phi_j - \bar{\phi}\right)\right]^2 \sum_{j=1}^{n}\left[\sin(\theta_j - \bar{\theta})\right]^2}$$

Where $\theta_j = 2\pi|\hat{a}|x_j\ (mod\ 2\pi)$, to circularly transform the linear variable $x_j$, and $\bar{\phi}$ and $\bar{\theta}$ are the circular means of $\phi_j$ and $\theta_j$. To calculate a *P*-value for $\rho$, we consider the null hypothesis where $\rho = 0$. Under the null, the scaled correlation $z = \rho\sqrt{n(\hat{\lambda}_{2,0}\hat{\lambda}_{0,2})/\hat{\lambda}_{2,2}}$ is normally

distributed for large *n*, where $\hat{\lambda}_{i,j} = \frac{1}{n}\sum_{k=1}^{n}[\sin(\phi_k - \bar{\phi})]^i\,[\sin(\theta_k - \bar{\theta})]^j$. Thus via the cumulative normal distribution, $p = 1 - \mathrm{erf}(\frac{|z|}{\sqrt{2}})$.

**Histological Procedures**

Prior to perfusion, 40mA of current was passed through each electrode for 30s to aid in tetrode localization. Animals received an overdose of pentobarbital and were perfused intracardially with saline then 4% formaldehyde (vol/vol). After dehydration in 20% sucrose solution, the brains were frozen and sliced into 40µm coronal sections through the extent of the dorsal hippocampus. The remaining caudal portion of the brain was sliced into 40µm sagittal sections to allow visualization of fiber placement and viral expression in MEC. Half of the slides from both regions were Nissl-stained for tetrode and fiber localization. The second half was DAPI counterstained (Vector Laboratories) for fluorescence microscopy to observe viral expression in MEC and afferent projections into the hippocampus.

**Anaesthetized Recordings**

Two animals were anesthetized using Isoflurane and rAAV5-hSyn-JAWS-KGC-GFP-ER2 was infused into the MEC as described previously. The animals were then given 4 weeks to recover and for viral expression to develop. On the recording day animals were anesthetized using intraperitoneal injection of a cocktail of Ketamine (60mg/kg) and Xylazine (7.5mg/kg), with additional injections given as needed. Once anesthetized, the animals were placed in a stereotaxic frame and the craniotomy over MEC was reopened. Pulled glass pipettes were filled with saline solution and attached to micromanipulators with a recording electrode. A 200µm core fiber was attached to the recording pipette and positioned to deliver light to the recording site.

The fiber was coupled to a 635nm 300mW laser (Optoengine) and the power was calibrated to supply 8mW/mm$^2$ at the fiber tip. The recording pipette was positioned above dorsal MEC and lowered 2.8mm from the cortical surface before being slowly advanced until suitably high amplitude action potential voltage deflections were detected from a cell. We then proceeded to record 20 or 30 sweeps of 35 seconds in which the laser was used to supply light for 5 seconds. Upon completion of recording animals were sacrificed and processed for histology to confirm recording location was within MEC.

## QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical tests were performed in MATLAB (MathWorks). All statistical tests are described in the corresponding figure legends and results section, and comparisons were two sided. Non-parametric Wilcoxon Signed Rank tests, Kolmogorov-Smirnov tests, and Kruskal-Wallis one-way ANOVAs are used throughout. Summary data are presented as mean ± SEM. Box and whiskers are IQR and 1.5 x IQR respectively. Bootstrapped 95% confidence intervals were constructed from 1000 random resampling with replacement of the population of interest. Correlations were computed using Pearson's correlation coefficient.

## DATA AND SOFTWARE AVAILABILITY

Analysis-specific code and data are available by request to the Lead Contact.

**(in separate document)**

## KEY RESOURCES TABLE