In a temporally segmented experience hippocampal neurons represent temporally drifting context but not discrete segments.

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Abstract

There is widespread agreement that episodic memory depends on a representation of spatiotemporal context. Recent work suggests that people parse the flow of experience into discrete episodes separated by event boundaries. A complementary body of work suggests that context changes gradually as experience unfolds. We recorded from hippocampal neurons as rats performed 6 blocks of an object discrimination task in sets of 15 trials. Each block was separated by a one-minute delay to enable segmentation. The reward contingency reversed from one block to the next to incentivize segmentation. If animals segmented the blocks and learned the reversal contingency, we would expect the animal's choice to switch with the beginning of each block. Similarly, hippocampal ensembles should reflect the discrete alternating block structure. If the delay between blocks had no effect on memory, one would expect the animals to perseverate, starting each block by repeating the choice from the previous block. We observed neither of these patterns of results. Animals started each new block at chance as if they relied on a recency-weighted average of the reward experience. Hippocampal ensembles showed continuous contextual drift both within block and across blocks and showed no evidence of sensitivity to the alternating block structure. Despite clear boundaries between blocks, we saw no behavioral or neural evidence for event segmentation in this experiment. Rather, the

hippocampal ensemble drifted continuously. This continuous drift was consistent with the recency weighted averaging seen in the behavioral results.

Introduction

Episodic memory refers to the vivid recollection of a specific event situated in a particular place and time. Episodic memory requires the generation of an internal representation of an ongoing episode as it unfolds as a sequence of events in a unique place and time (Tulving & Madigan, 1970). The hippocampus is essential for episodic memory, and is thought to mediate this function by binding events to an ongoing spatiotemporal context (Eichenbaum, Yonelinas, & Ranganath, 2007). Electrophysiological evidence suggests that hippocampal cell ensembles represent a 'cognitive map,'(O'Keefe & Nadel, 1978) that acts as a contextual or relational scaffold onto which events may be bound together for later retrieval (Davachi, 2006). In this way, the contextual relationship between events dictates their association: greater similarity in the hippocampal representation of context between events results in a greater likelihood of their association (Cai et al., 2016; Hsieh, Gruber, Jenkins, & Ranganath, 2014; Manns, Howard, & Eichenbaum, 2007). Thus the way spatiotemporal context is represented in the hippocampus governs the organization of episodic memory.

There are two complementary hypotheses for how spatiotemporal context is organized in the hippocampus. Event segmentation accounts posit that our experiences are segmented into discrete situational contexts by which experiences may be associated and across which experiences can be differentiated (Baldassano et al., 2017; DuBrow, Rouhani, Niv, & Norman, 2017; Muller & Kubie, 1987; Zacks, Tversky, & Iyer, 2001). Temporal context accounts postulate that the hippocampal representation evolves continually, and therefore temporal

context is a continuous timeline (Howard & Eichenbaum, 2013; Howard, Fotedar, Datey, & Hasselmo, 2005). There is behavioral, neuroimaging and animal neurophysiology evidence consistent with both hypotheses, and the two hypotheses are not mutually exclusive.

Consistent with the event segmentation hypothesis, abrupt changes in environmental or contextual cues can cause a behavioral separation in memory traces (Ezzyat & Davachi, 2011; Sols, Dubrow, Davachi, & Fuentemilla, 2017; Zacks et al., 2001). Hippocampal BOLD activity increases when a border between contexts is perceived, which suggests that the hippocampus plays an active role in segmenting context (Baldassano et al., 2017). The representation of visual stimuli in the hippocampus shifts when participants shift tasks, suggesting the representation of stimuli themselves crucially depends on context, and that contextual segments affect the representation of stimuli (Mack, Love, & Preston, 2016). This suggests a hippocampal-based mechanism to parcellate experience into contextual chunks, such that events within a context are perceived to be more similar, and events across contexts are discriminated (DuBrow et al., 2017).

If event segmentation is reflected in neural firing fields in rodents, we would expect individual hippocampal cells to remap from one contextually relevant segment to another. Indeed, individual hippocampal units globally remap in response to contextual changes such as the physical attributes of an environment, or the behavioral goals in that context, or even the recent past leading up to that context (Brandon, Koenig, Leutgeb, & Leutgeb, 2014; Komorowski, Manns, & Eichenbaum, 2009; Leutgeb et al., 2005; Markus et al., 1995; Markus, Barnes, McNaughton, Gladden, & Skaggs, 1994; Wills, 2005). In a particularly dramatic example that is analogous to the present experiment, Komorowski et al. trained rats to perform a context-dependent object discrimination task. In this task two objects were presented in each of two spatial contexts, and which object was rewarded depended on the context in which the

objects were presented. Hippocampal units showed different firing patterns to the same object across the spatial contexts, as if the experiences had been segmented based on the behaviorally relevant spatial context (Komorowski et al., 2013, 2009). This is analogous to event segmentation results in which human participants segment experience according to changing behavioral goals of an actor (Swallow et al., 2011). Furthermore, the separation in the representations of objects developed with learning and was eliminated by muscimol injection into the PFC suggesting event segmentation depends on the cooperative function of the hippocampus and PFC (Komorowski et al., 2013; Navawongse & Eichenbaum, 2013; Place, Farovik, Brockmann, & Eichenbaum, 2016).

The temporal context hypothesis proposes that context in the hippocampus includes a gradually-drifting temporal signal, providing a scaffold for episodic memories (Howard & Eichenbaum, 2013). In this model, a slowly evolving representation is leveraged by the hippocampus to associate events in close temporal proximity. This model was inspired by psychological observations during list recall in humans that suggest list items are associated on a temporally graded continuum (Howard, Youker, & Venkatadass, 2008; Kahana, 1996; Unsworth, 2008). This interpretation is bolstered by fMRI data that show representational similarity in the hippocampus during recalled events reflects a broad continuum of relatedness that maps onto the temporal and spatial proximity of those (Deuker, Bellmund, Navarro Schröder, & Doeller, 2016; Nielson, Smith, Sreekumar, Dennis, & Sederberg, 2015). The representational similarity in hippocampal BOLD activity between stimuli reflects their temporal relatedness, and this is evident even across temporally grouped communities (Lositsky et al., 2016; Schapiro, Kustner, & Turk-Browne, 2012; Schapiro, Turk-Browne, Norman, & Botvinick, 2016). Furthermore, the hippocampal representation of events includes information about past

stimuli such that activity in response to an item reflects the conjunctive representation of that event and preceding events in a temporal sequence (Allen, Salz, Mckenzie, & Norbert, 2016; Hsieh et al., 2014; Wood, Dudchenko, Robitsek, & Eichenbaum, 2000).

At the single cell level, a gradually changing representation of context would manifest as temporal autocorrelations in neural firing. Such temporal autocorrelation has been observed in a range of species using a range of recording techniques over a range of time scales from seconds up to hours and days (Folkerts, Rutishauser, & Howard, 2018; E. A. Mankin et al., 2012; Emily A. Mankin, Diehl, Sparks, Leutgeb, & Leutgeb, 2015; Manns et al., 2007; Mau et al., 2018; Paz et al., 2010; Ziv et al., 2013). For instance, one study using tetrode recordings found that ensembles drift during shorter time intervals of seconds to minutes as animals memorized novel sequences of odors (Manns et al., 2007). Similarly a recent study using calcium imaging and immediate early gene expression found that the activity of CA1 ensembles drifted gradually over hours and was correlated with the mouse's ability to discriminate between events across that interval (Cai et al., 2016).

To summarize, previous work shows that a) hippocampal representations in the rodent segment spatial environments with distinct behavioral requirements as predicted by the event segmentation hypothesis and b) hippocampal representations drift across time as predicted by the temporal context hypothesis. In order to determine the boundary conditions on event segmentation and temporal context, we designed a task in which distinct behaviors were reinforced in different temporal blocks of trials in the same spatial environment. We cued the boundary between blocks by shuttling the animal to a separate chamber for 1 minute to create a cue to segment the blocks of trials but there were no overt cues present to signal the new behavioral context at the time of the choice. A representation of temporal context that changes

continuously across blocks would be behaviorally suboptimal in this experiment. Rather, the strategy to maximize reward would be to segment the experiment into behaviorally meaningful contexts.

We trained rats to perform a blocked object discrimination task while we recorded extracellularly from dorsal CA1 ensembles. As in earlier work that showed evidence for spatial event segmentation (Komorowski et al., 2013, 2009), rats were presented with pairs of pots containing unique odors and digging media and the rat was rewarded for choosing the correct pot from the pair. The identity of the rewarded pot was consistent for each block of 15 trials after which a 1-minute inter-block delay was imposed; for the next 15 trials the other pot was rewarded. The event segmentation hypothesis predicts two stable mappings of objects and places. In contrast, the temporal context hypothesis predicts a continuous decorrelation of neural representations that was unaffected by the blocked structure of the experience.

Experimental Methods

***Subjects*.** Subjects were 5 male Long-Evans rats (Charles River) weighing between 350 and 450 grams and between the ages of 6 months to 1 year for the duration of the experiment. All animals were single housed and maintained on a 12 h light/dark cycle (lights on 8:00 A.M. to P.M.). Behavioral training and testing were conducted exclusively during the light phase. Animals were maintained at a minimum (85%) of their *ad libitum* feeding body weight during all behavioral training and testing periods. Procedures were conducted in accordance with the requirements set by the National Institutes of Health and Boston University Institutional Animal Care and Use Committee (IACUC).

***Behavioral Apparatus*:** The behavioral training and testing environment was a custom-built wood apparatus (60 l x 60 w x 40 h cm) consisting of a 40 cm x 40 cm box, and a 20 cm wide side alleyway. The objects consisted of identical circular terra cotta pots (10 cm high with an internal diameter of 9 cm), each with their own unique digging media and odors (e.g., purple beads with grapefruit scent). The pots were distinguishable only by their scent and digging media, requiring the animal to overtly sample before choosing to dig. In order to prevent the animals from being guided by odor of the Froot Loop (Kellogg's) cereal reward, finely crushed Froot Loops were sprinkled into all digging media.

Surgery. Anesthesia was induced by inhalation of 5% isoflurane (Webster Veterinary Supply) in oxygen and then a stable plane was maintained at 1.5%-3% throughout the entirety of surgery. Before surgery animals were injected with the analgesic Buprenex (buprenorphine hydrochloride, 0.03 mg/kg i.m.; Reckitt Benckiser Healthcare), and the antibiotic cefazolin (330 mg/ml i.m.; West-Ward Pharmaceutical). The skin of the animal's head covering the skull was shaved and cleaned with alcohol swabs before then being placed in a stereotaxic frame (Kopf). A longitudinal incision was made to expose the skull and the bone, and underlying fascia was cleared in order to gain access to stereotaxic coordinates and locations for anchoring screws. Animals were implanted with microdrives containing 18-24 independently drivable tetrodes targeting the dorsal pole of the CA1 cell layer of the hippocampus (centered at 3.6 mm posterior and 2.6 mm lateral from bregma). Finally a screw was placed above the cerebellum to serve as a ground signal. Each tetrode was composed of four 12 um RO 800 wires (Sandvik Kanthal HP Reid Precision Fine Tetrode Wire; Sandvik). Tetrodes were plated with non-cyanide gold solution, via electrolysis in order to reduce impedance to between 180 and 220 k**Ω**. At the

conclusion of the surgery, all tetrodes were gradually lowered ~0.5 - ~1.5 mm into tissue. Upon recovery from anesthesia, animals underwent post-operative care for 3 days and received doses of Buprenex and cefazolin, as described above, two times a day (12 hour intervals). Animals were allowed to recover 1 week before behavioral testing commenced.

***Neural Recordings*.** Electrophysiological recordings for this project were collected on a 96 channel OmniPlex D Neural Acquisition System (Plexon). Each channel was amplified on head-mounted preamps by 1000x, and then amplified again 1000x before being digitized at 40 kHz. Spike data were bandpass filtered from 200 Hz to 8.8 kHz and local field potentials from 1.5 Hz to 400 Hz. Spike channels were referenced to a local electrode in the same region in order to remove both movement-related and ambient electrical noise. That local reference electrode was then referenced to ground and provided the LFP signal in the region. Action potentials of neurons were detected via threshold crossing and then sorted later using Offline Sorter (Plexon). Only putative pyramidal cells with an average firing rate of less than 10 Hz, and at least one spike for at least 5 sampling events were included in analyses. Cineplex Studio (Plexon) was used for capturing behavioral tracking data, and Cineplex Editor (Plexon) was employed to enter event markers and to verify animal tracking data. Between recorded training sessions tetrodes were advanced at a minimum of 40 um and positioned based on visual inspection of spike clusters in order to maximize neural unit yield.

***Histology*.** Upon completion of behavioral testing, rats were anesthetized with <5% isoflurane in oxygen. Anatomical recording sites were confirmed by creating a small lesion in the brain tissue by passing a 40 **μ**A current until the connection was severed. Immediately after

completion of electrolytic lesions, animals received an overdose injection (interperitoneal) of Euthasol (Virbac AH) and upon cessation of breathing were immediately transcardially perfused with ice cold 0.5% potassium phosphate buffered saline followed by 5% phosphate buffered formalin (VWR). Brains were then removed and placed in additional 5% formalin phosphate for at least 36 hours. Brains were then submerged in 30% sucrose for cryoprotection until sectioning into 40 µM thick sections via cryostat (CM 3050s; Leica Biosystems). Brain sections were processed using standard Nissl staining protocol in order to visually confirm tetrode-recording sites (Figure 1c).

Animal Training and Task. Once each animal recovered from surgery they were initially trained to dig for Froot Loop (Kellogg) bits in an aloe-scented pot over a cloves scented pot (both in sand). Once they reliably dug in the aloe pot and refrained from digging in the cloves pot, recordings began in the blocked-reversal task. Each recording session consisted of 6 blocks of 15 trials for a total of 90 trials. Within each 15 trial block the reward contingency was set so that one pot always had food and the other pot did not. Each trial started with the insertion of a divider so that the experimenter could place the pots in a pseudorandom position. Once pots were placed, the divider was removed and the animal was allowed to sample each pot, but only allowed to dig in one. Upon digging, the unchosen pot was immediately removed and the animal was allowed to dig until he found reward or gave up because he chose incorrectly. After either completion of reward consumption or a 3 second delay following pot removal, the rat was shuttled to the far half of the chamber and the divider replaced. The next trial commenced immediately until trial 15 was reached. After the last trial of each block, the rat was shuttled into

the side alley to wait for 60 seconds. After the break, the reward contingency was reversed and the next block of 15 trials was performed.

Quantitative Analyses: All analysis of the collected data were performed using custom scripts from MATLAB (MathWorks). ANOVAs were performed using the 'anovan' function in MATLAB under a standard type 3 sum of squares. For individual unit analyses, peri-event histograms were generated from 120 to 160 millisecond bins and smoothed using a moving average of a three bin span. Units with an average firing rate of >10 Hz across the whole recording session were assumed to be interneurons and were removed from all population analyses. For population analyses, trial rate vectors were constructed for each cell by averaging the firing rate across the two seconds surrounding the first sampling event on each trial, and then z-normalizing the rate across trials for each individual neuron. This z-transform was employed to prevent overestimation of correlation values due to highly active units. A population vector correlation matrix was generated for each rat by calculating the Pearson correlation of the population vector for each trial to each other (Supplemental Figure 2). That correlation matrix was then averaged across all sessions to generate the 'super rat' matrix observed in Figure 5. All measures of drift were constructing by first generating a mean value for each session onto which statistics were performed, and the mean +/- SEM across sessions was plotted. Bayes factors were obtained by inputting summary statistics into an online engine accessible at http://pcl.missouri.edu/.

Results

Rats performed a Temporally Blocked Object Discrimination Task

We recorded from 768 putative pyramidal cells across 25 sessions from 5 rats each implanted with a 24-tetrode hyperdrive aimed at dorsal CA1 (Figure 1C). Rats were trained to perform a blocked object discrimination task designed to segment memory into six 15 trial blocks (Figure 1A). Within each block of 15 trials, one of the two distinguishable pots was accompanied by reward. After each 15 trials, a temporal delay signaled the end of a block; the reward contingency was reversed to the other pot for the subsequent block of trials. Between blocks the rat was shuttled into a side chamber for one-minute so that the end of a block was signaled not only by an increased trial duration *per se* but also by an intervening experience. Individual trials took on average a little under 30 seconds to perform (26.0 +/- 0.40 sec), each block of trials took about 6 minutes (6.04 +/- 0.56 min), and a session lasted roughly 45 minutes (45.44 +/- 1.1 min).

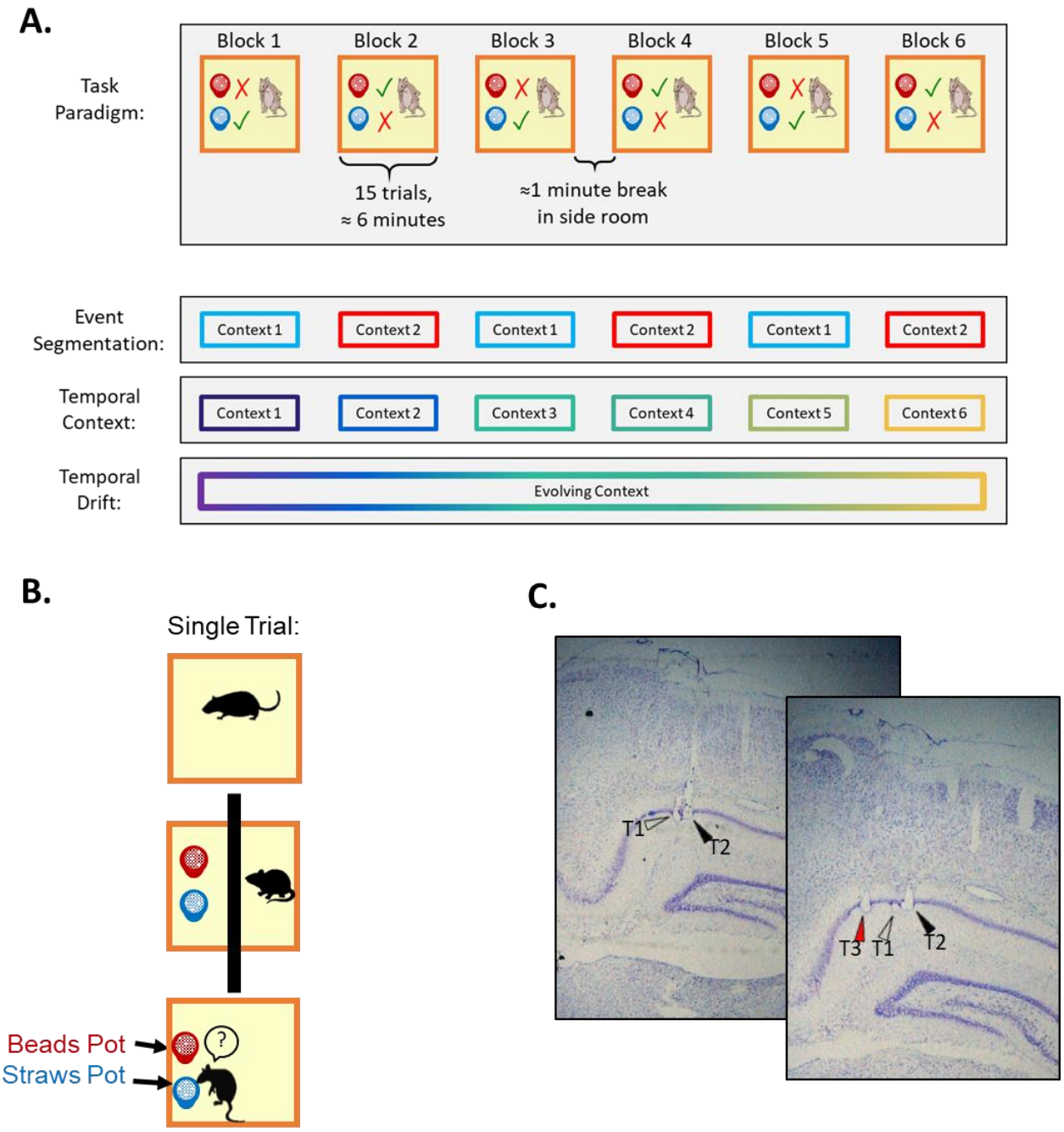

Figure 1. Task Design. A. Rats performed a blocked object discrimination task in which the reward contingency was held constant for 15 trials in a row and then reversed for the next 15. Each block took roughly 6 minutes, and each delay between blocks was fixed at 1 minute. B. Each trial consisted of three phases, a short ITI, the pot setup phase, and the sample/choice phase. C. Recording locations of three individual tetrodes. Arrowheads indicate final tetrode locations in the pyramidal layer of dorsal CA1.

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Rats performed as though each block of trials was a new episode.

Following pre-training on simple pot discrimination, rats took roughly a week to reach a criterion of 70% correct within a given session. Recording began after criterion was reached. Once trained, all rats performed similarly to each other (Mean across rats=80% +/- 1.97%, ANOVA, F(4)=0.1), and all rats performed similarly across days (Mean across days=79% +/- 0.52% ANOVA, F(7)=1.61) and blocks within a day (Mean across blocks=79%+/-1.13% ANOVA, F(5)=1.73). Typically, errors were concentrated around the beginning of each block, but were not restricted to the beginning of the recording session (Figure 2). We generated two alternative hypotheses regarding behavior. Our first hypothesis was that the rats would recognize the block transitions as a cue to change their response strategy, and would begin each block by switching their response by digging in the now-correct pot. Alternatively, rats could have ignored the boundary cue. In this case the animals would begin each block by erroneously perseverating with the (now-incorrect) response from the previous block. We evaluated these hypotheses at the group level, but also at the individual animal level (if some rats respected the contextual cues while others did not, the group could perform at chance on average despite none of the individual rats performing at chance). To investigate both hypotheses, we compared each rat's performance for each trial across blocks to chance by performing a binomial test. A two-sided binomial test asks whether the probability of correct responses was *either* above *or* below chance. Contrary to both hypotheses, no rat performed different from chance for each of the first two trials in each block (Figure 2, trial 1: 45.6%+/- 5.7%, Two Sided Binomial Test, trial 2: 50.4%+/- 6.21%), but rats were better than chance for all subsequent trials in each block (Figure 2, trial 3: 60.1%+/- 2.9%, Binom. $P<0.05$, trials 4 to 15: 87.7% +/- 2.2%, Binom. $P< 0.005$).

Overall, rats appeared to begin each block of trials by guessing, and then rapidly learning the new rule contingency. Thus, the performance of each rat on each block could be well characterized by a recency-weighted average over recent experiences. This accounts for the chance performance following the long inter-block delay and also the learning of the new reward contingency within block (Figure 2 Right).

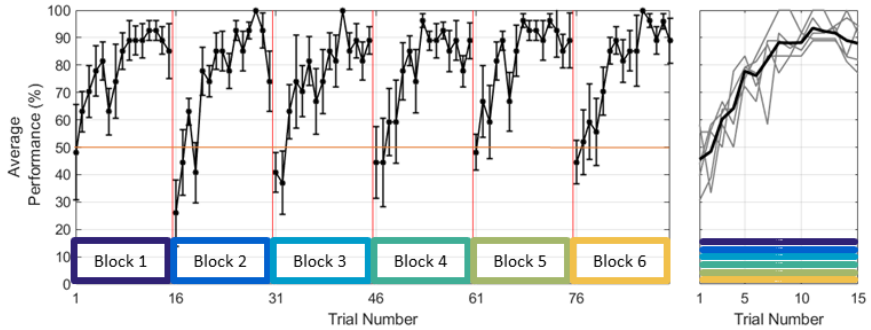

Figure 2. Rats Behaved in a Stereotyped Manner. **Left**: Mean +/- SEM performance throughout each recording session. Rats Began each block in a session at chance regardless of the position of that block in the session. **Right**: No rat performed Better or worse than chance at the beginning of each block. Each color represents one rat while black represents mean across all rats. All rats behaved as though each block represented a novel context in which to learn the rule contingency.

Single units code temporal structure.

We examined single unit activity by generating peri-event rasters and histograms centered on pot-sampling events. Figure 3 illustrates the firing fields we anticipated under two hypotheses. Event segmentation predicts that hippocampal cells would generate a stable firing field in response to one object in one place under one rule condition but a different firing field for the object in the other rule condition (Figure 3 left). Temporal context predicts that cells would respond to one object in one position but only for a transient number of trials (Figure 3 right).

Figure 4 illustrates typical examples of hippocampal single-unit responses to task features. Many cells fired robustly during sampling events but only responded during events in a

specific place, or when sampling a specific object. Individual units showed firing fields in response to multiple combinations of objects and places that were not captured by a single coding dimension. Example Cell 1 (Figure 4: panel 1) fired just before the rat sampled either object at the left location, and most robustly during the second block of trials. Example cell 6 (Figure 4: panel 6) exhibited one firing field during the third block just after the rat sampled object 2 on the right side, and then exhibited a second firing field in block 6 as the rat sampled either object on the right side. There were also cells that coded for behaviors consistently across blocks. Example cell 3 (Figure 4: panel 3) fired during sampling events on the right side during every block of trials. To exclude the possibility that cells were drifting due to acquisition error, we carefully examined the spike clusters from each tetrode as a function of time, and excluded any tetrodes that included clusters that were not stationary with regard to time (Manns et al., 2007). Supplemental Figure 3 shows typical unit clusters divided into each of the six blocks of the recording session. The single unit data therefore suggested that the hippocampal representation of the task exhibited a slowly evolving code for temporal context.

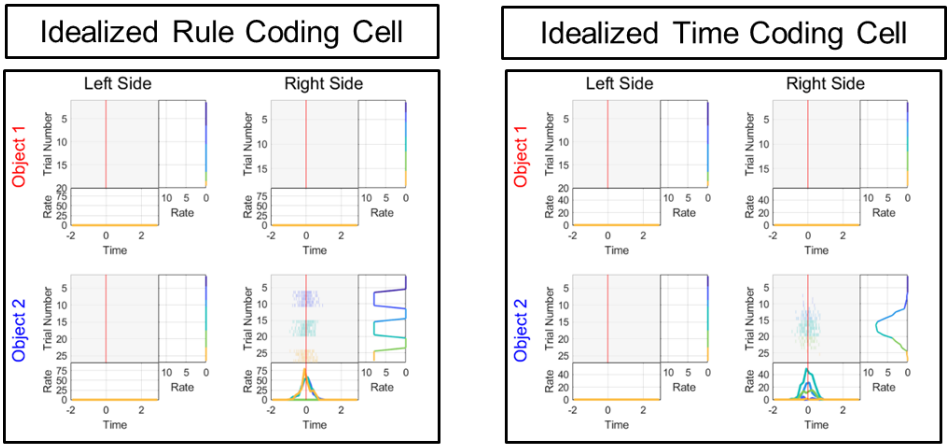

Figure 3. Idealized firing fields of dCA1 units in the temporally blocked object discrimination task. Left: Ideal cell that responds selectively to one object-position combination, and does so only under one rule contingency. Right: Idealized object-position conjunctive cell that responds only during one period of time during the task. Each idealized cell provides information about the object, position, and temporal structure of the task.

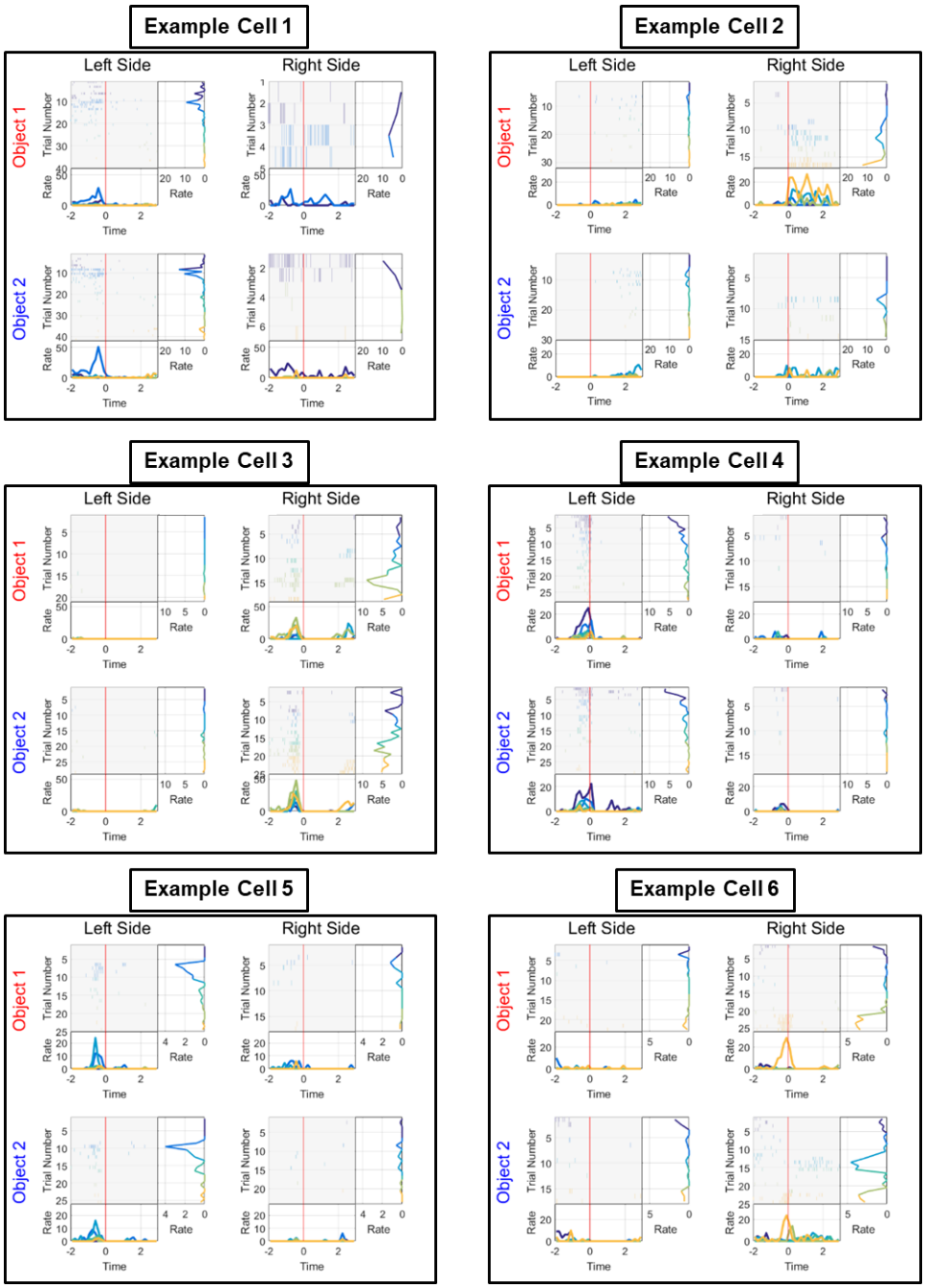

Figure 4. Single units code for places, objects, and temporal context. Peri-event rasters and histograms for six example cells. Time refers to seconds relative to the start of object sampling, and firing rate is in Hz. Rasters and line plots are color-coded by the block in which the trial occurred. Each of these cells responds to specific combinations of time, position and object in a complex manner. For example, example cell 1 responds just before samples on the left side, and does so most rigorously during the second block of trials. This cell exemplifies conjunctive coding for place and block. Similarly, example cell 2 responds to object B on the right side, and only does so later in the session. This cell conjunctively codes for what, where, and when.

Ensemble activity suggests hippocampal activity patterns slowly drift.

Event segmentation predicts that the hippocampus generates two stable representations, one for each rule condition (Fig 5 top left). Temporal context predicts that ensembles slowly change, and new representations would be continually generated (Figure 5 bottom left). To compare these hypotheses, we constructed ensemble firing rate vectors from the average firing rate during the two seconds surrounding the sampling event on each trial. We then z-normalized each units rate across trials, and then calculated the Pearson correlation of the population vector from each trial to all others to generate a correlation matrix for each rat (Supplemental Figure 2). We then averaged that matrix across all animals to generate a grand mean correlation matrix (Figure 5 Right). The empirical pattern of ensemble activity showed strong support for the drift characteristic of temporal context but no apparent evidence that representations from the distant past were reinstated. We then quantified the strength of drift in the HPC population as it related to factors including behavior, response outcomes, and temporal interval.

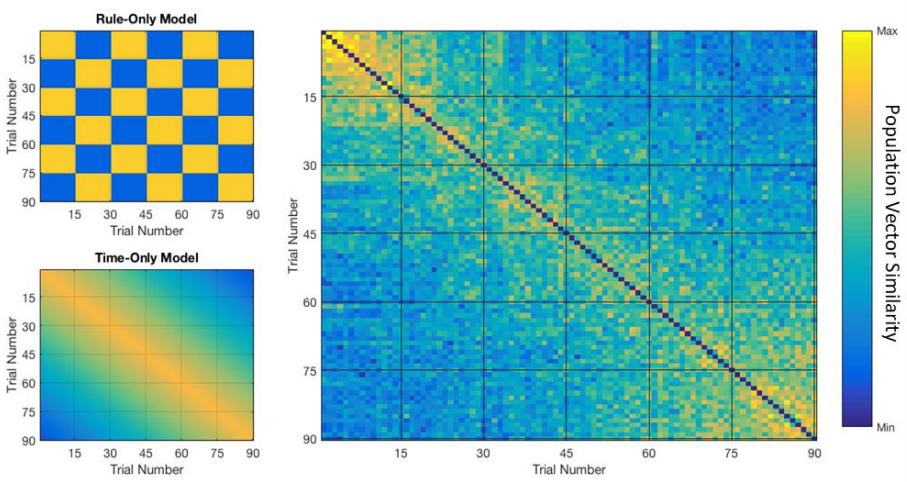

Figure 5. Observed correlation matrix suggests continuous temporal drift. Left above: Predicted correlation matrix if cells map two stable rule states. Left bottom: Predicted correlation matrix if cells map time. Right: Empirical correlation matrix more closely resembles predicted matrix under the hypothesis that cells track time even though task conditions repeated every 15 trials. Firing rates for each cell were constructed using a 2 second sampling window surrounding the first sampling event on each trial and then z-normalizing across trials. A correlation matrix was generated from the Pearson correlation between ensemble rate vectors on each trial to each other trial for each recording session, and the average across all recording sessions is shown.

Hippocampal Populations showed drift within block

The behavioral recency effect would predict a memory signal that drifts within a block of trials. We assayed the change in population activity at each trial lag within the 15 trial blocks (Fig 6A). We found significant temporal drift in the representations of the same object and position combination within the 15 trial blocks, (Figure 6A) (Observed slope= -0.011, observed data exceeded all 10,000 perms, perm. =-7.28 $x10^{-6}$, σ=1.8$x10^{-3}$). This drift was apparent in the active ensemble regardless of which object the rat was sampling or where he was (observed slope=-7.8$x10^{-3}$, observed data exceeded all 10,000 perms, perm =-8.16 $x10^{-6}$, σ=8.4$x10^{-4}$). This drift occurred individually in each rat tested except for Rat 3, likely because of too few data points (Rat 3 included 2 sessions with 12 units total, observed slope=-0.011, observed data exceeded 716/1000 perms, perm x=-1.24 $x10^{-4}$, σ=0.0076).

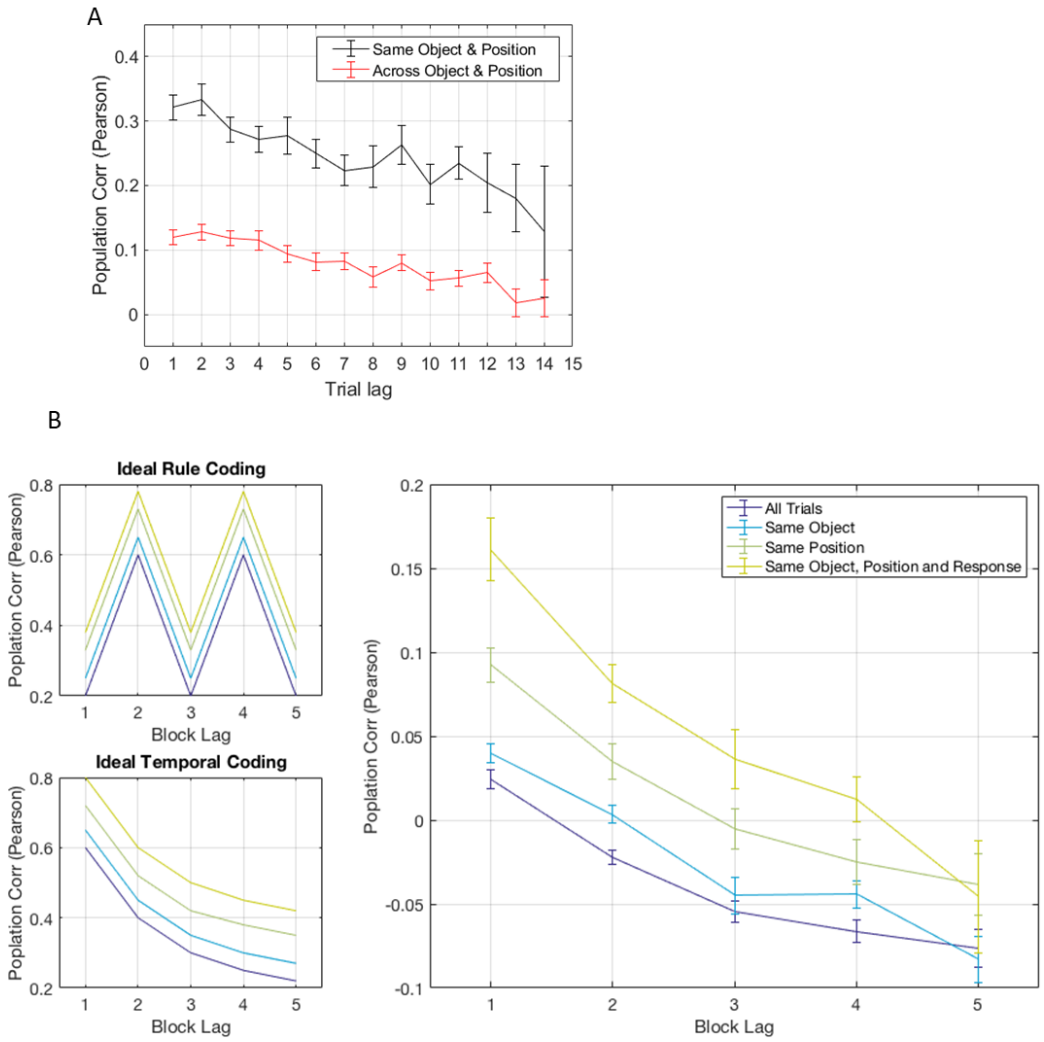

Figure 6. Ensembles drifted within each block and across blocks without segmenting experience. A. Ensemble correlations reliably fell as trial lag within each block increased. This effect was evident both in the overall population activity (red line) and in the ensemble coding the same object and position (black line). B. Top left. Ideal curves under event segmentation hypothesis. Event segmentation predicts alternation in correlations resembling a zig-zag. Bottom Left. Ideal curves under the contextual drift hypothesis. Contextual drift predicts a monotonic decrease in each curve. B. Right. Observed curves for ensemble correlations fell as the block lag between the trials grew. This was true for the overall population (purple line) as well as in the ensemble coding the same object (blue) or position (green) or same object, position and behavioral response (yellow). All curves show a systematic decrease from small to large lags with no obvious alternation. To the extent that there are fluctuations in the curves, these fluctuations were small and not consistent across different types of comparisons.

Hippocampal Populations showed drift across blocks

We then sought to quantify the manner at which the population of hippocampal units changed between each block of trials. If hippocampal ensembles reflected the two rule states, hippocampal activity would be more similar between blocks that shared a rule condition, versus those that involved opposing rule conditions (figure 6B top left). Conversely, if hippocampal ensembles tracked time, then hippocampal activity would become progressively less similar between blocks at increasing lag (Figure 6 bottom left.) We found that the overall population correlation between blocks consistently fell as the block lag grew (Figure 6 B Right, purple line)(across all trials slope=-0.025, Observed slope exceeded all 10000 perms, permutation mean $-2.38\text{x}10^{-7}$, σ=0.0034). The representation for the same behavior (e.g. same object, position, and response) also progressively decorrelated with block (Figure 6B, right, yellow line) (Permutation test: slope=-0.048, the observed slope exceeded all 10000 bootstrapped permutations, permutation x=$-1.5\text{x}10^{-5}$, σ=0.0073). All empirical curves resembled the predictions of ideal temporal coding. Note that deviations from the smoothly decreasing curves are small-any contribution from discrete event coding would have to be much smaller than the contribution due to gradually-changing temporal context. Moreover, event segmentation would predict that the deviations from a smooth curve should be consistent from one type of comparison to the other, resulting in the parallel curves Figure 6B (top left). However, to the extent there were deviations in the different empirical curves, they were not systematic across the type of comparison. Thus, consistent with behavior, we saw no evidence that hippocampal populations coded blocks into discrete segments based on the two rule conditions.

Finally, the representation of the delay in between blocks also progressively changed across time (Slope of correlation vs. lag=-0.076, observed slope exceeded all 10000 perms, perm

mean=-3.6 x 10-3, σ=0. 017). We observed strong temporal drift in the whole population as well as in the representation of the same object and position that persisted into the inter-block-interval, and there was no sensitivity to the repetition of the rule conditions.

Hippocampal shifts between blocks can be accounted for by time.

We observed continual drift in the hippocampal population that seemed to ignore the contextual cues. However, behavior indicated that the animals responded to the contextual boundaries because they did not perseverate across the block transitions. This could either be because the boundary cue induced a separation in hippocampal representations or that the separation in representations was merely a consequence of the extended time between the two trials spanning the delay. We first sought to assay whether block transitions induced a separation in representations by correlating trials in adjacent blocks and comparing those to trial pairs in the same block. To do this we compared representations of the same object and position and plotted each pair by their trial lag, and then colored them based on block distance (Figure 7A). The correlation between trials in the same block was significantly greater than that in adjacent blocks but at the same trial lag (JZS Bayes T-Test yielded a Bayes Factor strongly in favor of the alternative, odds ratio 400:1). This suggests that the transition between blocks induced a separation between representations of trials that happened in different blocks. However, this separation may be due to the long one-minute delay between the trials that would not reflect active segmentation of experience. To address whether the extended time fully accounted for the apparent boundary effect on the hippocampus, we plotted the correlation between trials by their temporal lag and then organized pairs by whether there was a block transition between them (Figure 7B). When we compared trial pairs in the same block with those in adjacent blocks in

this way, we found that the block break caused no more reduction in the population vector correlation than would be expected by elapsed time (JZS Bayes T-Test yielded a Bayes factor strongly in favor of the null, odds ratio 31:1). Thus the effect of the block transitions on hippocampal activity was completely eliminated by accounting for the extended time lag.

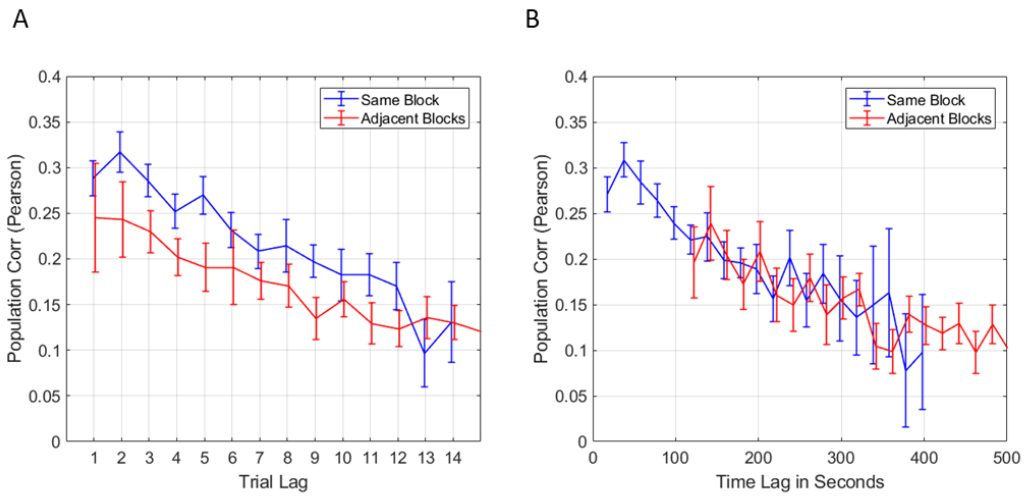

Figure 7. Time fully accounts for shifts in population state across blocks. A. Population vectors within a block (blue) were more similar than those in adjacent blocks (red) when trial lag was considered. B. Population vectors within block (blue) were *not* more similar than those in adjacent blocks (red) when time was considered. Population vector correlations were lower in adjacent blocks, time was sufficient to account for this decrease. Population vectors were constructed in the same manner as above.

Discussion

In this experiment we sought to determine how the hippocampus codes for behaviorally-relevant context in a task in which there are no external cues available to distinguish the rule contingencies. Event segmentation accounts predict that animals would have been able to parcellate experience into discrete episodes based on contextual boundaries; temporal context accounts predict that the hippocampal representation should provide a continuous relational metric that progressed consistently across time. We provided rats with a temporally blocked experience such that a boundary event (removal from the testing chamber) cued a change in the

reward contingency. Importantly, the context boundary was a transient cue that signaled a change in which object was rewarded, but was not associated with an external cue that would be available to the animals as they made the choice.

Behavior didn't show event segmentation, only memory for the recent past

Interestingly, well-trained animals neither perseverated across cued block transitions, nor did they anticipate the rule change. Instead, every rat we tested used a strategy that appeared to involve a memory of only trials in the recent past. During the block transitions, all animals behaved as if they forgot the past rule condition and began each new block at chance. As each block progressed, accuracy gradually increased, as if the rat remembered recently experienced rewarded events. Overall, the animals responses were based on a temporally graded average of past trial outcomes analogous to the recency effect observed in human memory experiments, wherein memory is strongest for items presented most recently (Glenberg & Lehmann, 1980; Murdock Jr., 1962).

The hippocampal map did not show evidence for segmentation, but changed gradually across time scales

Consistent with the behavioral results, hippocampal populations showed gradual drift across time. Gradual population drift was observed within blocks as well as across blocks. There appeared to be an increase in drift between trials separated by a block boundary, but the degree of drift could be accounted for by the passage of time between blocks. These data add to a growing body of literature that suggests that hippocampal representations change with time. Importantly, this type of temporal drift has been observed after ruling out tetrode drift and total

activity of the population in experiments employing a variety of recording methodologies (Cai et al., 2016; Emily A. Mankin et al., 2015; Manns et al., 2007; Mau et al., 2018; Rubin, Geva, Sheintuch, & Ziv, 2015; Ziv et al., 2013). Because drift is observed with recording techniques ranging from tetrode recording to calcium imaging to immediate early gene expression, this reduces the possibility that it is simply a measurement artifact---all of the recording methodologies would have to have independent artifacts that happen to produce the same results. This raises the possibility that population drift reflects a functional correlate of hippocampal processing.

There was no evidence of event segmentation

In this experiment we found no support for event segmentation in either behavior or in the activity of hippocampal ensembles. Experiments that did observe segmentation used spatially distinguishable contexts (Komorowski et al., 2013, 2009) or overt external cues to discriminate the context, such as an ambient sound, odor, or object (McNaughton et al., 1996; Zaremba et al., 2017). The task in the present experiment was specifically designed to be devoid of such ambient cues, as a neural response to that cue could be mistaken for a new context signal. Other tasks generated contexts consisting of separate behavioral tasks or event sequences in the same chamber (Bower, Euston, & McNaughton, 2005; Ferbinteanu & Shapiro, 2003; Markus et al., 1995). This experiment differed in that context was not designated by spatial environment, and the reward locations did not differ between contexts (Kobayashi, Nishijo, Fukuda, Bures, & Ono, 1997; Markus et al., 1995).

In some sense, the null results of the present experiment are comparable to those found in a separate experiment in which rats were unable to distinguish separate spatial contexts. Wood et

al found that when animals had trouble distinguishing between spatially similar contexts, the hippocampal representations across those contexts was also similar (Grieves, Jenkins, Harland, Wood, & Dudchenko, 2016). The congruent behavioral and neural data in this experiment suggest a similar interpretation: the rats failed to recognize there were two alternating rule conditions in this task, and hippocampal ensembles showed no rule based segmentation. The lack of such an effect across these two paradigms places an important boundary conditions on the circumstances under which event segmentation can occur.

Event boundaries failed to separate memory traces

We found that imposing a boundary cue, in this case the removal from the task environment caused a separation in memory traces such that performance fell to chance on the next trial. Studies in humans suggest this effect is caused by increased hippocampal activation coinciding with recognition of an event boundary (Baldassano et al., 2017; Swallow et al., 2011). This hypothesis has been recently confirmed in the rodent in a similar context-guided paradigm where space served to contextualize experience (Place et al. 2016). Other experiments have found contextually dependent firing in the same physical space when context was defined as the immediately preceding sequence of places or events (Allen et al., 2016; Wood et al., 2000). Insofar as such cues can induce event segmentation, one might have expected that leaving the testing chamber between blocks would have been a sufficiently salient change to induce event segmentation.

However, our animals failed to recognize that removal from the environment signified any change in context. In other words, in our experiment the boundary cue caused no extra separation in hippocampal traces over what occurred as a result of time. Our study aligns more

with those that conclude such context dependent firing is a subtle phenomenon that may be sensitive the location of the reward itself (Bower et al., 2005). Recent modeling work suggests a different interpretation: that event segmentation may occur when the actor detects shifts in the latent cause of a situation (Gershman, Monfils, Norman, & Niv, 2017). This suggests that a defining feature of context is the latent cause structure attached to that experience. The behavior suggests that our animals failed to track the latent causes in this experiment, and therefore we hypothesize they were unable to segment experience based on shifts in latent cause. This would explain our failure to observe either neural reinstatement or neural segmentation in this experiment.

Segmentation might be achieved across time by using more explicit or salient contextual boundaries. The shaping procedure in rodent experiments has a profound effect on context disambiguation in the hippocampus (Bower et al., 2005). We hypothesize that given the opportunity to explicitly learn the two rule states, rats may hold an internal representation of context after removal of explicit cues and therefore continue to exhibit event segmentation in their absence.

Time expressed in the MTL

The drifting representation found in this experiment provide a continuous dimension for relating experiences and is a viable mechanism for tracking the temporal relationships of events across macrotime (Howard Eichenbaum, 2017). In this experiment, many individual cells had firing fields at the point within a trial when the rat sampled the objects, but that firing field was also organized around a set of trials over the experimental session. Thus, objects and places were represented in a framework that was nested in time (MacDonald, Carrow, Place, & Eichenbaum,

2013). This temporal modulation of experience is analogous to what are called ‘time cells,’ that have been observed over shorter time scales, and may be read out in a similar manner. Each provide a map that includes not only the stimuli around the animal, but also a graded similarity to past instances of those stimuli. In other words, single unit firing fields provided information about the three components of episodic memory: events, places, and time. Furthermore, recent reports suggest that hippocampal activity may multiplex routines in time as well, such that the representation of small repeated routines can be nested in that on a larger scale via drift over hours to days (Mau et al., 2018). Indeed hippocampal activity may leverage this coding scheme to track hidden temporal structures between recurring events (Schapiro et al., 2016). Thus, temporally modulated drift may be a central mechanism for the generation of a spatiotemporal scaffold onto which events and places may be bound and related.

Acknowledgements:

This project was originally conceived and designed in part by Howard Eichenbaum. Sadly, he passed away before the results were obtained and therefore was unable to evaluate this manuscript. The authors gratefully acknowledge his many contributions to this experiment and inspiration we’ve derived from his leadership.

Competing interests

The authors cite no competing interests.

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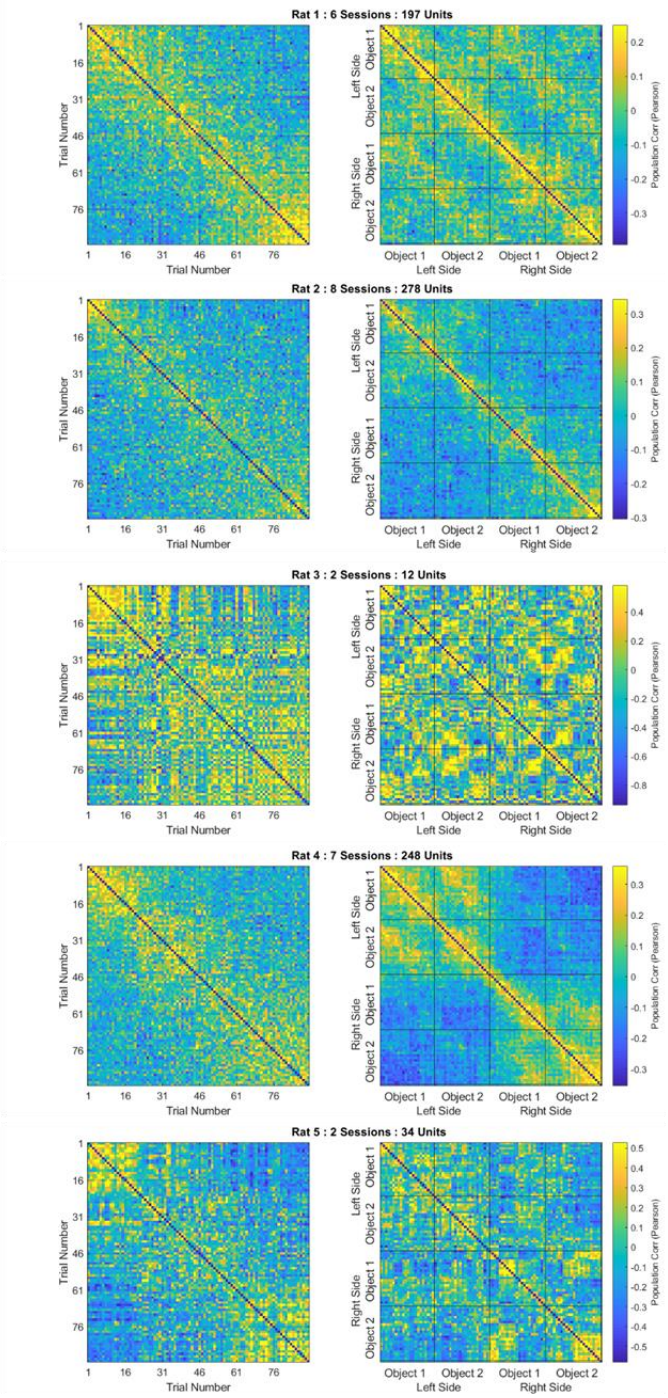
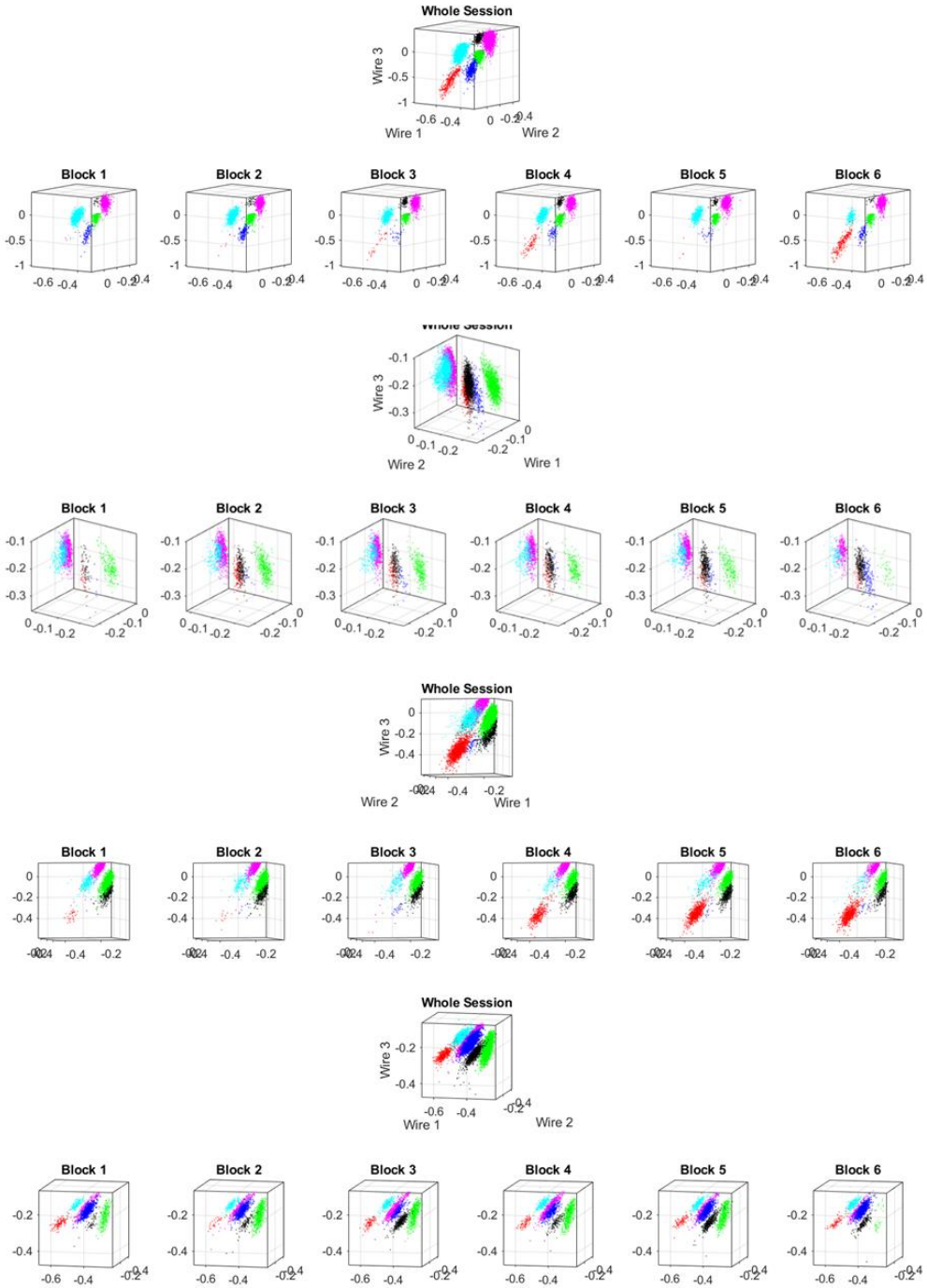
Supplemental Material



Supplemental Figure 2. Trial-by-Trial correlation matrix for each rat shows temporal drift. Similarity was calculated by generating z-normalized firing rate vectors for each cell across sampling events, and then calculating the spearman's rho correlation value between the population vectors on each trial. Left matrices were sorted by trial number, right matrices by position, then by object, then by trial number.



Supplemental figure 3. The drift observed in single units was not an artifact of clustering technique. Four example tetrodes containing units that contributed to temporal drift, but whose waveforms did not drift. Data points represent spike amplitude on each of three wires, and each color represents spikes originating from the same putative neuron. No clusters systematically moved in 3-D space.