Context-dependent incremental timing cells in the primate hippocampus

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We examined timing-related signals in primate hippocampal cells as animals performed an object-place (OP) associative learning task. We found hippocampal cells with firing rates that incrementally increased or decreased across the memory delay interval of the task, which we refer to as incremental timing cells (ITCs). Three distinct categories of ITCs were identified. Agnostic ITCs did not distinguish between different trial types. The remaining two categories of cells signaled time and trial context together: One category of cells tracked time depending on the behavioral action required for a correct response (i.e., early vs. late release), whereas the other category of cells tracked time only for those trials cued with a specific OP combination. The context-sensitive ITCs were observed more often during sessions where behavioral learning was observed and exhibited reduced incremental firing on incorrect trials. Thus, single primate hippocampal cells signal information about trial timing, which can be linked with trial type/context in a learning-dependent manner.

hippocampus | time | primate | associative learning | behavioral learning

A fundamental feature of episodic memory is recalling the temporal sequence of events within an episode. Many studies have shown that the hippocampus plays a critical role in such episodic memories in both humans (1–3) and animals (4, 5). For example, the hippocampus is preferentially active in humans tasked to remember the previous order of familiar objects (6, 7), encode sequences of word triplets (8), or learn sequences of key tapping during a reaction time task (9). Further, damage to the hippocampus in humans causes deficits in remembering the temporal order of contextual events in a virtual town (10) and the grouping of sets of words (11). Similarly, animal studies report selective bilateral damage to the hippocampus impairs memory for the sequential order of items in sequence (12–14).

Multiple studies have examined the neurophysiological underpinnings of memory for sequential order in the hippocampus. One study in rodents reported that a population of simultaneously recorded hippocampal CA1 cells signaled timing during an olfactory temporal order memory task by gradual changes in their population response (15). Similarly, populations of primate hippocampal cells signaled incremental timing across a delay interval during a task where subjects were required to remember both what items were shown and in what order (16).

Other work in rodents suggests that timing may be supported by individual hippocampal "time cells" that are tuned to particular time intervals within a delay period (17–21). One study attempted to disambiguate the influences of location, time, and distance of these time cells and found that many hippocampal neurons could be differentially separated into one group influenced by time and another group influenced by distance (18). Another study showed that time cells still tracked across a delay period even when rats were not actively locomoting (19).

In a previous study, we reported an incremental timing signal in a population of primate hippocampal cells during the performance of a visual temporal order memory task, where animals were required to encode the sequential order of known objects before and after a delay period (16). Here, we sought to characterize this signal further in the primate hippocampus by asking if similar incremental timing patterns were seen as subjects performed an object-place (OP) associative learning task (22). In this task, animals first saw one of four possible OP combinations (Fig. 1*B*). Each combination was made up of one of two possible novel objects (O1 or O2) in one of two possible spatial locations (P1 or P2) on a computer screen. Each OP combination was associated with a bar release in one of two temporal windows indicated by an orange (early) or green (late) dot on a screen (Fig. 1*A*). With trial and error, the animals learned which particular OP combinations were associated with which temporal window to release the bar for reward. We identified a subset of individual primate hippocampal cells that incrementally increased or decreased their firing across the delay period of this associative learning task. We refer to them as incremental timing cells (ITCs). A subpopulation of these ITCs was selective to particular trial contexts (i.e., release type, OP combination). We also explored how learning and task performance were encoded in these cells, and discuss the similarities and differences between the primate ITCs and rodent time cells (17–21).

Results

Definition and Classification of ITCs. We recorded from a total of 152 hippocampal cells during the performance of the OP associative learning task. Here, we focus on activity during correct trials for the 139 cells with a firing rate (FR) of at least 1 Hz during the delay period of the task (error trials are analyzed separately later). Initial inspection of this population showed that many cells changed their FR over time during the delay period (Fig. 2 *A* and *B*). We first asked if these delay-active cells incrementally increased or decreased in FR across the delay interval, similar to the pattern of activity previously shown to

Significance

Episodic memory refers to the ability to recall specifics of past events in our lives. An essential aspect of events is timing when things occur during an episode. A number of recent studies have shown that the hippocampus, a structure known to be essential to form episodic memories, possesses neurons that explicitly mark moments in time. We add a previously unidentified finding to this work by showing that individual primate hippocampal neurons not only track time, but do so only when specific contextual information (e.g., object identity/location) is cued. These time context-sensitive neurons represent a novel way in which the brain unites disparate streams that comprise an episode and will aid in our understanding of how we store and retrieve episodic memories.

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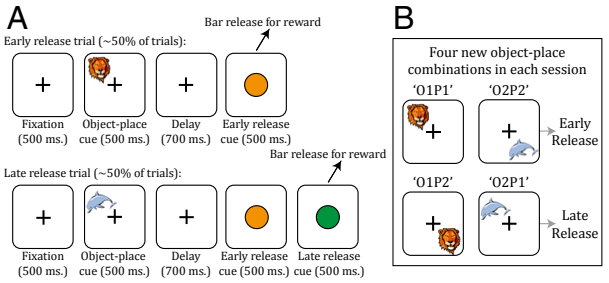

Fig. 1. Task outline. (*A*) Each trial of the OP association task begins by requiring the subject to fixate for 500 ms while holding a bar. One of four OP cues is then presented for 500 ms, which is followed by a 700-ms delay period. At the end of this delay, an orange dot is shown. The primate decides to either release the bar during this "early release" period (*Top*) or hold the bar throughout the 500 ms the orange dot is shown. (*Bottom*) In this case, a second, green dot is shown. Bar release during the 500 ms the green dot is shown constitutes a "late release" trial (*Bottom*). Animals learn which OP combinations are correct for early and late release by trial and error. (*B*) This diagram outlines the four OP combinations and the correct release required for reward. Two novel objects were presented in two novel places during each session. O1P1 and O2P2 were correct for early release, whereas O1P2 and O2P1 were correct for late release. Because the same object in a different place required a different bar release, the primate was required to learn the OP association and could not perform better than chance by memorizing objects or places separately.

represent relative time to and from cued events during the performance of a temporal order memory task (16). We hypothesized that similar neural activity from single cells recorded during the delay period of the OP associative learning task could provide accurate information about incremental timing. To explore this possibility, we constructed peristimulus time histograms (PSTHs; $\sigma = 20$ ms) for each cell and examined if they had similar firing patterns across the delay for all four trial types of the OP task (Fig. 1*B*) as this previous study found a population of ITCs with delay activity that did not differentiate between trial types (16). We used a Wilcoxon rank-sum test on the 35 time bins (700 ms) during the delay period between the four trial types for each cell and found 61 of 139 cells did not significantly change their FR between trial types ($P > 0.05$). We next tested if these cells that were nonselective to trial type incrementally increased or decreased their FR across the delay. To assess monotonicity, we used an F test with resampling methods on the PSTH of all trials to determine which were fit significantly better by a first-order polynomial compared with a zeroth-order polynomial ($F >$ 95% of F values for 2,000 randomly resampled distributions; *SI Methods*). Of the 61 cells, 16 exhibited a linear rise ($n = 9$) or fall ($n = 7$) in FR across the delay period (Fig. 2 *A* and *B* and Fig. S1 *A* and *B*). Like the population response reported in the temporal order memory task (16), these hippocampal cells incrementally changed their FR during the delay period similarly for all cues and trial types. We refer to these 16 cells as "agnostic" ITCs.

One possible alternative to the agnostic ITCs tracking time is that they signal anticipation of reward or a decision. Because previous such anticipatory neural signals all increased in FR (23–25) and seven of 16 of our agnostic cells decreased incrementally over the delay period, anticipation of reward/decision cannot explain all of the agnostic ITCs. However, we still considered the possibility that the remaining nine of 16 increasing agnostic ITCs could signal anticipation. To address this possibility, we took advantage of the fact that half of the trials are "early release trials" rewarded immediately when the animal made a choice after the delay interval, whereas the remaining trials were "late release trials" that required the animal to continue holding through the orange dot period until reward was given for a correct decision at the beginning of the green dot period (Fig. 1*A*). If these cells represented anticipation of reward, we hypothesized that on late release trials, these cells should continue firing incrementally through the duration of the orange dot period until reward was given at the beginning of the green dot period (hypothesis *i*), whereas on early release trials, incremental firing during the delay period of the task should peak when reward was given in the orange dot period (hypothesis *ii*). To test hypothesis *i*, we asked if activity on late release trials during the orange dot period was higher than activity of these same trials during the delay period. Three of nine cells showed significantly higher FRs during the orange dot period than during the delay (Wilcoxon rank-sum, $P < 0.05$) (23). If these three cells showed anticipatory signal as previously described (24), we would also expect the early release trials to decrease their FR after the 500 ms orange dot period immediately after the delay period because they no longer would be firing in anticipation of reward (hypothesis *ii*). Instead, as shown in Fig. S2, the early release trials (orange and red) for all of these cells continued to fire strongly through the orange dot period, making it unlikely these are true anticipatory neurons.

Another possible interpretation of the seven of 16 agnostic ITCs that decrease their FR during the delay period is that the incrementally declining activity represents a gradual dissipation of a prominent visual response during the OP presentation period (26, 27). If this were true, we would expect the FR across the cue period to be significantly stronger than during the delay period. We found only one of seven cells had a significantly higher FR (Wilcoxon rank-sum) during the cue period than during the delay. Therefore, on a whole, the majority of agnostic

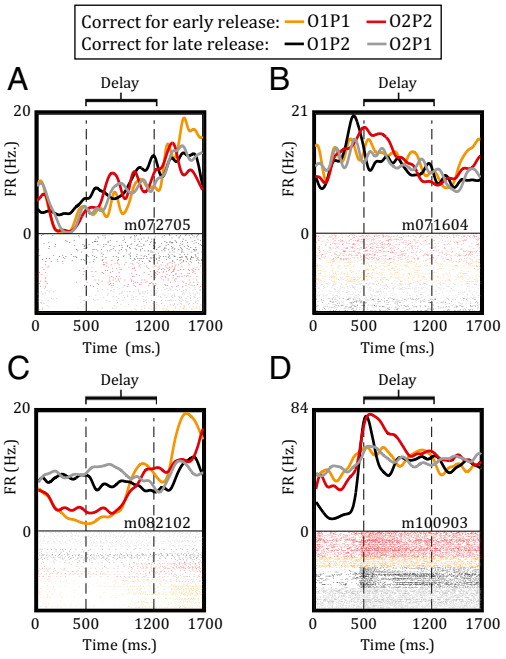

Fig. 2. Example ITCs. (*A* and *B*) Two example agnostic ITCs are graphed for the cue, delay, and 500 ms of the postdelay period divided by the vertical dashed lines. Shown are the probability density functions (PDFs) separated by each trial type with the corresponding raster plots of each trial organized by color on the bottom (trials earlier in the session start on the bottom of each color). Agnostic ITCs show a similar firing pattern for all trials across the delay period. (*C*) Example release-selective ITC. Trials correct for early release (O1P1 in orange and O2P2 in red) incrementally change their FR across the delay. Trials correct for late release (black and gray) also show an incremental change in FR, but with a declining pattern. (*D*) Example OP-selective ITC. Only the trials correct for the O2P2 trial type (red) show an incrementally changing response across the delay.

ITCs (12 of 16) cannot not be explained by either anticipatory or cue dissipating signals.

Next, we turned to an even larger population of 78 of 139 cells that changed their FR for different trial types. To identify which major task component (i.e., release type, object identity, place, specific OP combination) was most responsible for these trial-by-trial differences in FRs, a line was fit to a separate PSTH for each of the four trial types for each cell as a simple measure of changing FR across the 35 time bins of the delay and the absolute value of the slope was used to quantify the degree of change. Using a three-way repeated measures ANOVA on the resulting slopes, we found that release type (i.e., early vs. late) and the interaction between object and place (i.e., one of four individual trial types) were the two significant components responsible for the trial type–specific change in FR during the delay period (release, $P = 0.023$; object × place, $P = 0.023$; Fig. S3*A*). Two alternative statistical approaches that examined incremental timing signals in cells individually also showed more cells selective to release as opposed to object or place (Fig. S3 *B* and *C*).

We characterized the differential effects between release type and individual trial type using resampling methods (20, 28) because standard statistical tests were not able to differentiate among all possible changing firing patterns across the delay period PSTHs (Fig. S4). Of the 78 of 139 cells that differentiated between trial types, we found 43 cells with significantly different delay activity between early and late release trials but without significantly different firing patterns for the two OP combinations correct for each of these release types. We then performed an *F* test with resampling methods as described above to determine if this release-selective delay activity incrementally changed over time. These methods identified 21 "release-selective ITCs" that showed an incremental rise ($n = 10$) or fall ($n = 11$) in FR across the delay period selective to either early release trials or late release trials (Fig. 2*C* and Fig. S1 *C* and *D*). For example, as shown in Fig. 2*C*, OP-selective O1P1 (orange line) and O2P2 (red line) are both correct for early release and show similar rising firing patterns, whereas trials correct for late release (black and gray lines) show distinct firing patterns. Notably, two of the 19 neurons with a release-selective incremental timing signal showed this difference for both early and late release trials, totaling 21 total release-selective incremental timing signals. The two cells that display an incremental timing signal to both types of release trials have distinct, but also incrementally changing, firing patterns to each group of early and late release trials (e.g., Fig. 2*C* incrementally increases for early release trials and incrementally decreases for late release trials).

We also considered alternative interpretations of release-selective ITCs other than incremental timing (details are provided in *SI Methods*). Briefly, searching for possible anticipatory responses, we identified six release-selective ITCs that were selective to late release trials and continued to fire strongly through the orange dot period. We also found that none of 11 of the declining release-selective ITCs showed enhanced responses during the cue period, indicating that these ITCs do not show a dissipation of visual response. Therefore, the majority of release-selective ITCs (15 of 21) cannot be explained by anticipatory or cue dissipating signals.

Using the same resampling strategy as described for release-selective ITCs, we next characterized incremental firing changes unique to one of the four individual trial types. We identified 31 of 139 cells that fired significantly differently to particular OP combinations (with a minimum of 15 trials). Of these cells, 26 incrementally increased ($n = 10$) or decreased ($n = 16$) their FR across the delay for at least one of the four OP associations and were labeled "OP-selective ITCs" (Fig. 2*D* and Fig. S1 *E* and *F*). For example, Fig. 2*D* shows a cell that incrementally decreases its FR for only O2P2 trials. The majority of these OP-selective ITCs (20 of 26) only showed this incremental change for a single OP combination. For the remaining six of 26 OP-selective ITCs, distinct incremental timing signals were found for two OP associations for five of these cells and three OP combinations for one cell (e.g., Fig. S1*E* incrementally rises for O2P2 trials and incrementally falls for O1P1 trials). Summing these totaled 33 OP-selective incremental timing signals for the 26 OP-selective ITCs.

We looked at alternative interpretations of OP-selective ITCs other than incremental timing (details provided in *SI Methods*) and found that only one of 33 OP-selective ITCs showed a potential anticipatory response by rising in FR through the delay into the orange dot period. We also found eight of 33 OP-selective ITCs that decreased in FR from the cue period into the delay period, indicating possible dissipation of a cue-evoked response (an example of a possible cue-dissipating cell vs. a counter-example is shown in Fig. S1*F* vs. Fig. 2*D*). Therefore, the majority of OP-selective ITCs (24 of 33) cannot be explained by anticipatory or cue dissipating signals.

We next asked if there were differences in the distributions of the ITCs anatomically. We found no obvious spatial order of the ITCs (Fig. S5). In particular, there was no significant difference between each type of ITC along the anteroposterior axis of the primate hippocampus, which is analogous to the dorsoventral axis in rodents (29, 30) ($P > 0.05$, *t* test; Fig. S5). The lack of anatomical segregation is consistent with the previous population of time-related cells found in the primate hippocampus (16).

Learning-Related Neural Signals. We performed a series of analyses to determine if the three categories of ITCs altered their signals in response to behavioral learning. First, we asked what percentage of the three different categories of ITC cells was recorded when animals learned at least one of the four OP associations. Learning was defined by a Bayesian statistical criterion previously described for these data (22, 31). Whereas 62.5% (10 of 16) of the agnostic ITCs were recorded when at least one new association was learned, in striking contrast, we found 94.7% (18 of 19) of release-selective ITCs and 92.3% (24 of 26) of OP-selective ITCs were recorded when at least one new association was learned. Such a distribution would not be expected by chance ($P = 0.016$, 3×2 Fisher's exact test). Further, these latter two ITC groups were found significantly more often during sessions when learning was achieved as the animals learned at least one OP combination in 89 of 126 (70.6%) recording sessions (χ^2 test; $P = 0.48$, agnostic vs. all sessions; $P = 0.021$, release-selective vs. all sessions; $P = 0.015$, OP-selective vs. all sessions). These tests suggest that context-selective ITCs were more likely to be found in the hippocampus when new associations were learned. We examined this relationship in a different way by asking how many of the four possible OP combinations were learned during sessions in which agnostic, release-selective, and OP-selective ITCs were recorded. We found a significantly greater number of the four OP combinations were learned during sessions in which release-selective and OP-selective ITCs were recorded compared with sessions in which agnostic ITCs were recorded (2.2 ± 0.3 combinations, release-selective vs. 1.1 ± 0.3 combinations, agnostic ITCs, $P = 0.030$, *t* test; 2.2 ± 0.3 combinations, OP-selective vs. 1.1 ± 0.3 combinations, agnostic ITCs, $P = 0.016$, *t* test; Fig. 3 *A* and *B*). This distinction was particularly evident during sessions when three or four OP combinations were learned, which provides the clearest indication of the animals grasping the OP association structure, because release-selective and OP-selective ITCs were found in greater frequency than agnostic ITCs (Fig. 3*A*). Therefore, hippocampal ITCs that conjunctively represent time and specific task information were more likely to be found if the primate successfully learned more OP associations during a session. Despite this relationship between ITCs and learning, we were unable to show that any of these ITC types changed their firing responses throughout the session, as previously shown in the primate hippocampus (22, 32), as the animals learned new associations (Fig. S6).

To examine signals related to task performance, we compared the responses of ITCs on correct vs. error trials. Because there were fewer error than correct trials (36.7% of trials with a bar release were answered incorrectly across all recording sessions

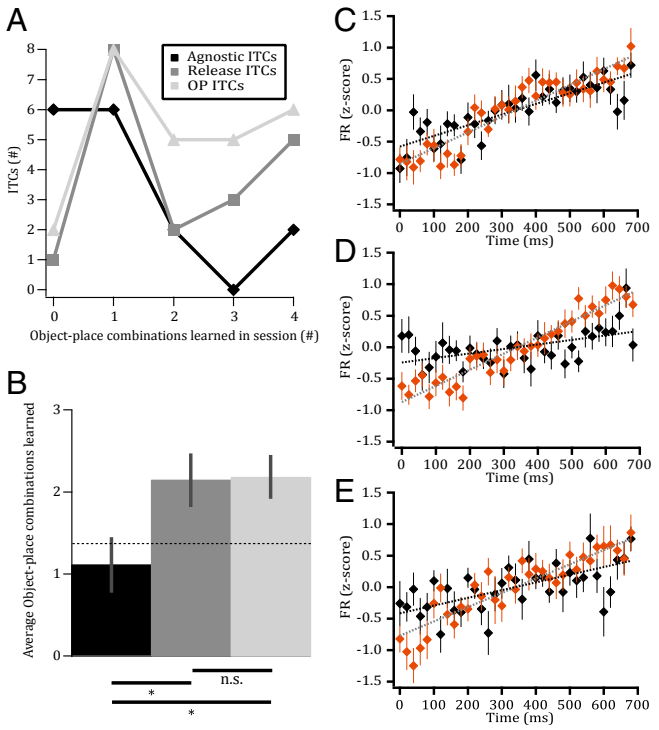

Fig. 3. Population analysis of ITC classes. (*A*) Number of OP associations learned to statistical criterion by the primate during recording was pooled for each ITC type. For example, in sessions when the animal learned all four OP combinations, six OP-selective, five release-selective, and two agnostic ITCs were found. (*B*) Average number of OP associations learned to the statistical criterion for each ITC type. Error bars show SE; $P < 0.05$ significance (shown by asterisks) was assessed via a heteroscedastic *t* test (agnostic vs. release, $P = 0.030$; agnostic vs. OP, $P = 0.016$; release vs. OP, $P = 0.934$). The dotted line shows the average of 1.35 OP associations learned for all 128 sessions. n.s., not significant. (*C*) Population graphs of correct vs. error trials for agnostic ITCs. Orange diamonds are correct trials, whereas black diamonds show error trials. Each agnostic ITC was individually z-scored and then averaged together with other cells of this type (cells that incrementally sloped downward were multiplied by −1 before averaging). The final graphs show the normalized, population-averaged correct and error responses across the 35 bins of the delay. Significance was assessed between correct and error trials by analysis of covariance, which is equivalent to the "time_bins × correct" interaction using linear regression. The dotted lines are linear fits, with gray fit to correct trials and black fit to error trials. (*D*) Same as in *C*, for release-selective ITCs. (*E*) Same as in *C*, for OP-selective ITCs.

for the 139 cells), we used population statistics to compare neuronal signals between these trials. For each ITC with ≥10 error trials in the three different ITC categories, we split the correct and error trials, created PSTHs for the delay period firing of each, and converted to z-scores (negative sloping cells were multiplied by −1). Early and late release PSTHs were created for each category and were then averaged together to illustrate the population response (Fig. 3 *C*–*E*). This grouping allowed us to compare incremental timing signal between correct and error trials through the relative slopes of the different ITC types. We found a general trend where incremental timing signal was significantly reduced across the delay period when the monkey subsequently answered the trial incorrectly (analysis of covariance: agnostic ITCs, $P = 0.0015$; release-selective ITCs, $P = 2.9E\text{-}10$; OP-selective ITCs, $P = 8.5E\text{-}5$). These results include errors in early release-cued trials, which occur when the monkey incorrectly held the bar through the early release period, as well as errors in late release-cued trials, when the monkey incorrectly released the bar during the early release period. To identify if any of the ITC types showed a larger difference in correct vs. error signal, we measured the correct × time_bins × ITC_type interaction between each using linear regression. Only the agnostic vs. release-selective ITCs showed a significant difference (agnostic vs. release-selective, $P = 0.0072$; agnostic vs. OP-selective, $P = 0.30$; release-selective vs. OP-selective, $P = 0.15$), indicating that release-selective ITCs showed a significantly greater difference in incremental timing signal when comparing correct and error trials.

Discussion

We examined delay activity of individual hippocampal cells during the performance of an OP associative learning task and identified a substantial number of recorded cells that linearly increased or decreased their FR across the delay of the task. Changes in FR across such a period devoid of stimulation suggest that these cells are providing an estimate of time passage during the mnemonic delay period (16). The OP associative learning paradigm allowed us to differentiate ITCs into three distinct categories. Agnostic ITCs tracked time for all trial types. The last two categories of ITCs conjunctively signaled both time and trial context: Release-selective ITCs signaled time depending on the release-type required for a correct response on a particular trial, and OP-selective ITCs tracked time only for trials that began with the presentation of a specific OP combination. Release-selective and OP-selective ITCs were found significantly more often than agnostic cells during sessions when learning occurred, whereas all three ITC types showed a decrease in the strength of the incremental timing signal on error trials.

These findings are consistent with previous work in the primate hippocampus using a temporal order memory task (16), suggesting that incremental timing is a common signal in this region. Unlike this previous temporal order study, we also found cells modulated by stimulus-selective firing in our release-selective and OP-selective ITCs that were selective to particular trial types. We believe the different stimulus demands between the temporal order and OP task likely account for this discrepancy. The same eight objects were used throughout all training and recording sessions in the temporal order task. Because animals were only tasked to recognize the order of these stimuli on each trial, but not to learn new contextual associations involving them, the representations of these well-known objects themselves were unlikely to require the hippocampus (33). In the task presented here, new objects were presented in new places during each day and had to be associated with an early or late bar release. As a result, animals were not only required to remember specific contextual information in the form of new objects in defined places but had to associate these combinations with specific time points for response. This unity of context and time in the OP task might explain the presence of these unique ITCs that code for time only on specific trials.

The monkey hippocampal ITCs described here represent a previously unidentified means of linking temporal signal to the contextual encoding typically ascribed to the hippocampus. This work also adds to the foundational work of time cells in the rodent hippocampus, which have been shown explicitly to provide an estimate of timing (18) even when animals were stationary (19). Although this study provides additional evidence of how time is represented in the hippocampus, ITCs show both similarities and differences with time cells described in the rodent hippocampus. The most obvious difference is in the specific pattern of neural activity seen during the delay interval. Individual rodent time cells respond at specific time points within a 10- to 20-s delay period and, as a population, temporally "tile" or span the duration of the entire delay interval (17–21). In contrast, for monkey ITCs, we observed incremental increases and decreases in activity during the relatively short 700-ms delay interval. Another difference is the exclusion of interneurons in rodent time cell work. However, we do not believe their inclusion explains or unduly biases our results because the three categories of ITCs remained if we excluded putative interneurons from our analysis (Figs. S7 and S8). One possible interpretation of these

divergent signals is that there is a fundamental difference in how temporal information is conveyed by hippocampal time cells in monkeys (incremental timing) and rodents (specific time marking). Another possibility is that monkey and rodent hippocampal time cells are more similar than these studies suggest and the distinct firing patterns might be due to the different delay intervals used between species. Consistent with this interpretation, we note that the absolute durations of the elevated FRs in delay-active primate hippocampal cells (typically hundreds of milliseconds for all types; Fig. S8) are within the range of the length of activity of individual rodent time cells (17–21). This latter hypothesis raises the possibility that monkey ITCs would tile the entire duration of a long delay interval and rodent hippocampal time cells would incrementally fire when the delays are short. To address this possibility, it will be important to use similar task parameters and delay intervals in both species to compare the pattern of ITC/time cell signals directly in each.

Despite these apparent differences in the pattern of firing of the monkey and rodent time cells, there are also similarities in their firing characteristics. For one, consistent with the release-selective and OP-selective ITCs, a previous rodent study used a generalized linear model (GLM) to show time-context conjunctive coding in rodent hippocampal time cells that fired differentially during the delay period when a particular object began each trial (20). Similarly, another study used GLM analysis to show that the activity of large proportions of hippocampal neurons could be explained by combinations of temporal and spatial information (18). Combinatorial coding of unified representations as shown for the OP-selective and release-selective ITCs could provide a framework for the hippocampus to unite the many contextual details that comprise relational memories (34). Such conjunctive encoding of object, place, time, and performance information in single hippocampal neurons provides a previously unidentified way in which this structure can combine disparate streams of information in the service of memory. Conjunctive coding has been theorized for hippocampal function (35, 36), as opposed to a more distributed representation expected in the cortex (36, 37).

Our study also provides new information about the relationship between time and learning in the hippocampus. Whereas a previous study in rodents recorded time cells during an associative learning task (20), this study did not explore how behavioral learning influenced the time cell signals. Here, we report that release-selective and OP-selective ITCs were found almost exclusively during sessions in which at least one new association was learned (94.4% and 92.3% of sessions, respectively) and also that the number of associations learned by the animal was significantly increased when these selective ITCs were recorded (Fig. 3 *A* and *B*). Therefore, because time-context conjunctive cells were enriched when the animal learned new contextual associations, these cells might participate in encoding for specific combinations of information (e.g., objects, places) and linking them within a temporal frame. This relationship between time and learning in the hippocampus is supported by recent functional MRI (fMRI) work in humans examining pattern similarity, wherein hippocampal activity was selective for sequences of presented objects in learned positions but not when unlearned combinations of the same objects in different positions or different objects in the same positions were shown (38). A model of associative learning in the hippocampus supports the idea that context-sensitive cells can rapidly learn new associations through persistent firing mechanisms (39). In this model, "context fields," akin to place fields, through which a neuron fires throughout a gap to unite sequentially presented information, are proposed (39, 40). The incremental timing signal shown here would unite both the cue and release period through a persistent firing signal and provide a means of estimating the relative time from one cued event to the next through the incremental changes in firing (16). Such incremental timing would provide the hippocampus with a baseline temporal structure (41), whereas differential contextual components could be identified by the more specific time-context conjunctive cells.

Previous work has found that incrementally increasing neuronal FRs in primate lateral intraparietal cortex were indicative of decreasing uncertainty in a decision over time (42). In particular, monkeys were required to differentiate if a test cue was shorter or longer than a standard cue, and incrementally rising signals [but not falling signals, as found for 57% (35 of 61) of ITCs here] were shown to reflect a mechanism for this relative time perception. However, incorrect responses by the primate showed a reduction in this neural signal compared with correct trials during the delay before response (42), possibly indicating increased uncertainty before an error. Similarly, we found a general trend of incremental timing signal decreasing during the delay period on trials that the monkey subsequently answered incorrectly (Fig. 3 *C–E*), although our results show a reduced change in signal for both incrementally increasing and decreasing ITCs. The reduced cellular encoding of temporal order on error trials has previously been shown for populations of rodent hippocampal cells when animals were tasked to recall the sequence of odor-place events (15). A more recent study found that populations of rodent time cells showed less correlated firing during a mnemonic delay period when the animals subsequently answered incorrectly (19). This confluence of results suggests that reductions in strength of timing signal across a mnemonic delay might reflect a cellular correlate in the hippocampus of the uncertainty in the animal's response. These results provide a link between the time-context conjunctive cells and task performance, potentially supporting how temporal memories for correct associations are preferentially encoded. The differential signal between correct and error trials shown here is also reminiscent of fMRI work in humans that showed significantly greater hippocampal activation during encoding of sequences of words that were successfully remembered compared with encoding of sequences that were subsequently misordered (8).

Our findings show that incremental timing signals are not specific for tasks requiring specific temporal order memory (16). Similarly, a recent study showed rodent time cells still tracked time across a delay period in a delayed match to sample task with no temporal component (19). These parallel findings support the idea that temporal coding is a general signal present in the hippocampus during a mnemonic delay interval even when animals are not required explicitly to track time. The potentially omnipresent maintenance of an organizational structure in the hippocampus is reminiscent of the common finding that rodent place cells encode space even when there is no explicit spatial demand for reward (e.g., ref. 43). It will be important for future work to explore this timing signal through hippocampal recordings during additional task designs. In particular, changing the length of the delay period on a trial-by-trial basis will be essential to determine how ITCs respond in the face of changing task parameters. This work will also help identify how similar the timing signal in the monkey hippocampus is to the timing signal described in the rodent hippocampus.

Methods

A previous publication on these data (22) provides full details on subjects, behavior, learning, electrophysiology, anatomy, and other task-related information.

Subjects. One male rhesus macaque (14.7 kg) and one male bonnet macaque (8.1 kg) were used for the experiments. All procedures and treatments were done in accordance with NIH guidelines and were approved by the New York University Animal Welfare Committee.

Task. Primates were shown one of two novel objects (changed daily) in one of two potential places (also changed daily) on a computer screen during a 500-ms cue period (Fig. 1*A*). This cue period was followed by a 700-ms delay period when the primate held a bar while fixating on a central target. The primate was cued to release the bar when presented with either an orange dot or (500 ms afterward) a green dot. Bar release to the correct association was followed by a positive auditory feedback tone and juice reward. On

each day, two of four possible OP combinations were associated with either the early (orange dot) or late (green dot) bar release (Fig. 1*B*). The two OP combinations correct for early release trials were O1P1 and O2P2, whereas the two OP combinations correct for late release trials were O1P2 and O2P1 (Fig. 1*B*). We only analyzed the delay period of this task as this is the critical period during which the primate must hold the OP information in memory until the cue for bar release.

Behavioral Training. Animals were trained in a primate testing chair (Crist Instruments) located 0.54 m away from a 19-inch cathode ray tube monitor. The task was run using CORTEX software (National Institute of Mental Health) while eye movements were tracked using an IR camera (IScan, Inc.). Primates were trained on separate practice tasks, as well as on the primary task presented here. The practice tasks include a fixation-only variety of the task and a reference version of the OP task with previously used OP combinations to motivate the subject. Data from these practice tasks are not analyzed here.

Behavioral Learning. A Bayesian state-space model for learning of simultaneous problems was used to determine how many of the four OP combinations were learned in each session. This model takes into account the response bias, calculates the learning curves for each combination, and defines the criterion of a specific OP combination as learned when the probability of a correct response is >0.95 for the next trial of that type (31).

Electrophysiology. Individual tungsten microelectrodes (UEWLEFSM4N1E; FHC) were driven by hydraulic microdrive (Naguchi) into hippocampal regions that were guided by MRI. A chamber and grid system (Crist Instruments) was used to target penetrations of electrodes at the beginning of each session. The first 21 sessions used an online spike-sorting system (MSD) to isolate the activity of individual neurons. The remaining 107 sessions used a Plexon (Plexon, Inc.) online spike sorting system that was later isolated in offline sorter software. Individual cells were isolated using principal components, height, and time as primary parameters. Only stable clusters above background were kept for analysis.

Statistical Classification. A series of tests was done to differentiate cells into three classes of ITCs based on the firing pattern of their delay period PSTH. Cells were divided into classes by first determining if they fired similarly for all four trial types during the delay with a Kruskal–Wallis test and then if they fired similarly for each trial type correct for each release type using resampling methods. Neurons in each class were then analyzed with a permutated *F* test to determine if the FR significantly changed over the course of the delay. A detailed description of the statistical processes used is provided in *SI Methods*.

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