


RESEARCH ARTICLE

Higher Neural Functions and Behavior

Decoding working memory information from neurons with and without persistent activity in the primate prefrontal cortex

Lilianna Thrower,^{1*} Wenhao Dang,^{1*} Rye G. Jaffe,¹ Jasmine D. Sun,¹ and  Christos Constantinidis^{1,2,3}

¹Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee, United States; ²Neuroscience Program, Vanderbilt University, Nashville, Tennessee, United States; and ³Department of Ophthalmology and Visual Sciences, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Abstract

Persistent activity of neurons in the prefrontal cortex has been thought to represent the information maintained in working memory, though alternative models have challenged this idea. Theories that depend on the dynamic representation of information posit that stimulus information may be maintained by the activity pattern of neurons whose firing rate is not significantly elevated above their baseline during the delay period of working memory tasks. We thus tested the ability of neurons that do and do not generate persistent activity in the prefrontal cortex of monkeys to represent spatial and object information in working memory. Neurons that generated persistent activity represented more information about the stimuli in both spatial and object working memory tasks. The amount of information that could be decoded from neural activity depended on the choice of decoder and parameters used but neurons with persistent activity outperformed non-persistent neurons consistently. Averaged across all neurons and stimuli, the firing rate did not appear clearly elevated above baseline during the maintenance of neural activity particularly for object working memory; however, this grand average masked neurons that generated persistent activity selective for their preferred stimuli, which carried the majority of stimulus information. These results reveal that prefrontal neurons that generate persistent activity maintain information more reliably during working memory.

NEW & NOTEWORTHY Competing theories suggest that neurons that generate persistent activity or do not are primarily responsible for the maintenance of information, particularly regarding object working memory. Although the two models have been debated on theoretical terms, direct comparison of empirical results has been lacking. Analysis of neural activity in a large database of prefrontal recordings revealed that neurons that generate persistent activity were primarily responsible for the maintenance of both spatial and object working memory.

decoding; neuron; neurophysiology; prefrontal cortex; working memory

INTRODUCTION

The ability to maintain and manipulate information in memory over a period of seconds is commonly referred to as working memory (WM) (1). Early neurophysiological experiments in nonhuman primates identified neurons that not only respond to sensory stimuli but remain active during a period after the stimuli were no longer present, thus providing a neural correlate of working memory (2). This persistent activity has since been widely replicated, including in human recordings (3). Computational models typically simulate persistent activity through neural networks with recurrent connections

between units with similar tuning for spatial location (4, 5). Activation in the network behaves as a continuous attractor (6), with a stimulus generating a bump (peak) of activity in the network. This activity bump is maintained through the delay period, hence the term bump attractor, but may drift randomly, with the peak at the end of the delay period determining the location that is ultimately recalled by the subject (7). Drifts in neuronal activity thus account for deviations in behavior.

Persistent activity is not universally accepted as the neural correlate of working memory (8–10). Alternative mechanisms have been proposed that do not depend on persistent

*L. Thrower and W. Dang contributed equally to this work.
Correspondence: C. Constantinidis (Christos.Constantinidis.1@vanderbilt.edu).
Submitted 31 July 2023 / Revised 24 October 2023 / Accepted 24 October 2023



activity to represent information during the delay period of working memory tasks (11). For example, experimental studies have shown that the identity of a stimulus maintained in memory can be decoded from a population of neurons whose activity is not elevated above baseline levels of firing rate, but whose pattern of activity may vary dynamically over time, with different neurons becoming active at different time points (12). Computational models have also been proposed that could account for such changes via synaptic strength modification instead of spike generation (13, 14), which are generally referred to as “activity-silent” mechanisms. Moreover, recurrent neural networks have been used successfully to model complex cognitive tasks, including those related to working memory (15–18), demonstrating that it is possible to perform working memory tasks by virtue of rapid changes in synaptic weights after a stimulus presentation.

We were, therefore, motivated to compare the predictions of the bump-attractor and dynamic-coding models, using a database of recordings obtained from the prefrontal cortex of monkeys as they performed spatial and object working memory tasks. We examined how effectively stimulus information was represented from neurons that do and do not generate persistent activity. We also compared working memory for spatial location and object information.

METHODS

Animals

Data obtained from male rhesus monkeys (*Macaca mulatta*), ages 5–9 yr old, weighing 5–12 kg, as previously documented (19), were analyzed in this study. None of these animals had any prior experimentation experience at the onset of our study. Monkeys were either single-housed or pair-housed in communal rooms with sensory interactions with other monkeys. All experimental procedures followed guidelines set by the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and the National Research Council’s Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the Wake Forest University Institutional Animal Care and Use Committee.

Experimental Setup

Monkeys sat with their heads fixed in a primate chair while viewing a monitor positioned 68 cm away from their eyes with dim ambient illumination and were required to fixate on a 0.2° white square appearing in the center of the screen. During each trial, the animals maintained fixation on the square while visual stimuli were presented either at a peripheral location or over the fovea, to receive a liquid reward (typically fruit juice). Any break of fixation immediately terminated the trial, and no reward was given. Eye position was monitored throughout the trial using a noninvasive, infrared eye position scanning system (model RK-716; ISCAN, Burlington, MA). The system achieved a <0.3° resolution around the center of vision. Eye position was sampled at 240 Hz, digitized, and recorded. The visual stimulus display, monitoring of eye position, and synchronization of stimuli with neurophysiological data were performed with in-house software implemented in the MATLAB environment (MathWorks, Natick, MA), using the Psychophysics Toolbox (20).

Behavioral Task

Monkeys were trained to complete spatial and shape working memory tasks. The stimuli shown in the spatial task were white 2° squares, presented in one of nine possible locations arranged in a 3 × 3 grid with a 10° distance between adjacent stimuli. Here, we analyzed responses to only 8 stimuli, shown in the periphery of the grid (thus omitting analysis of the central, foveal stimulus). The stimuli shown in the shape task were white 2° geometric shapes, drawn from a set comprising a circle, a diamond, the letter H, the hashtag symbol, the plus sign, a square, a triangle, and an inverted Y-letter. These stimuli could also be presented in one of nine possible locations arranged in a 3 × 3 grid with a 10° distance between adjacent stimuli.

The presentation began with a fixation interval of 1 s where only the fixation point was displayed, followed by 500 ms of stimulus presentation (hereafter referred to as cue), followed by a 1.5 s delay interval where, again, only the fixation point was displayed. A second stimulus (hereafter referred to as sample) was subsequently shown for 500 ms. In the spatial task, this second stimulus would be either identical in location to the initial stimulus or diametrically opposite to it. In the feature task, this second stimulus would appear in the same location as the initial stimulus and would either be an identical shape or not. Only one nonmatch stimulus was paired with each cue so that the number of match and nonmatch trials were balanced in each set. In both the spatial and feature tasks, this second stimulus display was followed by a second delay period of 1.5 s where only the fixation point was displayed. The monkeys were thus required to remember the spatial location and/or shape of the first presented stimulus and, after the second delay period, report whether the second stimulus was identical to the first or not, by making an eye movement to one of two target stimuli (green for matching stimuli, blue for non-matching) that appeared after the second delay period. Each target stimulus could appear at one of two locations orthogonal to the cue/sample stimuli, pseudo-randomized in each trial so that the location of the correct target could not be predicted before the end of the trial.

A conjunction task was also used, which combined the active spatial and feature tasks, using the same shape stimuli and presented at the same possible locations, with the same timing. In a single recording session, only four shape-location combinations involving two shapes and two locations were used. The conjunction task was the most complex in the current study, as the monkeys were required to match two different types of information: location and shape.

Surgery and Neurophysiology

A 20-mm diameter craniotomy was performed over the primate prefrontal cortex (PFC), and a recording cylinder was implanted over the site. The location of the cylinder was visualized through anatomical magnetic resonance imaging (MRI) and stereotaxic coordinates postsurgery. For two monkeys, the recording cylinder was moved after an initial round of recordings in the post-training phase to sample an additional surface of the PFC.

Anatomical Localization

Each monkey underwent an MRI scan before neurophysiological recordings. Electrode penetrations were mapped onto the cortical surface. We recorded from a posterior-dorsal region that included area 8A, a mid-dorsal region that included area 8B and area 9/46, an anterior-dorsal region that included area 9 and area 46, a posterior-ventral region that included area 45, and an anterior-ventral region that included area 47/12.

Neuronal Recordings

Neural recordings were carried out in the aforementioned areas of the PFC both before and after training in each WM task. Subsets of the data presented here were previously used to determine the collective properties of neurons in the dorsal and ventral PFC, as well as the properties of neurons before and after training in the posterior-dorsal, mid-dorsal, anterior-dorsal, posterior-ventral, and anterior-ventral PFC subdivisions. Extracellular recordings were performed with multiple microelectrodes that were either glass- or epoxylite-coated tungsten, with a 250 μm diameter and 1–4 M Ω impedance at 1 kHz (Alpha-Omega Engineering, Nazareth, Israel). A Microdrive system (EPS drive, Alpha-Omega Engineering) advanced arrays of up to 8 microelectrodes, spaced 0.2–1.5 mm apart, through the dura and into the PFC. The signal from each electrode was amplified and band-pass filtered between 500 Hz and 8 kHz while being recorded with a modular data acquisition system (APM system, FHC, Bowdoin, ME). Waveforms that exceeded a user-defined threshold were sampled at 25 μs resolution, digitized, and stored for off-line analysis. Neurons were sampled in an unbiased fashion, collecting data from all units isolated by our electrodes, with no regard to the response properties of the isolated neurons. A semi-automated cluster analysis relied on the KlustaKwik algorithm, which applied principal component analysis of the waveforms to sort recorded spike waveforms into separate units. To ensure a stable firing rate in the analyzed recordings, we identified recordings in which a significant effect of the trial sequence was evident at the baseline firing rate (ANOVA, $P < 0.05$), e.g., due to a neuron disappearing or appearing during a run, as we were collecting data from multiple electrodes. Data from these sessions were truncated so that analysis was only performed on a range of trials with stable firing rates. Less than 10% of neurons were corrected in this way. Identical data collection procedures, recording equipment, and spike sorting algorithms were used before and after training to prevent any analytical confounds.

Data Analysis

Data analysis was implemented with the MATLAB computational environment (MathWorks, Natick, MA). Neuron analysis involved, initially, determining the mean firing rate of each neuron in each trial epoch. Next, we compared the responses of each neuron in the 1-s fixation period with each of the task epochs that followed (cue presentation, first delay period, sample presentation, second delay period). Any neuron that displayed a significant increase in mean firing rate in a particular epoch over the firing rate recorded in the 1-s baseline fixation period (evaluated with a one-tailed, paired

t test at the $\alpha = 0.05$ significance level) was identified as a task-responsive neuron. Neurons with persistent activity were defined here as those whose mean firing rate in the first delay period was significantly elevated over the fixation period (evaluated with a one-tailed, paired t test at the $\alpha = 0.05$ significance level), similar to previous studies (21–23).

Decoding analysis was performed as follows: the mean firing rate of each neuron was computed at successive time points spanning 1 s before to 5 s after cue onset. For most analyses, a 400-ms-wide window stepped every 100 ms was used. A smaller sliding window (100 ms) was also employed (Fig. 5) to demonstrate the robustness of our results to parameter selection. We additionally examined decoder performance for a 1-s interval in the center of the delay period (250 ms after the cue offset). Spike count vectors from multiple neurons were used to create pseudo-populations of 50, 100, and 200 randomly selected neurons with persistent activity or not. Five decoding algorithms were implemented using the MATLAB `fitc` function to decode stimuli location: support vector machine (SVM) with a Gaussian kernel, SVM with a linear kernel, discriminant analysis, classification trees, and naive Bayes. Decoder performance was estimated via 10-fold and 5-fold cross-validation methods. The decoding baseline was 12.5% for both tasks as we used eight possible locations for the spatial task and eight possible shapes for the feature task. For the cross-temporal decoding analysis, an SVM decoder was trained independently for each 400 ms sliding window time point of the trials, and then tested on all sliding window time points across the trials.

RESULTS

Extracellular neurophysiological recordings were collected from the lateral PFC of three monkeys trained to perform the match/nonmatch WM tasks (19, 23). The task required them to view two stimuli appearing in sequence with an intervening delay period between them and, after a second delay period, to report whether or not the second stimulus was identical to the first. The two stimuli could differ in terms of their location (spatial task, Fig. 1A), shape (feature task, Fig. 1B), or both (conjunction task, Fig. 1C). If the second stimulus matched with the first, monkeys would saccade towards a green target during a subsequent interval. Otherwise, they would saccade to a blue target at the diametrically opposite location, which differed randomly from trial to trial.

A total of 1,066 cells from three monkeys and 885 cells from two monkeys were collected while the animals were performing the spatial and shape tasks, respectively, and had sufficient numbers of trials for analysis. In the spatial task, 414 neurons exhibited persistent activity in the delay period (“persistent neurons” for simplicity, thereafter). We additionally identified 207 task-responsive, but nonpersistent neurons (i.e., neurons that had significantly raised activity in a stimulus presentation period, but not the delay period). Another 371 neurons exhibited no significantly elevated activity in any task interval. We refer to the latter two groups of 578 neurons as “all non-persistent” neurons. Finally, a group of 74 neurons exhibited significantly inhibited activity during the delay period of the task, as we have described previously (24); these were analyzed separately.

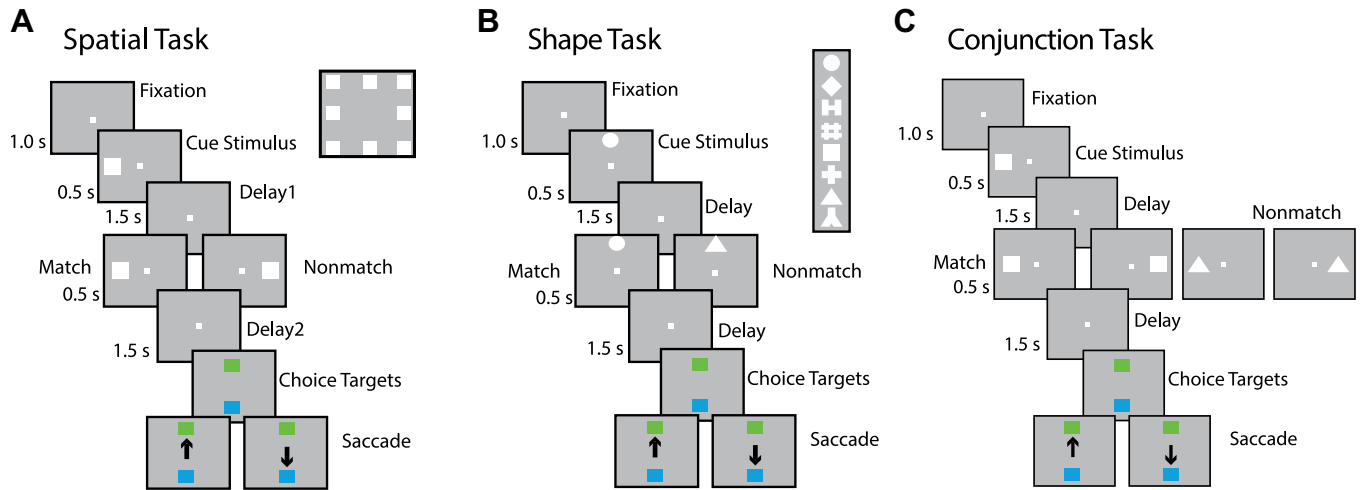


Figure 1. Task structure and stimuli used. The animals were required to maintain center fixation throughout the trial. When the fixation point was turned off, monkeys were required to make a saccade to a green target if two of the stimuli presented were identical (matched) or to a blue target if the stimuli were different (did not match). **A:** spatial location match-nonmatch task. Eight cue locations used for analysis are shown in the inset. **B:** shape feature match-to-sample task. Eight shapes used for analysis are shown in the inset. **C:** spatial-shape conjunction task. Two locations and two stimulus shapes were used for any single session.

The grand average across all neurons and stimulus conditions in the spatial task revealed only a modest increase in firing rate during the stimulus presentation periods (which are represented by the black horizontal lines at the bottom of Fig. 2A). Elevated activity in the delay period of the task is barely noticeable in this plot, as most prefrontal neurons do not generate persistent activity. Delay period activity is more noticeable in the neurons that generate persistent activity (red trace in Fig. 2A), but even for those it is important to note that persistent activity is tuned so that each neuron generates persistent activity for only a narrow range of locations (21) and averaging responses across all stimulus conditions will inevitably mask this stimulus-selective activity (25).

Decoding Information from Persistent and Nonpersistent Neurons

We compared the classification accuracy of decoding the stimulus location with a support vector machine (SVM) for neurons with persistent activity and for task-responsive neurons without persistent activity. We performed this analysis across the time course of the spatial match-nonmatch task (Fig. 2B). Mean classifier performance for decoding the location of the first (cue) stimulus with 10-fold cross-validation is shown in Fig. 2B. Persistent neurons exhibited higher classification performance at all task intervals, including the delay period. By contrast, classification

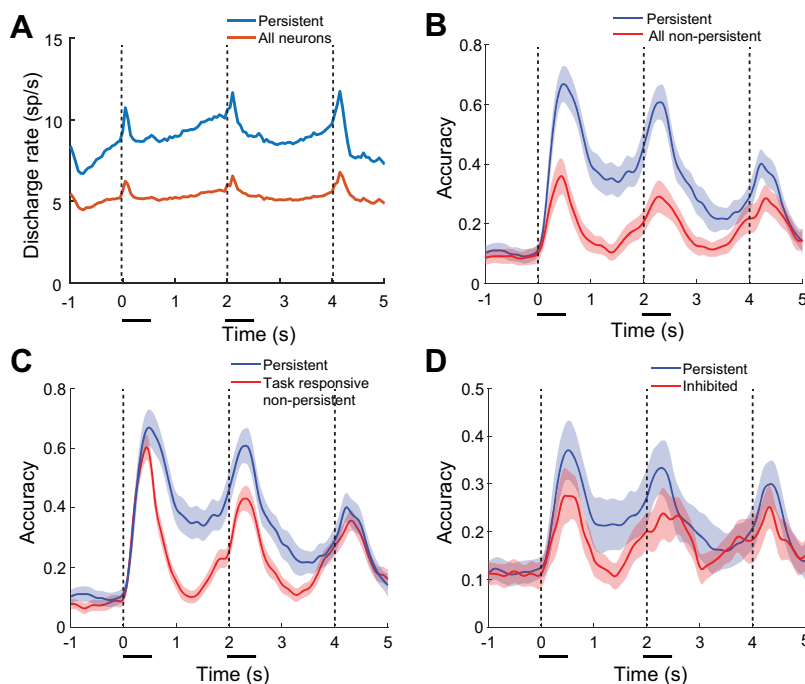


Figure 2. **A:** average, population peristimulus time histogram (PSTH) for all recorded neurons, and for neurons that generated persistent activity (persistent neurons) in the spatial working memory task. The three dashed vertical lines represent the times of the two stimulus and choice target presentations. Solid horizontal lines indicate the duration of the two stimulus presentations. **B:** SVM decoding performance for the spatial task as a function of time relative to the onset of the cue. Accuracy for populations of persistent and all nonpersistent neurons with $n = 200$ neurons and 10-fold cross-validation. The shaded area represents the standard deviation calculated over 100 repetitions. **C:** decoding accuracy for populations of persistent and task-responsive but nonpersistent neurons ($n = 200$ neurons). **D:** decoding accuracy for populations of persistent and inhibited neurons ($n = 50$ neurons). SVM, support vector machine.

accuracy essentially returned to chance levels at ~125 ms after the beginning of the delay period for nonpersistent neurons.

We also compared the classification accuracy of persistent neurons against the accuracy of task-responsive but non-persistent neurons, using equal sample sizes (Fig. 2C). Both populations reached a similar peak shortly after the presentation of the first (cue) stimulus, indicated by the horizontal line in Fig. 2. However, decoding accuracy diverged during the delay period, with the largest difference between populations being seen during the first delay period (0.5–2.0 s). A similar difference in decoding performance was also present during the second delay period, although it should be noted that at this point in the task, maintenance of the first stimulus appearance is no longer required; the monkeys could make a judgment about whether or not the two stimuli were matching after the appearance of the second stimulus, without needing to continue maintaining the location of either the first or second stimulus in memory. Neurons with significantly reduced delay period activity also failed to match the decoding performance of persistent neurons (inhibited neurons in Fig. 2D). The analysis that follows involves primarily comparisons of persistent neurons with task-responsive, nonpersistent neurons, to better understand the difference in properties in the delay period for neurons that carry comparable information during the stimulus presentation period.

To determine whether persistent and task-responsive, nonpersistent neurons could maintain spatial location information with equal accuracy during the delay period, and to avoid neurons with potentially long response latencies that might carry over at the first part of the delay interval, we calculated SVM decoding accuracy for the middle of the first delay period (0.75–1.75 s). Mean decoding accuracy was higher for persistent populations (0.47 s) than nonpersistent populations (0.17 s) for 100 population subsamples (Fig. 3), with essentially no overlap between the two distributions. A statistical comparison based on 100 simulations for each condition revealed a significant difference (t test, $t_{198} = 72.6$, $P < 0.001$). This suggests that persistent neurons encode

information substantially more accurately during the delay period. However, it was also notable that nonpersistent neurons did achieve a classification accuracy greater than chance in this period (t test, $t_{99} = 21.5$, $P < 0.001$).

Effect of Sample Size and Classifier Parameters

The absolute performance achieved by classifiers depends on the amount of neural data used, as well as the choice of parameters. It was therefore important to examine whether the difference between persistent and nonpersistent neurons was sensitive to these factors. We first varied the population size. Populations of 50, 100, and 200 neurons showed time courses consistent with the spatial task with the SVM decoding algorithm. Higher decoding accuracy for persistent populations during the first delay period was consistent across population sizes (Fig. 4, A–C). We saw increased accuracy at the first timepoint in the delay period as a function of the number of neurons, for both persistent and non-persistent populations (Fig. 4D). However, persistent neurons achieved consistently higher performance.

Decoding performance was also consistent between different sliding window sizes and cross-validation parameters of the decoder (Fig. 5, A–D). It is possible that by integrating firing rate over a fairly long (400 ms) period, we are underestimating the decoding ability of the non-persistent population, particularly if different population of neurons are engaged with fast dynamics, as postulated by dynamic-coding theories (11, 12). We therefore used a 100 ms window (Fig. 5C) and repeated the decoding analysis. For both nonpersistent and persistent populations, we once again saw the maxima when the stimuli were present and saw the minima during the delay periods when the stimuli were not present. Importantly the decoding advantage of persistent neurons was maintained. Higher accuracy during delay periods for persistent neurons was also seen for both 10-fold and fivefold cross-validation (Fig. 5, B and D).

Furthermore, to test if our findings with SVM decoder generalize to other types of decoders, we compared the performance of different classifiers against each other, including SVM, Discriminant Analysis, Naïve Bayes, and Classification trees (Fig. 6). SVM generally performed the best in our data set. However, for every classifier, persistent neurons consistently outperformed nonpersistent neurons in the delay period of the task.

Finally, we investigated the coding stability during the delay period of two populations, by using cross-temporal decoding (Fig. 7). Consistent with the results shown in the previous figures, decoding performance was much lower on the diagonal line in the nonpersistent populations, compared with the persistent population. Importantly, though not perfectly generalizing across time points during delay, the decoding performance for the persistent population was significantly over the chance level off the diagonal, indicating some stable coding.

Decoding Object Working Memory

The results of the prior sections suggest that neurons that generate persistent activity can maintain spatial working memory better than those without persistent activity. However, alternative theories of working memory have

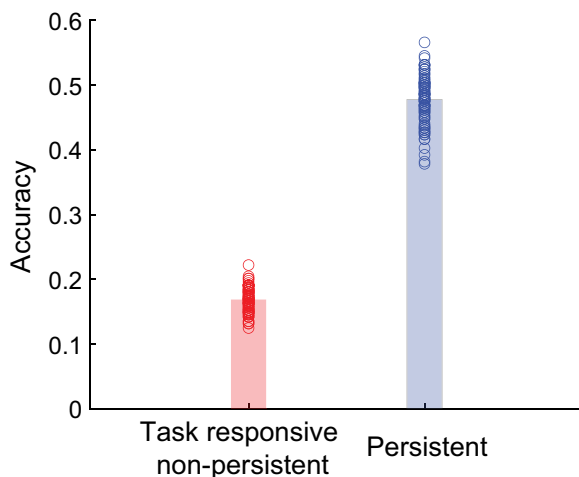


Figure 3. SVM decoding performance for the spatial task. Accuracy for the middle 1 s of the first delay period. Simulations are shown separately for persistent and (task-responsive) nonpersistent neurons with $n = 200$ neurons and 10-fold cross-validation. SVM, support vector machine.

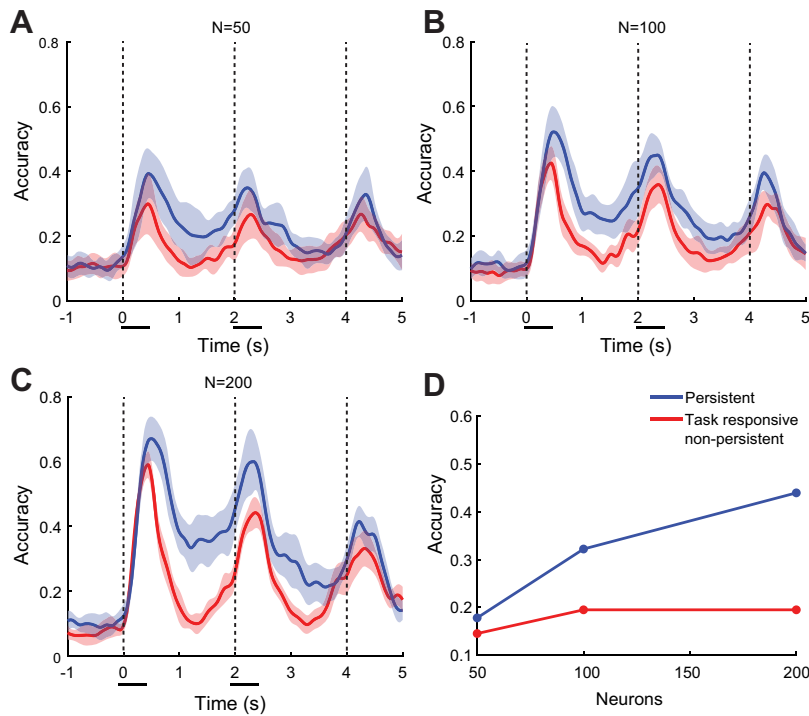


Figure 4. SVM decoding performance in the spatial task, for different sample sizes of persistent and (task-responsive) nonpersistent neurons. A–C: sample size of $n = 50$, 100, and 200 neurons, respectively. The shaded area represents the standard deviation. D: accuracy of data shown for the first time point of the delay period. SVM, support vector machine.

argued that the role of the prefrontal cortex is to “highlight” the spatial location of remembered stimuli, perhaps playing a supervisory or control role, while sensory areas maintain the actual features of stimuli in memory (26–29). Indeed, the findings that put forth activity-silent theories relied on the general

lack of increased persistent activity during the delay periods of object working memory tasks (11, 12). It was therefore critical to compare results from our shape working memory task, which required monkeys to remember the shapes of stimuli that were always presented at the same location (Fig. 1B).

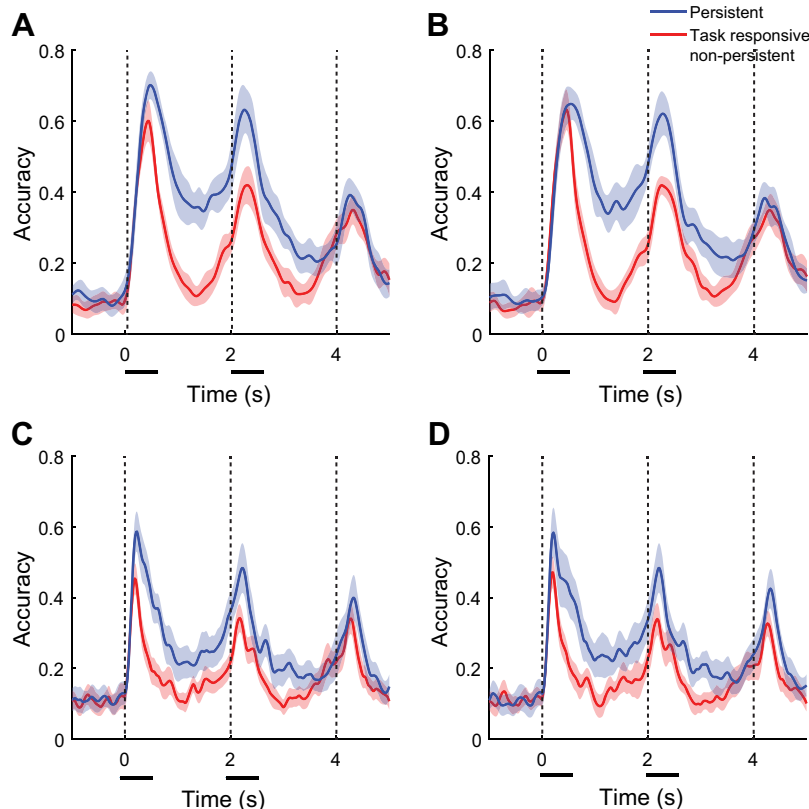


Figure 5. SVM decoding performance in the spatial task, for different parameters of cross-validation, sliding window size, and sliding window steps, for persistent and (task-responsive) nonpersistent neurons. Fivefold (A) and 10-fold (B) cross-validation, for 400 ms sliding average window. Sample size $n = 200$ neurons, for all cases. The shaded area represents the standard deviation across 10 iterations of random samplings. Fivefold (C) and 10-fold (D) cross-validation, for 100 ms sliding average window. Sample size $n = 200$ neurons, for all cases. The shaded area represents the standard deviation across 10 iterations of random samplings. SVM, support vector machine.

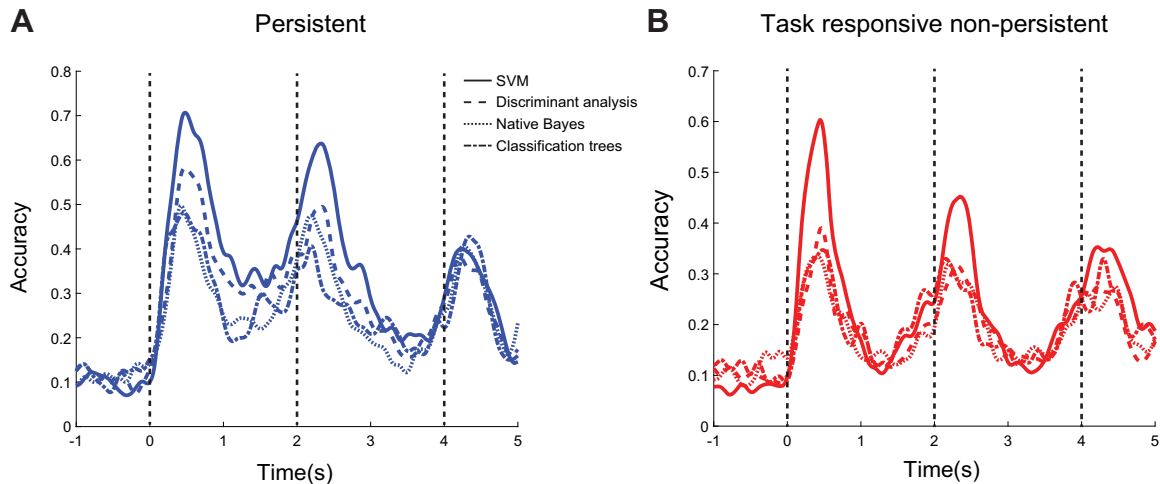


Figure 6. Decoding performance in the spatial task, for persistent (A) and task responsive, non-persistent neurons (B), using different classifiers. Sample size $n = 200$ neurons, for all cases.

Similarly to the spatial task, we identified 315 neurons that generated persistent activity (“persistent neurons”), 183 task-responsive neurons that did not generate persistent activity, and 312 neurons that did not respond to the task. The latter two groups again made up 495 “all nonpersistent” neurons. Finally, we identified 75 neurons whose activity was inhibited in the delay period. As was the case for the spatial task, the grand average firing rate across all neurons and stimulus conditions revealed very little delay period activity (Fig. 8A). This result essentially replicates the findings of previous studies that reported a lack of increased persistent activity during the delay periods of object working memory tasks (11, 12). This apparent lack of delay activity is due to the fact that only a minority of cells show persistent activity in the whole population. In addition, those cells are generally selective and only increase their firing rates to one or few particular stimuli rather than to all stimuli used. Thus, a grand average masks again the elevated activity of persistent cells for specific stimuli.

Results of SVM decoder classification accuracy are shown in Fig. 8B. Persistent and nonpersistent neurons reached similar classification accuracy during the stimulus presentation period. Importantly, persistent neurons consistently outperformed non-persistent neurons during the delay periods of the task (Fig. 8C). A statistical comparison based on 100

simulations for each condition revealed a significant difference (t test, $t_{198} = 33.1$, $P < 0.001$). This suggests that persistent neurons encode object information substantially more accurately during the delay period. Nonpersistent neurons also achieved a classification accuracy greater than chance in this period (t test, $t_{99} = 10.6$, $P < 0.001$). In this respect, the results of the spatial and object working memory tasks were qualitatively similar, although we note that the absolute value of classification was lower in the object task compared to the spatial task, as a result of fewer neurons being engaged by any shape stimulus.

Decoding Both Spatial and Shape Information from Working Memory

We have so far investigated the cases where one type of information was required to be maintained in the working memory and revealed that the persistent neurons contain more information than the nonpersistent cells. However, it is still unknown if this is true in more complex situations, like when combinations of different features are remembered. We thus analyzed 273 persistent neurons and 141 non-persistent (task responsive) neurons in the conjunction task (Fig. 1C). Results are shown in Fig. 9. The result was qualitatively similar compared with the tasks involving maintenance of one type of information at a time. Despite of similar

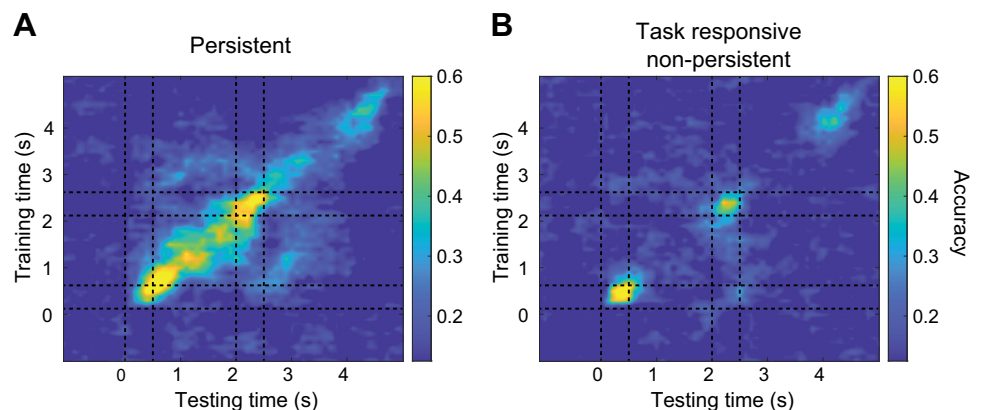


Figure 7. Cross-temporal decoding performance in the spatial task for the persistent (A) and task-responsive nonpersistent (B) populations. 0 s is the cue onset time.

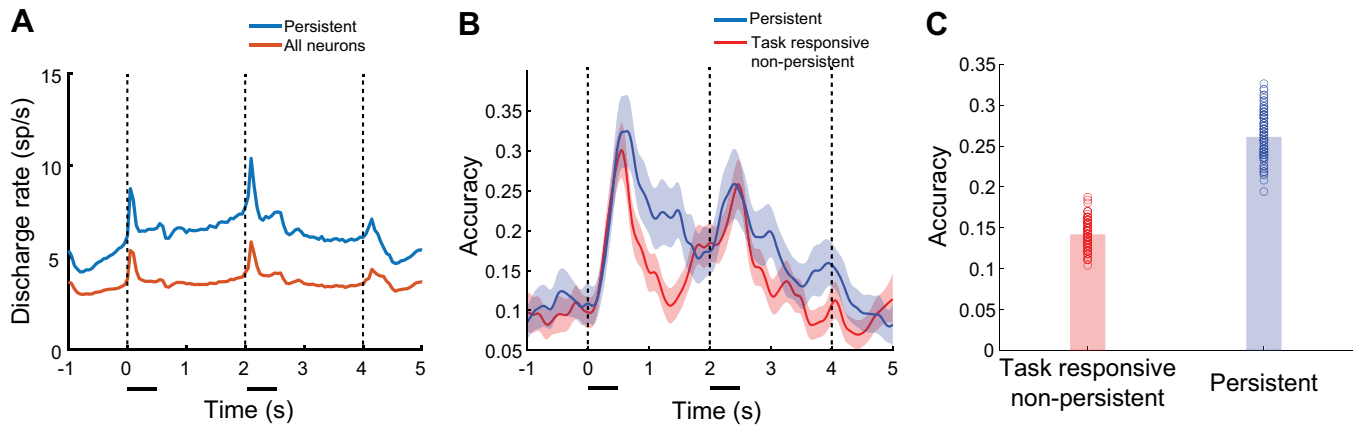


Figure 8. A: average PSTH for all recorded neurons and persistent neurons in the shape working memory task. B: decoding performance in the shape working memory task, for persistent and (task responsive) nonpersistent neurons, using the SVM classifier, with a 10-fold cross-validation. Sample size $n = 180$ neurons, for both cases. Time course of decoding accuracy during the duration of the trial. Conventions are the same as in Fig. 2. C: decoding accuracy for (task responsive) nonpersistent neurons during the middle 1 s of the delay period (0.75–1.75 s). Conventions are the same as in Fig. 3. PSTH, peristimulus time histogram; SVM, support vector machine.

level of decoding during the stimulation presentation epochs (the cue and sample period), cells without persistent activity in the delay period contained less information on stimuli identity during the delay period. A comparison of mean decoding accuracy in the center 1 s of the delay period indicated a significantly higher accuracy for neurons with persistent activity (t test, $t_{198} = 41.9$, $P < 0.001$).

DISCUSSION

The role of persistent activity has been reevaluated recently (11, 30, 31). Although strong evidence links behavior with persistent activity of neurons primarily in the prefrontal cortex but also in areas interconnected with it (32, 33) in some working memory tasks, objections have been raised about the general role of persistent activity across all working memory functions. It has thus been suggested that persistent activity in spatial tasks may represent motor

preparation rather than the remembered spatial location of the stimuli per se (10). Alternatively, the prefrontal cortex may play a supervisory role, “highlighting” the locations of stimuli, but not maintaining information about object identity (28, 34, 35).

Our study identified neurons that generate readily persistent activity in a spatial working memory task that separated the stimulus location from the eventual motor response. Our results showed that most information about the remembered stimulus location—which was necessary to perform the match-nonmatch task—was maintained by neurons that generated persistent activity. This persistent activity was also shown to be informative about the identity of stimuli held in memory, not just their spatial location. Our results therefore demonstrate that neurons that generate persistent activity maintain information more reliably across working memory stimulus domains in the primate prefrontal cortex.

Information Decoding from Persistent and Nonpersistent Neurons

Our analysis identified neurons with persistent activity, classically defined as activity that is elevated by a statistical criterion in the delay period compared to the fixation period of the task (21). The visual displays that our subjects observed during these two task epochs were identical, with only the fixation point appearing on-screen (see Fig. 1). The difference between these epochs thus lay in the stimulus presentation between them, which the subjects were taught to maintain in memory. Neurons that generated persistent activity accounted for most of the spatial and object information that could be decoded by the prefrontal population during the delay period. The effect was consistent across differently sized sets of neurons used to implement classification, across different classification parameters, and across different kinds of classifiers. An important caveat for this analysis is that neurons without persistent activity still exhibited some information, as predicted by activity silent theories (11). On the other hand, it is also possible that some of the neurons which failed to meet our statistical criterion generated some traces of persistent activity.

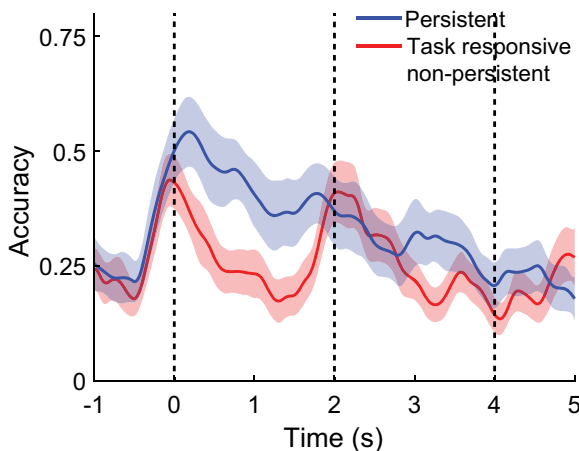


Figure 9. Decoding performance in the conjunction working memory task, for persistent and (task responsive) nonpersistent neurons, using the SVM classifier, with a 10-fold cross-validation. The stimuli identity (cue-shape combination) in the cue period was decoded. Sample size $n = 100$ neurons, for both cases. Time course of decoding accuracy during the duration of the trial. SVM, support vector machine.

Neurons with persistent activity comprised the minority of neurons in the shape working memory task. In fact, overall neuronal activity, averaged across all neurons and stimuli, barely suggested an increased firing rate in the delay period of the task. However, classification based on non-persistent neurons barely exceeded chance performance. This result thus provides direct evidence in favor of the idea that prefrontal neurons maintain stimulus-related information in working memory by virtue of persistent discharges (36).

Dynamic versus Stationary Encoding

Over the past decade, theoretical and experimental work has emphasized that working memory can be maintained even if neurons do not generate stable, persistent activity. Specifically, it has been proposed that the precise pattern of activation of an ensemble of neurons at each time point during a working memory task can be used to decode the identity of the stimulus, even though overall activity during the delay period is not significantly elevated above the baseline with the pattern of neuronal activation varying dynamically over time (12, 37). Such a pattern of activation appears to be the norm in rodent models, with individual frontal cortical neurons becoming active at different time points and “tiling” the delay period, rather than generating continuous persistent activity (38, 39).

Our present study replicated some of these findings and qualified some of the conclusions of previous studies. We show that averaged across all neurons and stimuli persistent activity was barely detectable in the primate prefrontal cortex, particularly for object stimuli (Fig. 8A). This result essentially replicates the result of the Stokes study, which popularized the idea of coding information in the absence of stable persistent activity (12). However, this result hides the existence of stimulus-selective persistent activity in a population of neurons. In fact, our work demonstrates that it is the latter population that disproportionately represents the remembered information.

Dynamic activity in a population of neurons is not mutually exclusive with persistent activity. Previous studies have identified neurons that generate persistent activity and are informative about the stimulus identity and task rules, which also exhibit some dynamics (40, 41). Information about the location of a stimulus in spatial working memory tasks evolves dynamically in time after the cue presentation, while different locations remain constrained in separable subspaces (42–44). Our current results also replicated this finding, showing the existence of some dynamics, which made it difficult to decode stimulus information from activity earlier or later in the trial. However, the dynamics of neurons with nonpersistent activity was essentially confined during the stimulus presentation periods.

Synaptic Mechanisms of Working Memory

Computational models have been proposed in which working memory could also be maintained through mechanisms that depend on synaptic strength modifications instead of spike generation (13, 14). Such mechanisms may be mediated by calcium availability at the presynaptic terminal, whose kinetics have a time constant in the scale of seconds (13). Another possible mechanism can be seen in the processes

of long-term potentiation, which represents memories as changes in the synaptic architecture, rather than neuronal activity (45). Synaptic modification in the sub-second timescale has thus been proposed as a mechanism that could maintain information in memory by some models (46, 47).

Strong theoretical and experimental evidence exists for some types of activity-silent mechanisms operating during working memory tasks. For example, activity elicited after repeated presentation of the same stimulus is typically reduced for many neurons, a phenomenon termed repetition suppression, even though persistent activity may not be present in most of these neurons (48). The difference in firing rate for match and nonmatch stimuli is diminished in error trials compared with correct ones, suggesting that behavioral performance is sensitive to this difference, and raising the possibility of working memory maintenance is being influenced by this activity (8, 49). Recurrent neural networks relying on rapid changes in synaptic weights after a stimulus presentation are able to perform working memory tasks (15–18). However, these studies have also suggested that synaptic mechanisms alone are inadequate to maintain information for more demanding tasks, such as tasks involving multiple sequential stimuli (16).

Serial bias is another phenomenon attributed to silent mechanisms. Specifically, the location of a previous trial's stimulus biases the response in the current trial. Recent spatial working memory studies have confirmed the existence of such mechanisms but have largely reconciled them with persistent activity mechanisms. When the appearance of the fixation point at the beginning of the trial increases neural excitability before the stimulus presentation, the previous trial's activity bump undergoes a transient reactivation, which then merges with the bump corresponding to the new stimulus (50). The merging of these two bumps of activation then causes the overall peak of firing rate activity to shift between the location of the previous and current stimulus location. In essence, synaptic mechanisms influence working memory to the extent that they influence spiking generation (36).

Working Memory for Complex Tasks

Prefrontal neurons generate persistent discharges in memory tasks that require the maintenance of stimulus features such as shape, color, direction of motion (51–56), the identity of objects, faces, and abstract pictures (57–64). These findings are also complicated by the fact that prefrontal persistent activity represents information beyond the characteristics of stimuli, including the rules of the cognitive task (65, 66), categories (59, 67), numerical quantities (68), and other abstract factors. It has, thus, been suggested that persistent activity may be mostly driven by task processes, rather than stimulus maintenance (69). Indeed, training to perform working memory tasks generally increases firing rate, particularly in delay periods, and induces a variety of other changes in factors such as variability and correlation (70, 71).

Our current results argue against the interpretation that the activity of prefrontal neurons entirely represents task and rule information. On the contrary, we identified stimulus-selective, persistent activity that maintained information about the remembered stimuli themselves during the delay period of the task within the prefrontal cortex. The

generation of persistent activity in the prefrontal cortex thus appears to be a critical mechanism of working memory maintenance, as predicted on theoretical grounds (25).

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

We wish to acknowledge Chrissy Suell for technical help; Kai Malcolm for initial simulations that led to this project; and Junda Zhu, and Zhengyang Wang for helpful comments on the manuscript.

GRANTS

Research reported in this paper was supported by the National Institutes of Health National Eye Institute under award numbers R01 EY017077, P30 EY008126, and F31 EY035546, and by the National Institute of Mental Health under award number R01 MH116675.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

Christos Constantinidis is an editor of *Journal of Neurophysiology* and was not involved and did not have access to information regarding the peer-review process or final disposition of this article. An alternate editor oversaw the peer-review and decision-making process for this article.

AUTHOR CONTRIBUTIONS

C.C. conceived and designed research; C.C. performed experiments; L.T., W.D., J.D.S., and C.C. analyzed data; L.T., W.D., R.G.J., J.D.S., and C.C. interpreted results of experiments; L.T., W.D., J.D.S., and C.C. prepared figures; L.T. and C.C. drafted manuscript; L.T., W.D., R.G.J., J.D.S. and C.C. edited and revised manuscript; L.T., W.D., R.G.J., J.D.S. and C.C. approved final version of manuscript.

REFERENCES

1. **Baddeley A.** Working memory: theories, models, and controversies. *Annu Rev Psychol* 63: 1–29, 2012. doi:10.1146/annurev-psych-120710-100422.
2. **Fuster JM, Alexander GE.** Neuron activity related to short-term memory. *Science* 173: 652–654, 1971. doi:10.1126/science.173.3997.652.
3. **Kamiński J, Sullivan S, Chung JM, Ross IB, Mamelak AN, Rutishauser U.** Persistently active neurons in human medial frontal and medial temporal lobe support working memory. *Nat Neurosci* 20: 590–601, 2017 [Erratum in *Nat Neurosci* 20: 1189, 2017]. doi:10.1038/nn.4509.
4. **Hart E, Huk AC.** Recurrent circuit dynamics underlie persistent activity in the macaque frontoparietal network. *eLife* 9: e52460, 2020. doi:10.7554/eLife.52460.
5. **Compte A, Brunel N, Goldman-Rakic PS, Wang XJ.** Synaptic mechanisms and network dynamics underlying spatial working memory in a cortical network model. *Cereb Cortex* 10: 910–923, 2000. doi:10.1093/cercor/10.9.910.
6. **Seung HS, Lee DD, Reis BY, Tank DW.** Stability of the memory of eye position in a recurrent network of conductance-based model neurons. *Neuron* 26: 259–271, 2000. doi:10.1016/s0896-6273(00)81155-1.
7. **Wimmer K, Nykamp DQ, Constantinidis C, Compte A.** Bump attractor dynamics in prefrontal cortex explains behavioral precision in spatial working memory. *Nat Neurosci* 17: 431–439, 2014. doi:10.1038/nn.3645.
8. **Zaksas D, Pasternak T.** Directional signals in the prefrontal cortex and in area MT during a working memory for visual motion task. *J Neurosci* 26: 11726–11742, 2006. doi:10.1523/JNEUROSCI.3420-06.2006.
9. **Lara AH, Wallis JD.** Executive control processes underlying multi-item working memory. *Nat Neurosci* 17: 876–883, 2014. doi:10.1038/nn.3702.
10. **Lundqvist M, Herman P, Miller EK.** Working memory: delay activity, yes! Persistent activity? Maybe not. *J Neurosci* 38: 7013–7019, 2018. doi:10.1523/JNEUROSCI.2485-17.2018.
11. **Stokes MG.** ‘Activity-silent’ working memory in prefrontal cortex: a dynamic coding framework. *Trends Cogn Sci* 19: 394–405, 2015. doi:10.1016/j.tics.2015.05.004.
12. **Stokes MG, Kusunoki M, Sigala N, Nili H, Gaffan D, Duncan J.** Dynamic coding for cognitive control in prefrontal cortex. *Neuron* 78: 364–375, 2013. doi:10.1016/j.neuron.2013.01.039.
13. **Mongillo G, Barak O, Tsodyks M.** Synaptic theory of working memory. *Science* 319: 1543–1546, 2008. doi:10.1126/science.1150769.
14. **Sugase-Miyamoto Y, Liu Z, Wiener MC, Optican LM, Richmond BJ.** Short-term memory trace in rapidly adapting synapses of inferior temporal cortex. *PLoS Comput Biol* 4: e1000073, 2008. doi:10.1371/journal.pcbi.1000073.
15. **Yang GR, Joglekar MR, Song HF, Newsome WT, Wang XJ.** Task representations in neural networks trained to perform many cognitive tasks. *Nat Neurosci* 22: 297–306, 2019. doi:10.1038/s41593-018-0310-2.
16. **Masse NY, Yang GR, Song HF, Wang XJ, Freedman DJ.** Circuit mechanisms for the maintenance and manipulation of information in working memory. *Nat Neurosci* 22: 1159–1167, 2019. doi:10.1038/s41593-019-0414-3.
17. **Mante V, Sussillo D, Shenoy KV, Newsome WT.** Context-dependent computation by recurrent dynamics in prefrontal cortex. *Nature* 503: 78–84, 2013. doi:10.1038/nature12742.
18. **Kim R, Sejnowski TJ.** Strong inhibitory signaling underlies stable temporal dynamics and working memory in spiking neural networks. *Nat Neurosci* 24: 129–139, 2021. doi:10.1038/s41593-020-00753-w.
19. **Riley MR, Qi XL, Zhou X, Constantinidis C.** Anterior-posterior gradient of plasticity in primate prefrontal cortex. *Nat Commun* 9: 3790, 2018. doi:10.1038/s41467-018-06226-w.
20. **Meyer T, Constantinidis C.** A software solution for the control of visual behavioral experimentation. *J Neurosci Methods* 142: 27–34, 2005. doi:10.1016/j.jneumeth.2004.07.009.
21. **Funahashi S, Bruce CJ, Goldman-Rakic PS.** Mnemonic coding of visual space in the monkey’s dorsolateral prefrontal cortex. *J Neurophysiol* 61: 331–349, 1989. doi:10.1152/jn.1989.61.2.331.
22. **Constantinidis C, Franowicz MN, Goldman-Rakic PS.** Coding specificity in cortical microcircuits: a multiple electrode analysis of primate prefrontal cortex. *J Neurosci* 21: 3646–3655, 2001. doi:10.1523/JNEUROSCI.21-10-03646.2001.
23. **Meyer T, Qi XL, Stanford TR, Constantinidis C.** Stimulus selectivity in dorsal and ventral prefrontal cortex after training in working memory tasks. *J Neurosci* 31: 6266–6276, 2011. doi:10.1523/JNEUROSCI.6798-10.2011.
24. **Zhou X, Katsuki F, Qi XL, Constantinidis C.** Neurons with inverted tuning during the delay periods of working memory tasks in the dorsal prefrontal and posterior parietal cortex. *J Neurophysiol* 108: 31–38, 2012. doi:10.1152/jn.01151.2011.
25. **Constantinidis C, Funahashi S, Lee D, Murray JD, Qi XL, Wang M, Arnsten AFT.** Persistent spiking activity underlies working memory. *J Neurosci* 38: 7020–7028, 2018. doi:10.1523/JNEUROSCI.2486-17.2018.
26. **Lara AH, Wallis JD.** The role of prefrontal cortex in working memory: a mini review. *Front Syst Neurosci* 9: 173, 2015. doi:10.3389/fnsys.2015.00173.
27. **Curtis CE, D’Esposito M.** Persistent activity in the prefrontal cortex during working memory. *Trends Cogn Sci* 7: 415–423, 2003. doi:10.1016/s1364-6613(03)00197-9.
28. **D’Esposito M, Postle BR.** The cognitive neuroscience of working memory. *Annu Rev Psychol* 66: 115–142, 2015. doi:10.1146/annurev-psych-010814-015031.
29. **Tong F, Pratte MS.** Decoding patterns of human brain activity. *Annu Rev Psychol* 63: 483–509, 2012. doi:10.1146/annurev-psych-120710-100412.

30. **Riley MR, Constantinidis C.** Role of prefrontal persistent activity in working memory. *Front Syst Neurosci* 9: 181, 2015. doi:10.3389/fnsys.2015.00181.
31. **Buschman TJ, Miller EK.** Working memory is complex and dynamic, like your thoughts. *J Cogn Neurosci* 35: 17–23, 2022. doi:10.1162/jocn_a_01940.
32. **Qi XL, Elworthy AC, Lambert BC, Constantinidis C.** Representation of remembered stimuli and task information in the monkey dorsolateral prefrontal and posterior parietal cortex. *J Neurophysiol* 113: 44–57, 2015. doi:10.1152/jn.00413.2014.
33. **Jacob SN, Nieder A.** Complementary roles for primate frontal and parietal cortex in guarding working memory from distractor stimuli. *Neuron* 83: 226–237, 2014. doi:10.1016/j.neuron.2014.05.009.
34. **Sreenivasan KK, Vytlaclil J, D'Esposito M.** Distributed and dynamic storage of working memory stimulus information in the extrastriate cortex. *J Cogn Neurosci* 26: 1141–1153, 2014. doi:10.1162/jocn_a_00556.
35. **Ester EF, Anderson DE, Serences JT, Awh E.** A neural measure of precision in visual working memory. *J Cogn Neurosci* 25: 754–761, 2013. doi:10.1162/jocn_a_00357.
36. **Jaffe RJ, Constantinidis C.** Working memory: from neural activity to the sentient mind. *Compr Physiol* 11: 2547–2587, 2021. doi:10.1002/cphy.c210005.
37. **Scott BB, Constantinople CM, Akrami A, Hanks TD, Brody CD, Tank DW.** Fronto-parietal cortical circuits encode accumulated evidence with a diversity of timescales. *Neuron* 95: 385–398.e5, 2017. doi:10.1016/j.neuron.2017.06.013.
38. **Baeg EH, Kim YB, Huh K, Mook-Jung I, Kim HT, Jung MW.** Dynamics of population code for working memory in the prefrontal cortex. *Neuron* 40: 177–188, 2003. doi:10.1016/s0896-6273(03)00597-x.
39. **Bolkan SS, Stujenske JM, Parnaudeau S, Spellman TJ, Rauffenbart C, Abbas AI, Harris AZ, Gordon JA, Kellendonk C.** Thalamic projections sustain prefrontal activity during working memory maintenance. *Nat Neurosci* 20: 987–996, 2017. doi:10.1038/nn.4568.
40. **Meyers EM, Qi XL, Constantinidis C.** Incorporation of new information into prefrontal cortical activity after learning working memory tasks. *Proc Natl Acad Sci USA* 109: 4651–4656, 2012. doi:10.1073/pnas.1201022109.
41. **Crowe DA, Averbeck BB, Chafee MV.** Neural ensemble decoding reveals a correlate of viewer- to object-centered spatial transformation in monkey parietal cortex. *J Neurosci* 28: 5218–5228, 2008. doi:10.1523/JNEUROSCI.5105-07.2008.
42. **Murray JD, Bernacchia A, Roy NA, Constantinidis C, Romo R, Wang XJ.** Stable population coding for working memory coexists with heterogeneous neural dynamics in prefrontal cortex. *Proc Natl Acad Sci USA* 114: 394–399, 2017. doi:10.1073/pnas.1619449114.
43. **Spaak E, Watanabe K, Funahashi S, Stokes MG.** Stable and dynamic coding for working memory in primate prefrontal cortex. *J Neurosci* 37: 6503–6516, 2017. doi:10.1523/JNEUROSCI.3364-16.2017.
44. **Parthasarathy A, Tang C, Herikstad R, Cheong LF, Yen SC, Libedinsky C.** Time-invariant working memory representations in the presence of code-morphing in the lateral prefrontal cortex. *Nat Commun* 10: 4995, 2019. doi:10.1038/s41467-019-12841-y.
45. **Ranganath C, Cohen MX, Brozinsky CJ.** Working memory maintenance contributes to long-term memory formation: neural and behavioral evidence. *J Cogn Neurosci* 17: 994–1010, 2005. doi:10.1162/0898929054475118.
46. **Miller EK, Lundqvist M, Bastos AM.** Working memory 2.0. *Neuron* 100: 463–475, 2018. doi:10.1016/j.neuron.2018.09.023.
47. **Lundqvist M, Rose J, Herman P, Brincat SL, Buschman TJ, Miller EK.** Gamma and beta bursts underlie working memory. *Neuron* 90: 152–164, 2016. doi:10.1016/j.neuron.2016.02.028.
48. **Grill-Spector K, Henson R, Martin A.** Repetition and the brain: neural models of stimulus-specific effects. *Trends Cogn Sci* 10: 14–23, 2006. doi:10.1016/j.tics.2005.11.006.
49. **Qi XL, Meyer T, Stanford TR, Constantinidis C.** Neural correlates of a decision variable before learning to perform a match/nonmatch task. *J Neurosci* 32: 6161–6169, 2012. doi:10.1523/JNEUROSCI.6365-11.2012.
50. **Barbosa J, Stein H, Martinez RL, Galan-Gadea A, Li S, Dalmau J, Adam KCS, Valls-Solé J, Constantinidis C, Compte A.** Interplay between persistent activity and activity-silent dynamics in the prefrontal cortex underlies serial biases in working memory. *Nat Neurosci* 23: 1016–1024, 2020. doi:10.1038/s41593-020-0644-4.
51. **Mendoza-Halliday D, Torres S, Martinez-Trujillo JC.** Sharp emergence of feature-selective sustained activity along the dorsal visual pathway. *Nat Neurosci* 17: 1255–1262, 2014. doi:10.1038/nn.3785.
52. **Hoshi E, Shima K, Tanji J.** Task-dependent selectivity of movement-related neuronal activity in the primate prefrontal cortex. *J Neurophysiol* 80: 3392–3397, 1998. doi:10.1152/jn.1998.80.6.3392.
53. **Sakagami M, Tsutsui K, Lauwereyns J, Koizumi M, Kobayashi S, Hikosaka O.** A code for behavioral inhibition on the basis of color, but not motion, in ventrolateral prefrontal cortex of macaque monkey. *J Neurosci* 21: 4801–4808, 2001. doi:10.1523/JNEUROSCI.21-13-04801.2001.
54. **Inoue M, Mikami A.** Prefrontal activity during serial probe reproduction task: encoding, mnemonic, and retrieval processes. *J Neurophysiol* 95: 1008–1041, 2006. doi:10.1152/jn.00552.2005.
55. **Genovesio A, Tsujimoto S, Wise SP.** Feature- and order-based timing representations in the frontal cortex. *Neuron* 63: 254–266, 2009. doi:10.1016/j.neuron.2009.06.018.
56. **Averbeck BB, Chafee MV, Crowe DA, Georgopoulos AP.** Neural activity in prefrontal cortex during copying geometrical shapes. I. Single cells encode shape, sequence, and metric parameters. *Exp Brain Res* 150: 127–141, 2003. doi:10.1007/s00221-003-1416-6.
57. **Rao SC, Rainer G, Miller EK.** Integration of what and where in the primate prefrontal cortex. *Science* 276: 821–824, 1997. doi:10.1126/science.276.5313.821.
58. **Roy JE, Buschman TJ, Miller EK.** PFC neurons reflect categorical decisions about ambiguous stimuli. *J Cogn Neurosci* 26: 1283–1291, 2014. doi:10.1162/jocn_a_00568.
59. **Freedman DJ, Riesenhuber M, Poggio T, Miller EK.** Categorical representation of visual stimuli in the primate prefrontal cortex. *Science* 291: 312–316, 2001. doi:10.1126/science.291.5502.312.
60. **O Scalaidhe S, Wilson FA, Goldman-Rakic PS.** Areal segregation of face-processing neurons in prefrontal cortex. *Science* 278: 1135–1138, 1997. doi:10.1126/science.278.5340.1135.
61. **Rainer G, Asaad WF, Miller EK.** Selective representation of relevant information by neurons in the primate prefrontal cortex. *Nature* 393: 577–579, 1998. doi:10.1038/31235.
62. **Rainer G, Miller EK.** Effects of visual experience on the representation of objects in the prefrontal cortex. *Neuron* 27: 179–189, 2000. doi:10.1016/s0896-6273(00)00019-2.
63. **Wilson FA, Scalaidhe SP, Goldman-Rakic PS.** Dissociation of object and spatial processing domains in primate prefrontal cortex. *Science* 260: 1955–1958, 1993. doi:10.1126/science.8316836.
64. **Miller EK, Erickson CA, Desimone R.** Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *J Neurosci* 16: 5154–5167, 1996. doi:10.1523/JNEUROSCI.16-16-05154.1996.
65. **White IM, Wise SP.** Rule-dependent neuronal activity in the prefrontal cortex. *Exp Brain Res* 126: 315–335, 1999. doi:10.1007/s002210050740.
66. **Wallis JD, Anderson KC, Miller EK.** Single neurons in prefrontal cortex encode abstract rules. *Nature* 411: 953–956, 2001. doi:10.1038/35082081.
67. **Shima K, Isoda M, Mushiake H, Tanji J.** Categorization of behavioural sequences in the prefrontal cortex. *Nature* 445: 315–318, 2007. doi:10.1038/nature05470.
68. **Nieder A, Freedman DJ, Miller EK.** Representation of the quantity of visual items in the primate prefrontal cortex. *Science* 297: 1708–1711, 2002. doi:10.1126/science.1072493.
69. **Owen AM, Stern CE, Look RB, Tracey I, Rosen BR, Petrides M.** Functional organization of spatial and nonspatial working memory processing within the human lateral frontal cortex. *Proc Natl Acad Sci USA* 95: 7721–7726, 1998. doi:10.1073/pnas.95.13.7721.
70. **Qi XL, Constantinidis C.** Neural changes after training to perform cognitive tasks. *Behav Brain Res* 241: 235–243, 2013. doi:10.1016/j.bbr.2012.12.017.
71. **Constantinidis C, Klingberg T.** The neuroscience of working memory capacity and training. *Nat Rev Neurosci* 17: 438–449, 2016. doi:10.1038/nrn.2016.43.