Spatiotemporal encoding of search strategies by prefrontal neurons

Feng-Kuei Chiang^{a,1} and Joni D. Wallis^{a,b,2}

^aDepartment of Psychology, University of California, Berkeley, CA 94720; and ^bHelen Wills Neuroscience Institute, University of California, Berkeley, CA 94720

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Working memory is capacity-limited. In everyday life we rarely notice this limitation, in part because we develop behavioral strategies that help mitigate the capacity limitation. How behavioral strategies are mediated at the neural level is unclear, but a likely locus is lateral prefrontal cortex (LPFC). Neurons in LPFC play a prominent role in working memory and have been shown to encode behavioral strategies. To examine the role of LPFC in overcoming working-memory limitations, we recorded the activity of LPFC neurons in animals trained to perform a serial self-ordered search task. This task measured the ability to prospectively plan the selection of unchosen spatial search targets while retrospectively tracking which targets were previously visited. We found that individual LPFC neurons encoded the spatial location of the current search target but also encoded the spatial location of targets up to several steps away in the search sequence. Neurons were more likely to encode prospective than retrospective targets. When subjects used a behavioral strategy of stereotyped target selection, mitigating the working-memory requirements of the task, not only did the number of selection errors decrease but there was a significant reduction in the strength of spatial encoding in LPFC. These results show that LPFC neurons have spatiotemporal mnemonic fields, in that their firing rates are modulated both by the spatial location of future selection behaviors and the temporal organization of that behavior. Furthermore, the strength of this tuning can be dynamically modulated by the demands of the task.

prefrontal | working memory | macaque

The capacity of working memory was empirically defined by Miller as 7 ± 2 items (1), although more recently this limit has been revised down to four items (2). These capacity limits seem hard to reconcile with our everyday experience, where we appear to have little problem temporarily remembering numbers of items at or beyond the empirical limit. This is partly due to the rigorous laboratory conditions under which capacity limits are measured, while in everyday life we use a repertoire of behavioral strategies to overcome these limits. For example, dealing a hand of cards to players in random order would necessarily involve a large working-memory load, requiring the dealer to keep track of how many cards each player has been dealt. By instead selecting a behavioral strategy, such as dealing the cards to players in a clockwise order, the dealer reduces the working-memory load to only a single item (last player dealt a card), enabling the task of dealing cards to be completed at a lower computational cost.

It remains unclear how these high-level strategies interact with the contents of working memory. A likely source for the neural basis of this interaction is the lateral prefrontal cortex (LPFC). Neurons in this area selectively encode task-specific information across intervening delay periods and distractors (3), consistent with maintaining information in working memory. Neurons in this region also encode high-level information about rules (4), categories (5), and strategies (6). The spatial self-ordered search task taxes both working memory and strategy use. This requires subjects to search through an array of targets collecting rewards, requiring monitoring of which targets were previously visited.

Patients with prefrontal damage were impaired at the task (7) and experiments in monkeys showed that this was specifically due to damage of LPFC, as opposed to the neighboring frontal eye fields (8).

Neuroimaging studies have also revealed that LPFC shows greater activity as working-memory load increases (9, 10). This could simply reflect the increase in task difficulty as working-memory capacity is approached, since increasing task demands reliably increase activity in prefrontal cortex (11). However, when information is organized into a meaningful sequence, task difficulty decreases and behavioral performance increases, while blood oxygenation level-dependent activity in LPFC remains increased (12). Taken together these findings suggest that LPFC plays an important role in organizing information in working memory that is orthogonal to the working-memory demands of the task. In support of this, increasing the delay over which information must be remembered does not exacerbate the effects of LPFC damage on the self-ordered search task, whereas increasing the number of items that must be monitored does (13). To investigate how strategies and working memory interact at the single-neuron level in LPFC, we trained monkeys to perform a spatial self-ordered search task and recorded the activity of LPFC neurons.

Methods

We trained two rhesus monkeys to perform an oculomotor spatial self-ordered search task (Fig. 1A). Subjects were presented with a display of six targets and were required to select each target in turn. Subjects were allowed to select the targets in any order but only received a reward the first

Significance

Working memory is a capacity-limited system that is used by cognitive processes, such as planning, reasoning, and decision making, to temporarily store information for short periods of time. We often employ behavioral strategies to overcome the capacity limit, such as processing information in sequences, but how this is accomplished at the neural level is unknown. By recording the electrical activity of prefrontal neurons during working-memory tasks, we found that prefrontal neurons had spatiotemporal receptive fields, encoding both the spatial coordinates of behavioral targets and their organization within a sequence. Furthermore, the strength of this tuning could be dynamically modulated according to how much subjects relied on working memory or behavioral strategies.

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¹Present address: Department of Neuroscience and the Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029.

²To whom correspondence should be addressed. Email: wallis@berkeley.edu.

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Fig. 1. (A) Spatial self-ordered search task. (B) Each configuration consisted of six targets (green filled circles), which were selected from 36 possible locations (gray filled circles). We ensured that targets were approximately balanced across the display by requiring that the centroid of the configuration (red cross) be located within $\pm 3^\circ$ of the fixation window (black circle). The intertarget distances were $> 6^\circ$ to avoid overlap of the eye position detection window around the target. (C) Number of unique sequences of target selection per configuration.

time that a target was selected per trial. Revisiting a previously selected target resulted in a 500-ms time-out. Thus, subjects had to maintain in working memory which targets had already been selected. Once subjects received six rewards by selecting each target once, the current trial was terminated and an intertrial interval was initiated. The target-reward contingency reset at the beginning of a new trial.

Target configurations consisted of six identical targets, each 2° in diameter and located at one of 36 positions on the visual display (Fig. 1B). The subject had to saccade to each target in turn and fixate it for 500 ms. Fixation was defined as maintaining the eye position within $\pm 3^\circ$ of the center of the target. Any drift out of this window would reset the 500-ms fixation duration. After the successful fixation of a target, the outcome was determined according to whether or not the selected target had been previously selected on the current trial. In between each target selection, the targets disappeared and the fixation cue was presented. The subject had to fixate this cue for 1 s before the targets were presented again. To avoid excessive frustration, if the subject experienced more than 10 time-outs in a single trial then the trial was aborted. This happened on $< 1\%$ of trials. To ensure that subjects realized that they had found all six possible rewards, the color of the targets changed from trial to trial in a green-white-blue sequence. The target configuration remained stable for a block of 40 trials, of which subjects completed six per session. The spatial configuration of targets used for each block was pseudorandomly chosen from a pool of 20 a priori-designed spatial configurations. The same configuration was presented repeatedly within a block but used only once per session.

We implanted both subjects with a head positioner for restraint and a titanium recording chamber over each hemisphere. Acutely, we recorded from bilateral LPFC using arrays of 16–32 tungsten microelectrodes (FHC Instruments). All procedures were in accord with the National Institute of Health guidelines and the recommendations of the University of California, Berkeley Animal Care and Use Committee. Further details are in *SI Appendix*.

Results

Task Performance. Subject R completed 15 sessions and subject Q completed 10 sessions, with each session comprising six blocks of 40 trials. One block of trials was excluded from subject Q as there were fewer than 20 complete trials in that block. To quantify task performance, we tabulated the number of revisited targets on each trial. Subjects performed the task at a high level (Fig. 2A); about 80% of trials were completed with two or fewer incorrect saccades.

To complete a trial, the subject needed to select each of the six targets once. We refer to these correct target selections as T_i ,

where i is the i^{th} selected target in the sequence $T_1 \dots T_6$. We defined the success rate for each target selection T_i as the number of trials where the target was selected without any errors, divided by the total number of trials (Fig. 2B). We then used the average success rate across $T_1 \dots T_6$ to calculate the expected success rate at each T_i , assuming that the overall probability of making a mistake is a linear function of the number of potentially incorrect saccades that are possible (*SI Appendix*). We then calculated whether the actual success rate significantly differed from the expected success rate using binomial tests. Both subjects made fewer correct saccades in selecting the fifth and sixth targets compared with the expected success rate (Fig. 2B; binomial test, $P < 0.001$). This suggests that the working-memory demands of the task increased as the number of selected targets increased and began to exceed the subjects' working-memory capacity by the fifth and sixth target selections.

We defined the saccadic reaction time (sRT) as the time from the onset of the target configuration to the beginning of the 500-ms hold target epoch (Fig. 2C). Incorrect saccades were excluded from the calculation of sRT. The overall median sRT was 210 and 221 ms from subjects R and Q, respectively. Further, we calculated sRT separately for each target selection and performed a linear regression using sRT as the dependent variable and the selected target's position in the selection sequence as a predictor. For both subjects, we found that sRT significantly increased as the number of selected targets increased [Fig. 2B; subject R: $F(1, 20,596) = 2,400$, $r^2 = 0.10$, $P < 1 \times 10^{-18}$; subject Q: $F(1, 12,491) = 81$, $r^2 = 0.006$, $P < 1 \times 10^{-18}$]. This could be an effect of exceeding working-memory capacity, or it might reflect the increased response selection requirements that arise from the increased number of targets that must be avoided.

Behavioral Strategies. We next analyzed the patterns of target selection to gain insight into the behavioral strategies that the subjects employed to solve the task. Each of the 20 configurations was pseudorandomly selected four to six times for subject R and three times for subject Q across the recording sessions. For each block of 40 trials using a specific spatial configuration, we

Fig. 2. Behavioral performance. (A) Distribution of the number of incorrect saccades per trial. (B) Observed and expected success rates plotted as a function of which target in the sequence was selected. The observed success rate was significantly lower than the expected error rate for the fifth and sixth selected targets in both subjects (binomial test, $*P < 0.001$). (C) Reaction times as a function of saccade. Box plots indicate the 25th, 50th, and 75th percentiles of the sRT distribution. Red dots indicate the mean sRT.

Fig. 6. (A) Single-neuron examples showing the effects of the stereotype strategy on spatial tuning. Spike density histograms are plotted from two neurons, one recorded in subject R (left two panels) and one recorded from subject Q (right two panels). The color of the plots refers to the position of the selected target on the screen, as shown in the key. Plots on the top are from the three blocks in the session with the higher SI, whereas the plots on the bottom are from the three blocks with the lower SI. Less spatial tuning was observed when the animal was searching through the targets using a more stereotyped strategy. (B) Spatial tuning, as measured by the r^2 from the reduced model, as a function of SI. Stronger spatial tuning occurred in blocks with less stereotyped behavior.

studies have shown that progressively more anterior regions of the frontal lobe are responsible for integrating behavior across progressively more complex and temporally extended task structures (18, 19). Neurophysiological recordings have shown that LPFC neurons encode information about specific actions, but this encoding often depends on how this action is embedded within a sequence of actions (20). At a more abstract level, population analyses have shown that the main information encoded by prefrontal neurons during the performance of a cognitive task is the sequence of events in the task (21). Prefrontal neurons have also been shown to encode high-level information, such as categories (5) and rules (4).

The above studies are consistent with a role for prefrontal cortex in structuring behavior at a high level. Our current results

suggest a mechanism that helps to delineate a more precise role for prefrontal cortex in this process. LPFC neurons were strongly tuned for the spatial locations of targets, but this tuning was not restricted to just the current target, but rather included previous and upcoming targets, often several steps in the sequence from the current target. Furthermore, spatial tuning was more similar for targets at the same temporal phase of the sequence than for targets at different temporal phases. This was particularly the case for tuning related to the current location but was also apparent in the tuning for past and future targets. Upcoming actions tended to be represented more strongly than completed responses, which is consistent with previous studies that have also noted a bias toward prospective encoding in prefrontal neurons (22, 23).

Previous studies that have examined the contribution of LPFC neurons to working memory have concentrated on the ability of LPFC neurons to exhibit spatial tuning across intervening delays, so-called mnemonic receptive fields (22, 24–26). Our results extend this concept and suggest that a more complete description of prefrontal tuning is a spatiotemporal receptive field that encompasses both the spatial coordinates of the target behavior as well its temporal order within the behavioral structure. Our results are consistent with recent models of prefrontal cortex that have emphasized the high-dimensional nature of prefrontal encoding schemes, particularly the mixed selectivity models (27, 28), since the spatial selectivity of an individual prefrontal neuron is not fixed but rather depends on the temporal phase of the task.

The spatial self-ordered search task was developed by Petrides and Milner because patients with prefrontal damage performed remarkably well on short-term memory tests, such as digit span and story recall (7, 29). This contrasted with the severe deficits that these patients exhibited on tasks that required planning (16, 17) or flexible control of behavior (30). Indeed, recent studies have shown that prefrontal damage does not impair classic tasks of spatial working memory, such as the memory-guided saccade task, so long as the frontal eye fields are preserved (31). Petrides has argued that the critical feature of the self-ordered search task that makes it sensitive to prefrontal damage is the requirement to attend to all stimuli within the set and monitor successive choices, in other words, tracking which stimuli have been selected and which have not been selected. The spatiotemporal receptive fields that we observed in LPFC neurons contain precisely the information that would be needed for this process, encoding both the current target of behavior as well as which stimuli have been and will be selected.

Role of PFC in Cognitive Control. When subjects searched through the targets in a more stereotyped way, their behavioral performance improved, despite a drop in the amount of spatial information that was encoded in LPFC. Our previous studies looked at the effects of reward on LPFC spatial tuning (32). If a reward-predictive cue was presented before a memorandum, then LPFC neurons showed stronger spatial tuning and behavioral performance improved. However, presenting the reward-predictive cue after the memorandum was distracting. Under these conditions, LPFC neurons showed weaker tuning and behavioral performance declined. Thus, behavioral performance correlated with the strength of LPFC spatial tuning. Taken together with our current results, this shows that the prefrontal representation of space is highly dynamic and the strength of tuning can change in response to the cognitive demands of the task. However, our current results contrast with this previous study, in that loss of spatial tuning was correlated with an improvement in behavioral performance.

One possibility is that using a stereotyped strategy to search through the targets recruits other brain regions more involved in sequence learning, thereby reducing the need for prefrontal

working-memory mechanisms (33). Neuroimaging studies have shown a shift from a prefrontal-cerebellar network to a premotor-striatal network as a motor sequence becomes more stereotyped and performance more automatic (34, 35). Neural recordings in the supplementary motor area have revealed neurons that encode specific sequences of movements, while inactivation of this area impairs the performance of a motor sequence, but not the execution of individual movements (36). One advantage of automation with skill development is that it frees up attentional resources for other tasks, which would be consistent with a drop in prefrontal tuning, if LPFC is indeed responsible for the allocation of these resources.

In sum, our data show that prefrontal coding of information relevant to working memory can be dynamically modulated according to task demands. Consequently, the implementation of behavioral strategies to reduce performance demands can change the information content of prefrontal neurons. This has implications for computational accounts of working memory, which are described as bump attractors, whereby persistent population codes are maintained through a combination of local recurrent excitation and broader feedback inhibition (37, 38). The capacity limits of working memory are thought to arise

because drift in the attractors can cause adjacent bumps to merge or fade, which becomes more likely as the number of attractor states increases (39, 40). A read-out problem could arise if the size of the bumps can be modulated by other factors, such as task demands. If a downstream brain area were receiving information solely from LPFC, there would be ambiguity as to whether noise in the representation arose from the capacity limits of the prefrontal representation or whether it reflected a handing off of the storage functions to other brain areas. In reality, this may be less of a problem, since most areas are receiving information from many different brain areas, and could, in principle, “listen” to the area with the most robust response. Future research could test this hypothesis, by comparing the strength of the spatial representation in downstream areas, such as the striatum and premotor cortex, with that observed in LPFC during performance of the self-ordered search task.

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