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Timecourse of object-related neural activity in the primate prefrontal cortex during a short-term memory task

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Abstract

We studied the timecourse of neural activity in the primate (*Macacca mulatta*) prefrontal (PF) cortex during an object delayed-matching-to-sample (DMS) task. To assess the effects of experience on this timecourse, we conducted the task using both novel and highly familiar objects. In addition, noise patterns containing no task-relevant information were used as samples on some trials. Comparison of average PF ensemble activity relative to baseline activity generated by objects and noise patterns revealed three distinct activity periods. (i) Sample onset elicited a transient sensory visual response. In this sensory period, novel objects elicited stronger average ensemble activity than both familiar objects and noise patterns. (ii) An intermediate period of elevated activity followed, which began before sample offset, and continued well into the delay period. In the intermediate period, activity was elevated for noise patterns and novel objects, but near baseline for familiar objects. (iii) Finally, after average ensemble activity reached baseline activity at the end of the intermediate period, a reactivation period occurred late in the delay. Experience had little effect during reactivation, where activity was elevated for both novel and familiar objects compared to noise patterns. We show that the ensemble average resembles the activity timecourse of many single prefrontal neurons. These results suggest that PF delay activity does not merely maintain recent sensory input, but is subject to more complex experience-dependent dynamics. This has implications for how delay activity is generated and maintained.

Introduction

The prefrontal (PF) cortex contains many neurons that show elevated activity during delay periods in cognitive tasks (Fuster, 1993; Goldman-Rakic, 1995; Miller & Cohen, 2001). Studies have implicated delay activity in short-term memory for spatial locations (Funahashi *et al*., 1989; Funahashi *et al*., 1993) and objects (Fuster & Alexander, 1971; Miller *et al*., 1996; Rainer *et al*., 1998a). Delay activity has also been associated with anticipatory or prospective coding for object stimuli (Rainer *et al*., 1999) and reward (Kubota & Niki, 1971; Watanabe, 1996), as well as target selection (Rainer *et al*., 1998b; Hasegawa *et al*. 2000), behavioural rules (White & Wise, 1999; Asaad *et al*. 2000) and motor preparation (Bruce & Goldberg, 1985; Watanabe, 1986; di Pellegrino & Wise, 1993). PF neurons exhibiting delay activity thus play an important role in bridging the gap between a sensory stimulus and a temporally delayed response during cognitive tasks. Lesion and electrophysiological studies have also shown that the PF cortex plays an important role in learning (Petrides, 1985; Parker *et al*., 1998), and that PF neural response properties can be modified strongly by experience (Bichot *et al*., 1996; Rainer & Miller, 2000).

In addition to this *in vivo* work correlating PF delay activity with various cognitive functions, several recent computational studies have investigated by which mechanisms delay activity might be generated and maintained. Models used to describe delay activity have focused typically on persistent activity, i.e. the prolongation of a neural response to a sensory stimulus after the removal of sensory stimulation (Durstewitz *et al*. 2000a; Wang, 2001). Approaches used to model persistent activity include discrete attractor models based on recurrent excitation and inhibition (Amit & Brunel, 1997; Amit *et al*., 1997) and detailed biophysical models (Compte *et al*. 2000; Durstewitz *et al*. 2000b). Other work has focused on networks with bistable solutions with a resting and an active state based on long NMDA-channel (Wang, 1999) or short AMPA-channel (Laing & Chow, 2001) activation timescales. While these models have contributed substantially to our understanding of delay activity and the mechanisms that may give rise to it, the electrophysiological studies suggest that activity in the monkey PF cortex is far more varied and complex than simple persistence of sensory information. Here, our aim is to provide a comprehensive account of the timecourse of neural ensemble activity during a simple cognitive task requiring short-term memory for objects.

We examined neural activity in a delayed-matching-to-sample (DMS) task (Fig. 1). When objects were used as samples, monkeys needed to retain this object information for a short delay to correctly perform the task. On some trials, noise patterns were used as samples. Although these noise patterns had similar image statistical properties as the objects, they did not provide any task-relevant information and did not need to be retained over the course of the delay. This allowed us to compare activity specific to processing and maintenance of task-relevant object information, with activity elicited by noise patterns that needed to be processed but not maintained in short-term memory.

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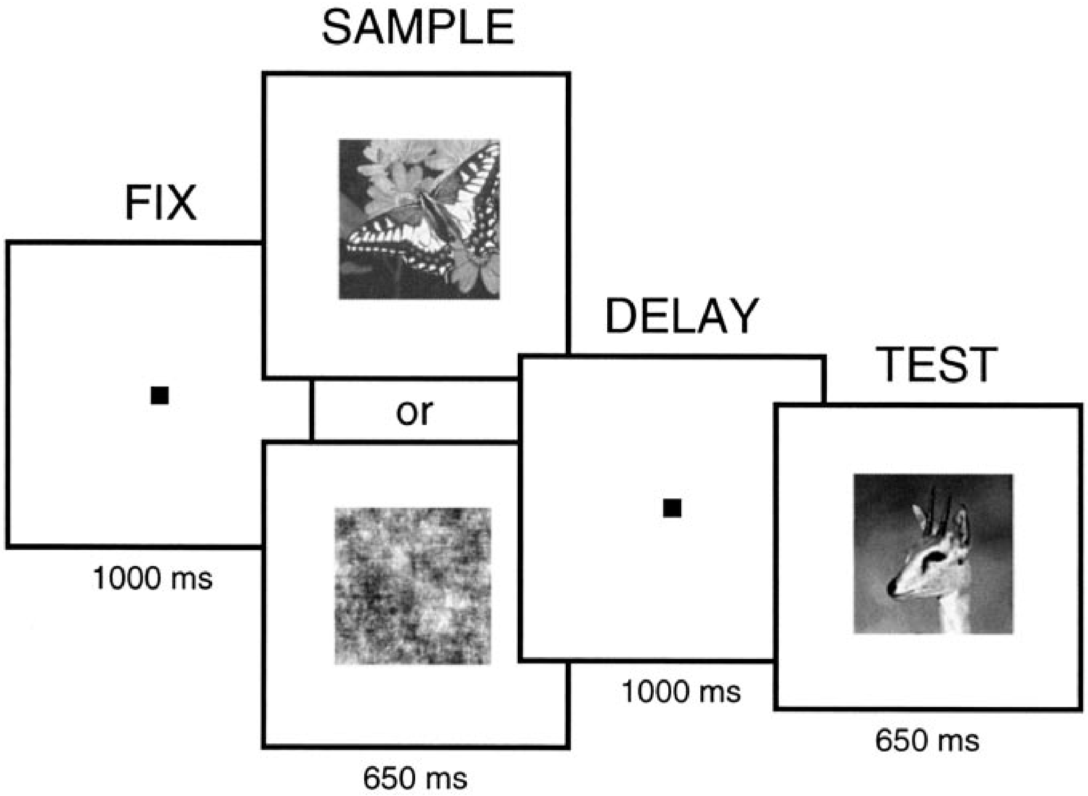


FIG. 1. Sequence of trial events. After a fixation period (FIX), a SAMPLE image (either one of five objects or one of four noise patterns) was presented. After a brief DELAY, a TEST image (one of the five objects) was presented, and monkeys had to release a lever if this TEST image matched the SAMPLE or in the case of a nonmatch hold the lever for the entire test duration and release to a subsequently presented correct match (not shown).

Furthermore, we addressed effects of experience on the timecourse of object processing by conducting the experiment with familiar as well as novel objects (see next section).

Materials and methods

Two adult rhesus monkeys, *Macacca mulatta* (monkey A, female, 8 kg; monkey B, male, 11 kg), participated in the experiments. All procedures were performed in accordance with National Institutes of Health guidelines and the recommendations of the Massachusetts Institute of Technology Animal Care and Use Committee.

Behavioural task

The behavioural paradigm was a modified version of delayed-matching-to-sample (see Fig. 1). Each trial began when the monkey grasped a metal lever. A fixation point ($0.3 \times 0.3°$) was then presented at the centre of a computer screen positioned in front of the animal. After attaining fixation, monkeys were required to maintain fixation within $\pm 1.25°$ of this fixation point throughout the rest of the trial. After 1000 ms of fixation, a sample object was presented for 650 ms. This sample object could be either one of five natural images (objects), one of four noise patterns (noise). During the experiment, intermediate images between objects and noise patterns generated by Fourier phase interpolation were also employed. The purpose of this was to study the ability of prefrontal neurons to communicate information about degraded images. Details about how these degraded images were generated as well as relevant behavioural and neural data have been described elsewhere (Rainer & Miller, 2000), and will not be further discussed here. After a brief delay period, one of the five natural images was presented as a test object. Monkeys had to release the lever if this test object matched the sample, or hold the lever for the entire test object duration in case of a nonmatch. In the nonmatch case, a brief second delay (200 ms) followed, which was always followed by a correct match object requiring a lever release. This second delay was included only to ensure that monkeys made a behavioural response on every trial and was not used in any of the analyses. Match and nonmatch trials occurred equally often. Monkeys received apple juice as a reward for correct performance on trials with object samples. On trials with noise patterns as the sample, half the trials were designated arbitrarily as match trials, the other half as nonmatch trials. Monkeys were thus rewarded randomly on half the trials, independent of whether they held or released the lever on trials with noise pattern samples. This reward protocol was chosen to ensure that monkeys were motivated to attempt identification of intermediate interpolated patterns. Both monkeys had extensive experience over several years with delayed matching tasks prior to participation in the present experiments.

Stimuli

Natural images were selected from a large database containing pictures of animals, faces, flowers and outdoor scenes. After adjusting the images to have equal mean intensity, we computed the Fourier amplitude spectrum for each image, and averaged the amplitude spectra to obtain a mean amplitude spectrum (MAS). The MAS had the spatial frequency ($f^{-\alpha}$) dependence characteristic of natural images (Field, 1987). The Fourier phase spectra of the images were then converted back into image space using the MAS. This ensured

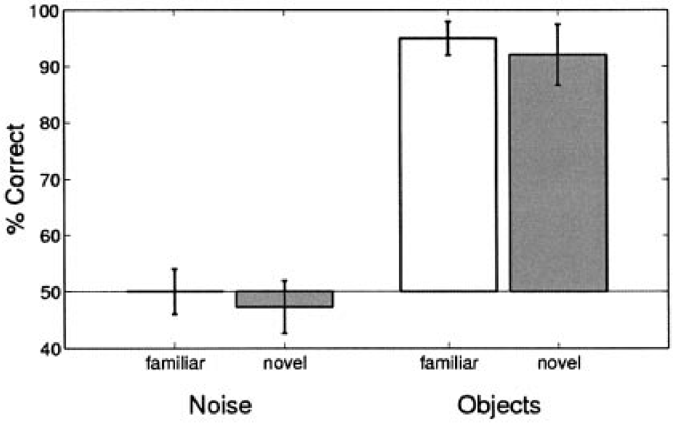


FIG. 2. Behavioural performance – behavioural performance pooled across a total of 25 sessions from two monkeys, with corresponding standard deviations.

that all five objects had identical power at all spatial frequencies. Noise patterns were created by first generating random phase spectra (i.e. each phase coefficient was randomly assigned within the range $-\pi$ to $+\pi$). These random phase spectra were then transformed to image space with the natural image MAS computed previously. Transformations were performed using custom-written software (MATLAB, Mathworks, Natick, USA). Noise patterns were thus matched to the natural images in terms of luminance and spatial frequency content. Stimulus size was 4° × 4°, and mean luminance of each entire image was 14 cd/m^2. Stimuli were always presented at the centre of gaze on a 17-inch computer monitor after appropriate gamma correction to ensure linearity of the display.

Novelty/familiarity

To assess effects of visual experience, monkeys performed the task using familiar and novel objects. During ‘familiar object’ sessions, we used objects that were highly familiar to the monkeys. They had

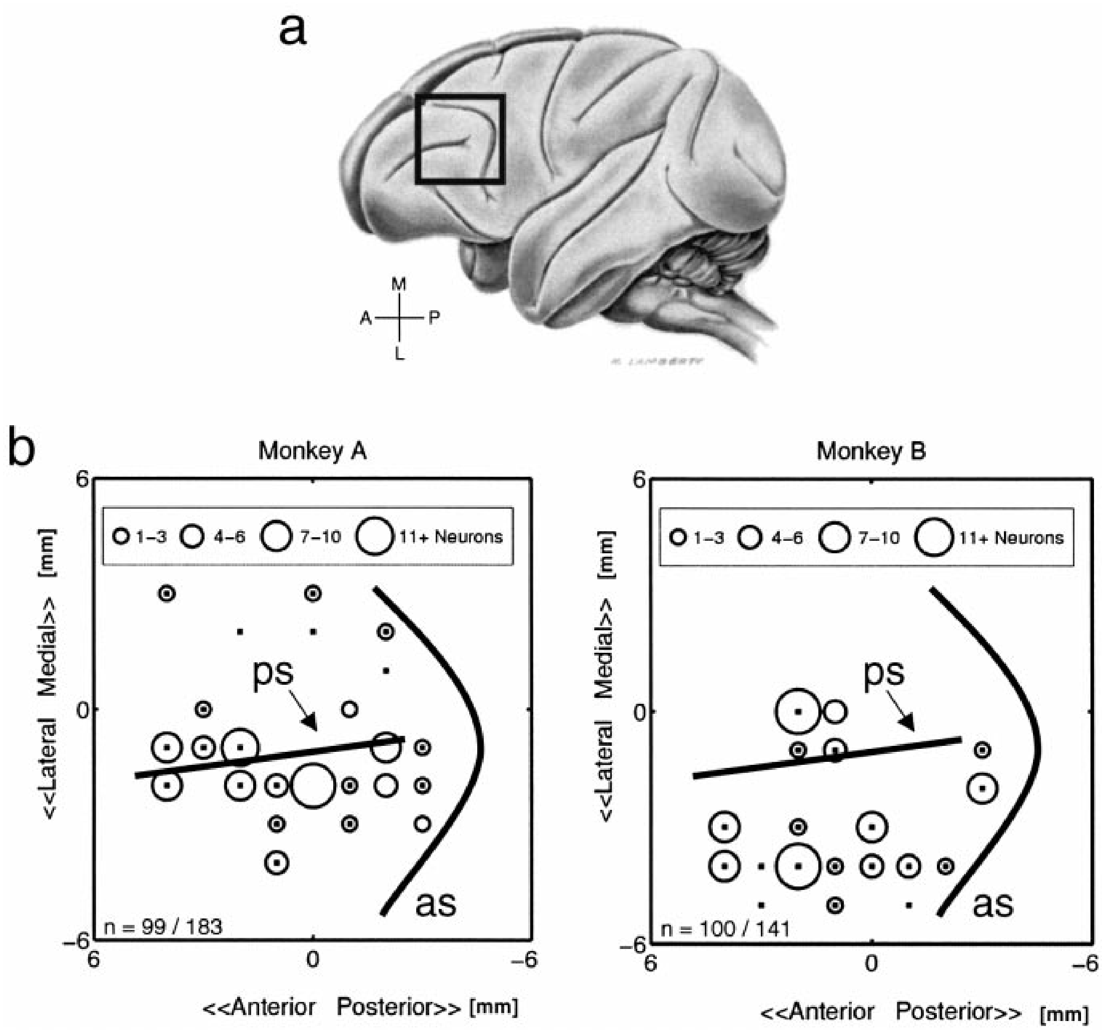


FIG. 3. Recording locations. (a) The box shows the general recording area on a lateral view of a generic *Macacca mulatta* brain. Abbreviations: M, medial; L, lateral; A, anterior; P, posterior. (b) Electrode penetration sites for each of the two monkeys are shown. Recordings were made from a region around and lateral to the principal sulcus (ps), anterior to the arcuate sulcus (as) of the prefrontal cortex. The size of the circles indicates the number of selective neurons recorded at that site; dots represent sites where nonselective neurons were isolated. A neuron was termed selective if it showed a significant difference in activity in response to objects vs. noise patterns in any of the three task periods (sensory, intermediate or reactivation). Significance was assessed using a Wilcoxon signed rank test (evaluated at $P < 0.01$, see Materials and methods). The number of selective neurons is shown at the bottom left for each of the monkeys, expressed as a fraction of the total number of neurons recorded in that animal with familiar and novel objects. (a) Drawing published with permission from The Max Planck Institute for Biological Cybernetics. Drawn by Mr. K. Lamberty for the Institute.

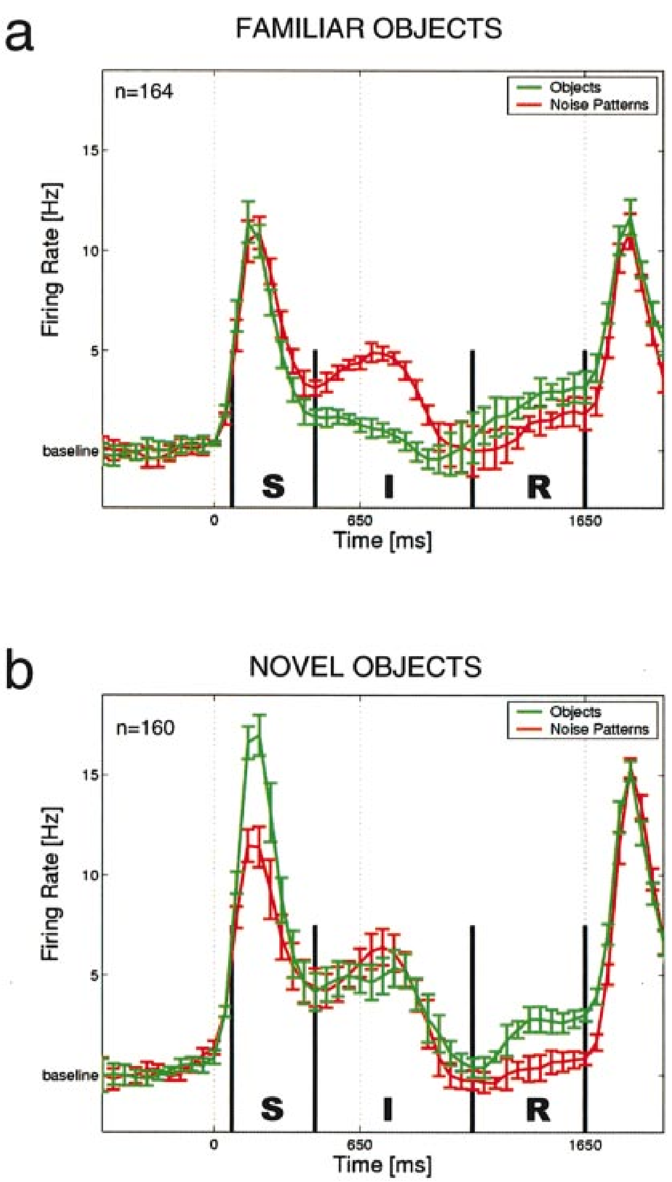


FIG. 4. Average population activity. Neural activity averaged across all recorded neurons in (a) the familiar and (b) the novel object experiments after subtraction of baseline activity. The vertical black bars represent the boundaries of the sensory (S, 80–450 ms), intermediate (I, 450–1150 ms) and reactivation (R, 1150–1650 ms) periods. Sample presentation occurred at 0 ms, the delay period started at 650 ms and ended at 1650 ms. Green curves represent activity to objects, and red curves represent activity to noise patterns. The number of neurons contributing to the graphs is shown on the upper left of each panel. Error bars represent standard deviations of mean activity to all five objects or all four noise patterns across the population, and are shown for illustrative purposes. Bin width, 50 ms.

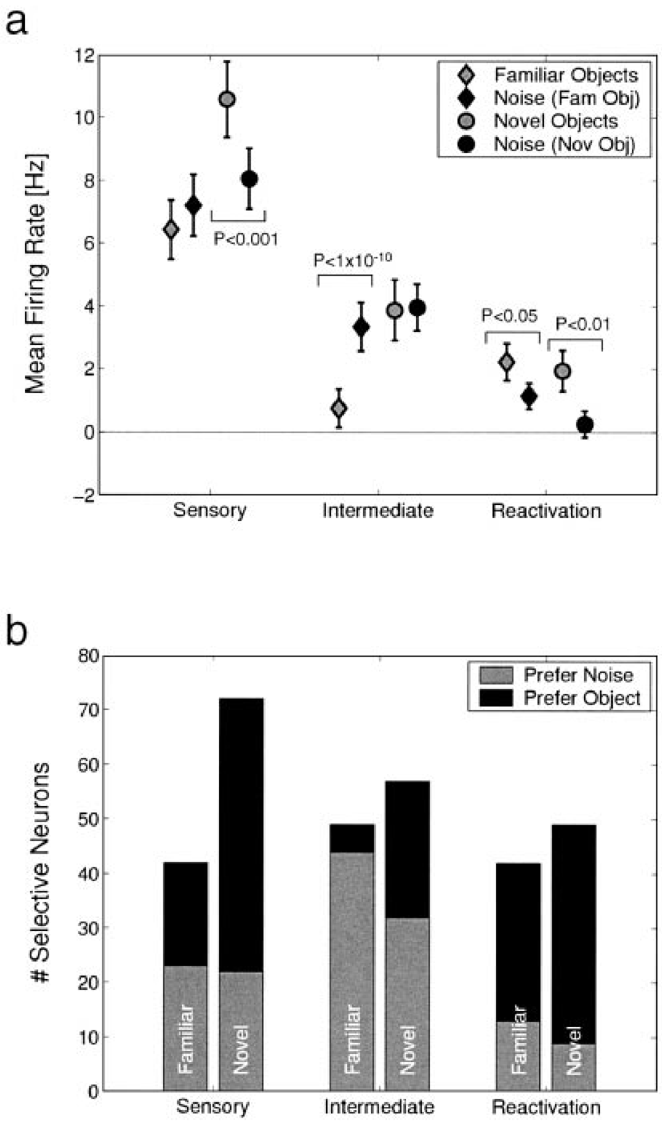


FIG. 5. Neural preference for objects or noise patterns. (a) Mean activity to objects and noise patterns is shown for each of the experiments in the three task periods for the entire population of neurons (familiar objects $n = 164$, novel objects $n = 160$) after subtraction of baseline activity. Standard errors (SEM) are shown for illustrative purposes (data are generally not distributed normally). A *W*-test ($P < 0.01$) revealed comparisons between objects and noise patterns that reached statistical significance. *P*-values are shown adjacent to the associated pair of datapoints. The abbreviations 'fam obj' and 'nov obj' refer to the familiar and the novel object experiments, respectively. (b) Each bar represents the number of neurons, a subset of the total population in each experiment that showed significant differences between objects and noise patterns during each of the three periods (*W*-tests, $P < 0.01$). The grey portion of each bar represents the fraction of neurons that preferred noise patterns (i.e. responded more on average to the four noise patterns than to the five objects), and the black portion represents the fraction of neurons that preferred objects.

extensive training with this particular set of objects for at least ten training sessions conducted prior to the experiments described here. A different set of familiar objects was used for each of the two monkeys. When familiar objects were used, noise patterns were always novel every session (i.e. we generated four new noise patterns for each session). During 'novel object' sessions, monkeys were presented with a new set of five objects, which they had never seen before. This set of novel objects did not change throughout the session. Thus, monkeys saw several repetitions of these novel objects during course of the session, but they did not have extensive prior experience with them, as was the case for familiar objects. For novel object sessions, noise patterns were kept constant across days such that the same four noise patterns were presented repeatedly while objects were changing across days.

Recording technique

A scleral search coil Robinson (1963), head restraint and recording chamber were implanted under aseptic conditions while the animals were anaesthetized using isoflurane. Postoperatively, the animals received analgesics and antibiotics and were kept alive for participation in further experiments. During recording sessions, monkeys were seated in primate chairs within sound-attenuating enclosures (Crist Instruments, Damascus MD, USA). Their heads were restrained, and a juice spout was placed near their mouth for automated delivery of reward (apple juice). For extracellular recordings of action potentials, we employed a grid system (Crist Instruments, Damascus MD, USA) with custom-made modifications that allowed us to use eight tungsten electrodes (FHC instruments, Bowdoin ME, USA) simultaneously. Penetrations were made perpendicular to the surface of the skull, and the minimum separation between adjacent electrodes was 1 mm. Recording sites near the principal sulcus of the lateral prefrontal (PF) cortex were localized using magnetic resonance imaging (see Fig. 3). We did not screen neurons for involvement in the task, but instead advanced the electrodes until the activity of one or more neurons was well isolated. After a suitable wait period of 1–2 h, we then commenced recording. This was performed to ensure an unbiased estimate of PF neural activity. Due to the number of conditions required and the limitations on the number of trials a monkey can work on a given day, it was not possible to complete both the familiar and the novel object experiment during a single recording session. However, care was taken to record neurons at similar locations in the two experiments in each of the monkeys, and at similar recording depths. Monkeys completed an average of 865 trials during 25 recording sessions (familiar objects, 14 sessions; novel objects, 11 sessions), resulting in on average over 20 repetitions for each of the five objects and about 30 repetitions for each of the four noise patterns. Analyses were conducted on data from all attempted trials (both correct and incorrect), excluding only trials on which the monkey broke fixation or failed to respond at all. We did this because behavioural choice occurred only upon test object presentation, which happened after the trial period that we analysed. Using all attempted trials yielded about

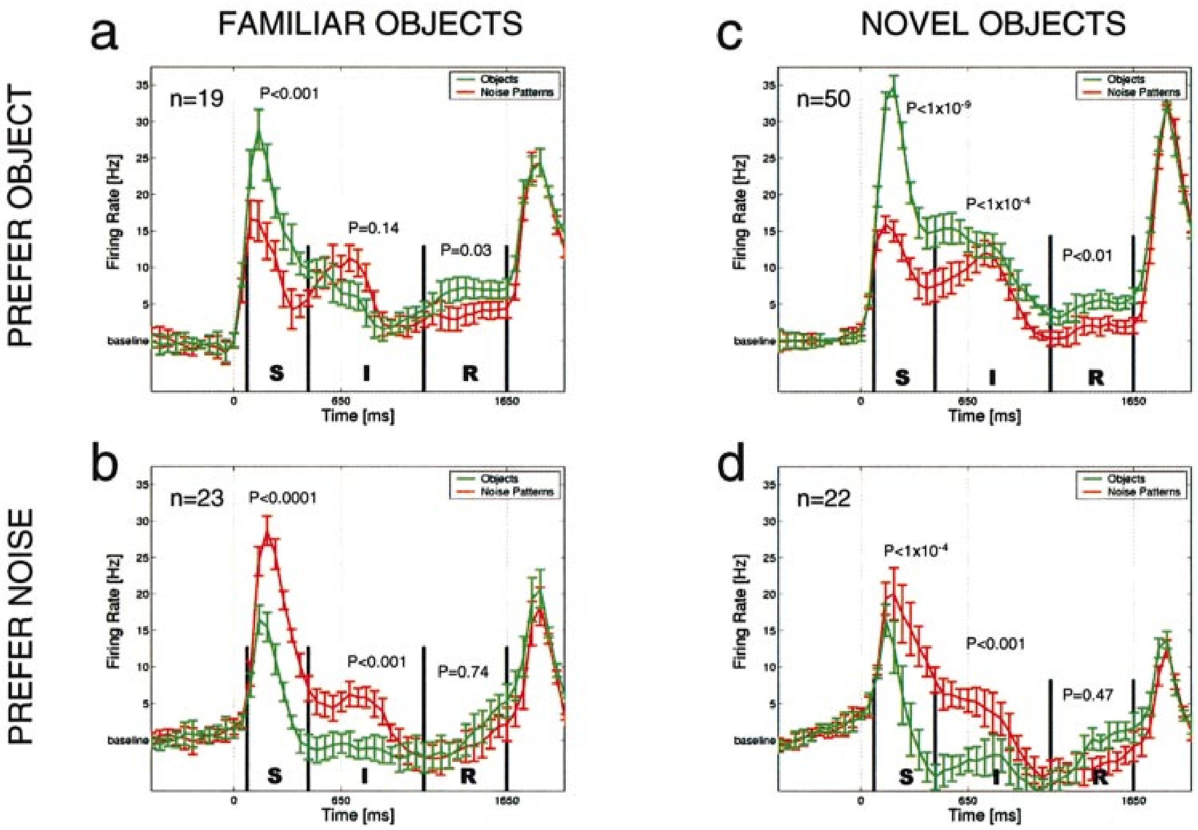
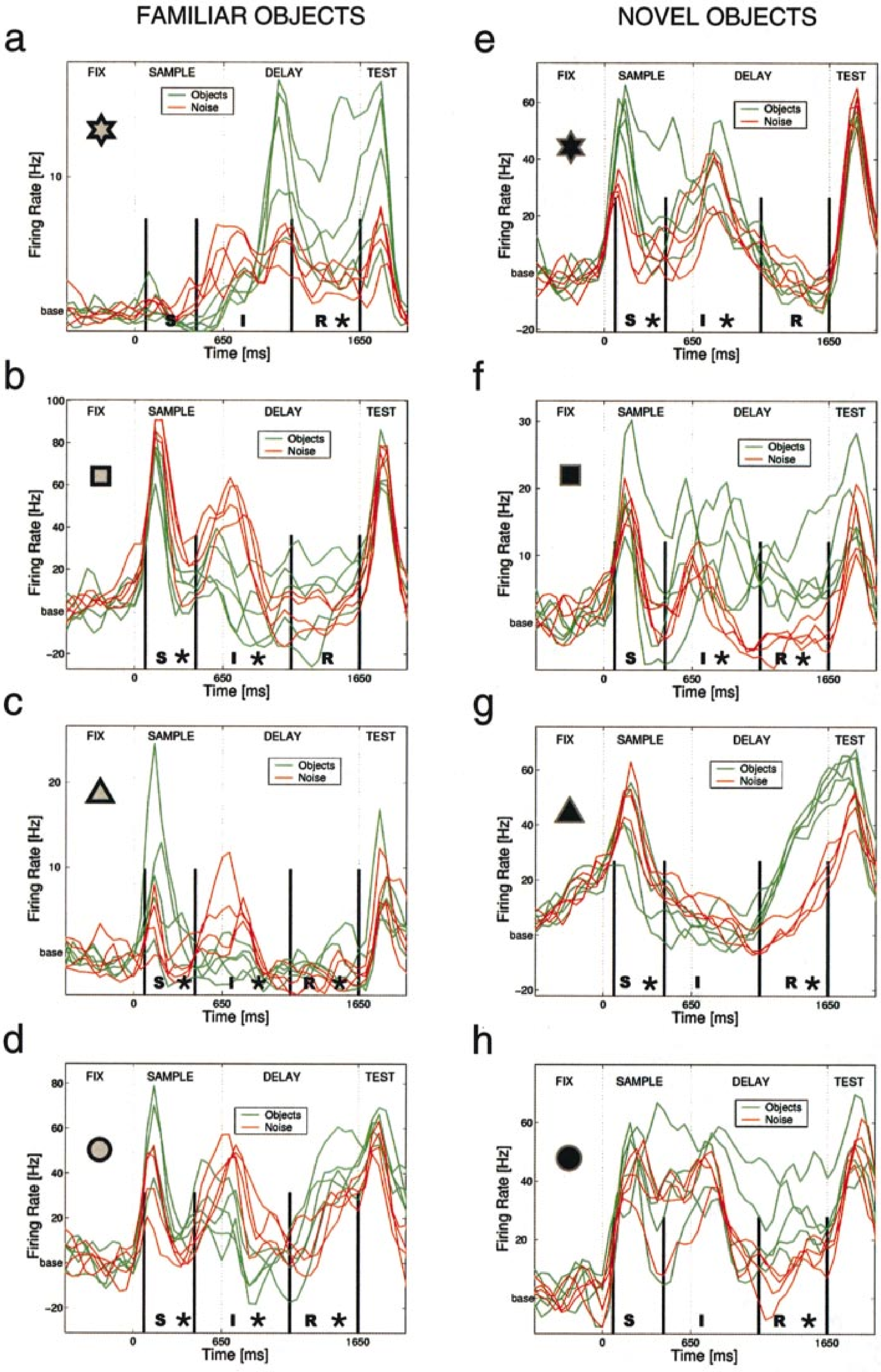


FIG. 6. Temporal dynamics of neural activity sorted by sample period preference. For each experiment, we selected neurons that during the sample period (S) showed statistically significant (*W*-test, $P < 0.01$) preference for (a and b) objects vs. noise patterns and (c and d) vice versa. Error bars represent standard deviations of the mean responses to objects or noise patterns across the population. The number of neurons contributing to each panel is shown in the upper left. Black vertical lines represent the boundaries of the sensory (S), intermediate (I) and reactivation (R) periods. The *P*-values represent significance of paired Wilcoxon tests comparing average activity to objects against average activity to noise patterns in each of these periods, for the neurons contributing to each graph.

FIG. 7. Single neuron responses to objects and noise patterns. Four single neuron examples from the familiar object experiment (a–d), and four single neuron examples from the novel object experiment (e–h) are shown. Responses to each of the objects and noise patterns are shown separately, with red curves representing the five objects and green curves representing the four noise patterns. Neural activity is shown relative to baseline firing rate. The symbols in the upper left part of each panel refer to the scatterplots showing activity for all neurons in Fig. 8. Black vertical lines represent the boundaries of the sensory (S), intermediate (I) and reactivation (R) periods. The top two rows represent single neuron examples from monkey A; the bottom two rows represent single neurons from monkey B. The stars drawn beside the period identity symbols (S, I or R) denote significant differences between average activity to objects relative to noise (*W*-tests, $P < 0.01$).


FAMILIAR OBJECTS
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twice the data for the noise pattern condition, allowing more accurate quantification of neural activity. However, our results did not depend on this choice; we repeated the major analyses performed in the present study using correct trials only, which yielded very similar results to the findings presented here.

Data analysis

Neural activity was analysed relative to a baseline of activity during the fixation period prior to sample object onset. Baseline activity was assessed during a period lasting 800 ms, starting 200 ms after onset of fixation (to exclude visual transients related to acquisition of fixation) and ending at sample onset. This baseline activity was subtracted from task-related activity for all the analyses in this study. This was performed to facilitate averaging activity for neurons with different baseline firing rates. Baseline firing rates were similar in the two experiments (familiar objects: median, 10.9Hz; range, 0.1Hz-39.2Hz; novel objects: median, 12.9Hz; range, 0.5Hz-37.1Hz).

Statistical tests

To evaluate whether there were significant differences in firing rate, to objects and noise patterns in the different task periods, we used the Wilcoxon signed rank test for equality of medians. Unlike the *t*-test, the Wilcoxon test makes no assumptions about the underlying distributions. To evaluate whether there was a significant difference in average activity between objects and noise patterns, we first computed average firing rates across all objects and all noise patterns for each neuron. Then we performed a matched pairs Wilcoxon signed rank test, assessing significance of the differences between these two values across the population. When assessing whether single neurons showed significant differences between objects and noise patterns, we grouped trials into two groups – the first one comprised of trials with one of the five objects as sample, and the second one comprised of trials with one of the four noise patterns as sample. We then performed an unmatched pairs (as object and noise pattern data is generated on different trials) Wilcoxon test, examining whether there were significant systematic differences in neural activity between these two groups of trials. In this text, we refer to these statistical tests as *W*-tests. Tests were performed on average activity during the three task periods: sensory (S), from 80 to 450 ms after sample onset; intermediate (I), from 450 to 1150 ms after sample onset and reactivation (R), from 1150 to 1650 ms after sample onset. For the major statistical analyses, *P*-values are given for the entire population as well as for each of the monkeys separately to demonstrate consistency of the results across the two animals.

To examine whether an observed fraction of neurons preferring objects to noise patterns was significantly different from even proportions, we performed a χ^2-test comparing the observed ratio to an even split. For example, for a case where 50 neurons preferred objects and 22 preferred noise patterns, we tested whether this [50 : 22] was a significantly different distribution from 36 : 36. When comparing fractions of selective neurons between the two experiments, we used a standard χ^2-test examining equality of distributions.

Results

Behaviour

Behavioural performance is summarized in Fig. 2. We found that monkey's behavioural performance was near ceiling when objects were used as sample stimuli in both the familiar and the novel object experiment (95% and 92% correct, respectively; *t*-test, $P > 0.1$). Thus, they were able to perform the DMS task equally well with novel objects as with highly familiar objects. However, when noise patterns were used as sample stimuli, monkeys' performance was near chance level of 50% correct in both experiments, because noise patterns did not contain any task-relevant information.

Neural activity

We recorded neural activity from 164 neurons in the familiar object experiment (monkey A, $n = 79$; monkey B, $n = 85$), and 160 neurons in the novel object experiment (monkey A, $n = 104$; monkey B, $n = 56$). Sites where neurons showing significant differences in activity in response to objects and noise patterns relative to baseline firing rate are shown in Fig. 3 for the two monkeys separately. As can be seen, most neurons were found near, as well as ventral to the principal sulcus.

Mean population response

The mean response of the entire unscreened neural population for each of the two experiments relative to baseline firing rate is shown in Fig. 4. Taking the experiments together, three distinct periods were evident in these average response histograms. Stimulus presentation evoked a transient sensory visual response (S), lasting from 80 to 450 ms after sample onset. This was followed by an intermediate period (I), lasting from about 450–1150 ms after sample onset. Finally, after a brief return to baseline activity, there was a reactivation period (R) during the last 500 ms of the delay characterized by an increasing 'climbing' activity profile. We observed several trends in these population histograms, which are quantified (paired Wilcoxon test, see Materials and methods) in Fig. 5a. (i) There was great similarity between neural responses to noise patterns in the two experiments, despite the fact that noise patterns were actually novel each day in the familiar object experiment and kept constant across days in the novel object experiment (*W*-tests, $P > 0.1$). Thus, familiarity of the noise did not affect the neural response. Accordingly, we henceforth use the term noise patterns regardless of whether they were employed in the familiar or novel object experiment. (ii) While during the sensory (S) period familiar objects elicited similar average activity as noise (*W*-test, $P > 0.1$; monkey A, $P = 0.06$; monkey B, $P > 0.1$), activity was greater in response to novel objects compared to noise patterns (*W*-test, $P < 0.001$; monkey A, $P = 0.047$; monkey B, $P = 0.007$). (iii) During the intermediate (I) period there was no difference in activity between novel objects and noise (*W*-test, $P > 0.1$; monkeys A and B, $P > 0.1$), while there was significantly less activity on average to familiar objects than to noise patterns (*W*-test, $P < 1 \times 10^{-10}$; monkey A, $P < 1 \times 10^{-6}$; monkey B, $P < 1 \times 10^{-5}$). (iv) During the reactivation (R) period, both familiar (*W*-test, $P < 0.05$; monkey A, $P = 0.052$; monkey B, $P < 1 \times 10^{-5}$) and novel (*W*-test, $P < 0.01$; monkey A, $P > 0.1$; monkey B, $P < 1 \times 10^{-5}$) objects elicited higher average activity than noise patterns, although this effect reached significance in only one animal.

These trends were confirmed by examining the number of neurons showing significant differences in activity between objects and noise patterns. In Fig. 5b we summarize for how many neurons this difference was significant in each of the task periods for both experiments. Significance was assessed using an unpaired Wilcoxon test (see Materials and methods). Comparing the familiar and novel object experiments, similar numbers of neurons showed significant differences in activity to objects and noise patterns during the intermediate and reactivation periods (χ^2-test, $P > 0.1$), whereas, during the sensory period, more neurons showed such differences in

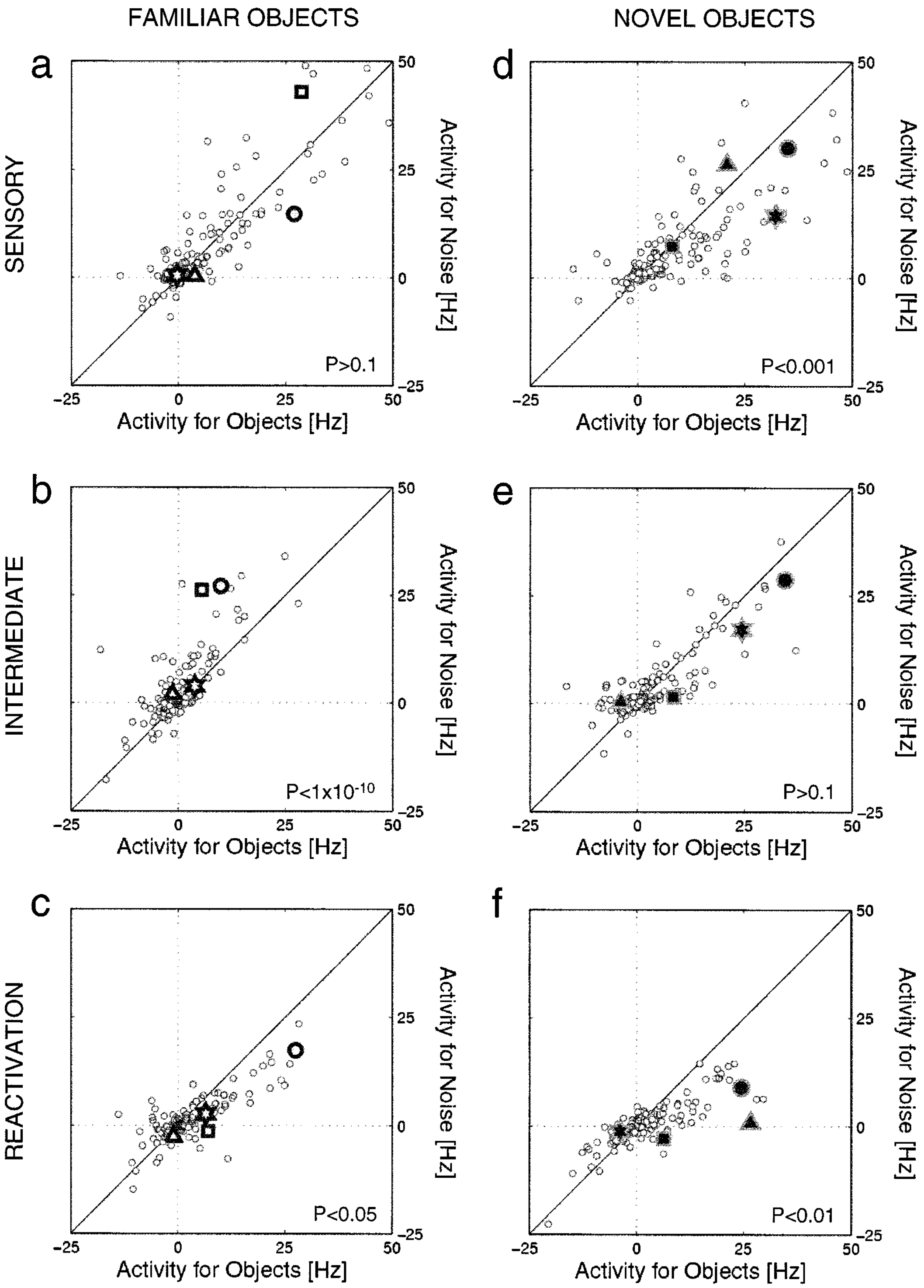

FIG. 8. Distribution of neural responses to objects and noise patterns. The columns display data from the familiar and novel object experiments. Each panel summarizes the response of the entire population of single neurons to objects and noise patterns during the sensory, intermediate and reactivation periods (familiar objects, $n = 164$; novel objects, $n = 160$). Each circle represents a single neuron, and large symbols represent the single neuron examples from Fig. 7. Neural activity is plotted relative to baseline response, such that positive values depict increases from the activity during the fixation period and negative values depict decreases. The significance level of a Wilcoxon test comparing mean activity between objects and noise patterns is shown at the bottom right of each panel for reference (same values as in Fig. 5a).

the novel object experiment (χ^2-test, $P < 0.001$). During the sensory period, preference for objects vs. noise patterns was evenly distributed in the familiar object experiment (χ^2-test, $P > 0.1$), while more neurons preferred novel objects to noise patterns (χ^2-test, $P < 0.05$). This indicates that although there was no systematic difference between activity to familiar objects and noise patterns during the

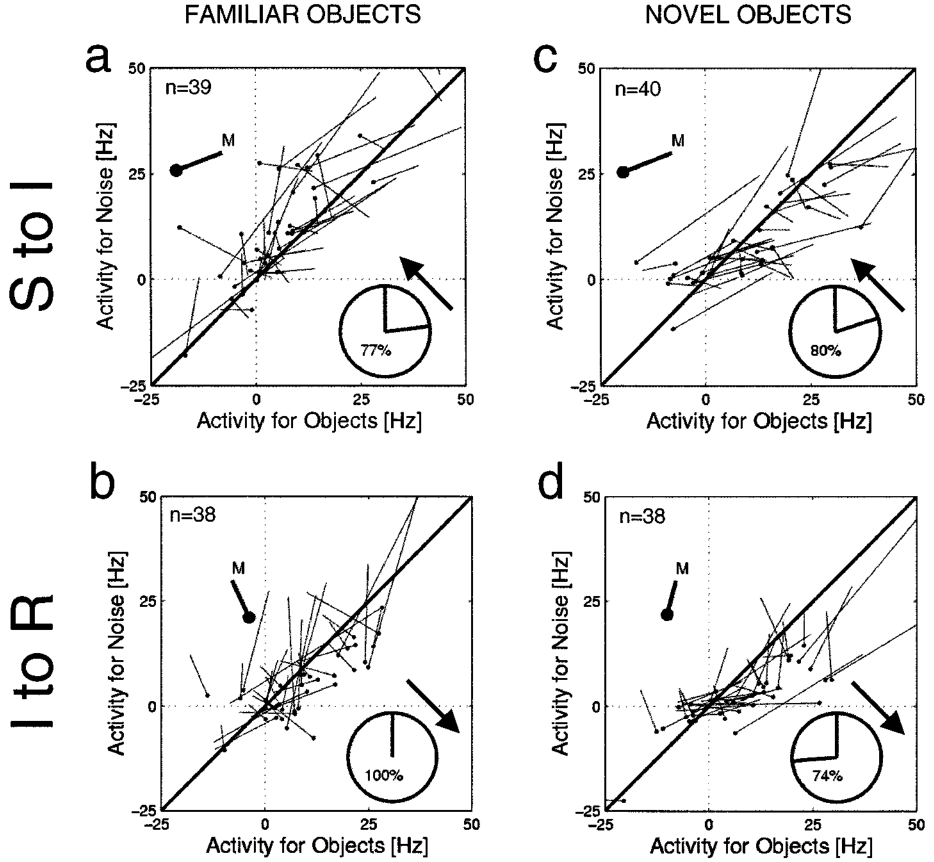

FIG. 9. Shifts in object preference. The vectors represent the shift in activity preference (a and c) from the sensory to the intermediate period (S→I) and (b and d) from the intermediate to the reactivation period (I→R), where the dots represent vector end points. Each vector represents a single neuron, and its' vertices represent that neuron's mean response to objects and noise patterns in the source and the target periods. For example, the vectors in Fig. 9a connect the vertices corresponding to each contributing neuron in Fig. 8a and Fig. 8b. Only neurons that showed shifts in preference between objects and noise patterns larger than 7 Hz are included in this analysis, i.e., neurons with a projection onto the bold vector beside the pie chart larger than 7Hz in length. The number of cases is shown in the upper left. The bold vector labelled 'M' represents the average shift for the population. The pie chart reports the fraction of neurons for which the vectors pointed in the same direction as the bold vector shown above the pie charts, which represents a systematic shift towards noise preference in (a and c), and a systematic shift towards object preference in (b and d).

sensory period, many single neurons actually did distinguish between familiar objects and noise. These subpopulations are analysed separately in the next section. During the intermediate period, a similar number of neurons preferred novel objects or noise patterns (χ^2-test, $P > 0.1$), while many more neurons showed greater activity to noise patterns than to familiar objects (χ^2-test, $P < 0.0001$). Finally, during reactivation, there was a trend for preference for objects over noise patterns in both experiments that reached significance only for novel objects (familiar objects, χ^2-test, $P = 0.12$; novel objects, χ^2-test, $P < 0.01$).

Mean response of neurons preferring objects or noise patterns

To examine the timecourse of the neural response in more detail, we plotted separately the temporal development of average activity for neurons that showed significant preference for objects over noise patterns and vice versa in each of the experiments. In Fig. 6a we show average activity for neurons that preferred familiar objects to noise patterns. Although these neurons were selected to show greater activity for objects during the sensory period, this activity preference was not maintained through the intermediate period but only reappeared during the reactivation period towards the end of the delay. During the intermediate period, neural activity was similar for objects and noise patterns (*W*-test, $P = 0.14$). For neurons that preferred novel objects to noise during the sensory period (Fig. 6b), this sensory preference was prolonged through the intermediate period and was also present during reactivation. Noise-preferring neurons showed similar trends in both experiments, as shown in Fig. 6c and d; preference for noise patterns during the sensory period was maintained during the intermediate period. During reactivation, these neurons did not show significant differences in response to objects and noise patterns (*W*-tests, $P > 0.1$).

Single neuron examples for familiar and novel objects

The above population results suggest that the temporal dynamics present in the average population do not appear to be a result of averaging together distinct neural populations, but rather might characterize response profiles of single neurons. Indeed, the development of neural activity over time seen in many single neurons resembled that of the average population. Single neuron examples for the familiar object experiment are provided in Fig. 7a–d, where each object and noise pattern is shown separately to allow assessment of the variability of neural activity among, as well as between, objects and noise patterns. In general, neurons were active during all three task periods, although Fig. 7a provides an example neuron that did not participate in sensory period processing. Consistent with the trends observed in the population, some single neurons preferred objects (e.g. Figure 7c), while others preferred noise patterns (Fig. 7b) during the sensory period. During the intermediate period, these neurons tended to prefer noise patterns, whereas, during reactivation, they tended to fire more vigorously to objects. Four examples for the novel object experiment are shown in Fig. 7e–h. During the sensory period, most neurons preferred objects (Fig. 7e and g) or showed no consistent preference (Fig. 7f and h). There was however, no consistent trend during the intermediate period, while there was systematic preference for objects during the reactivation period.

Distribution of neural preferences for objects or noise patterns

Scatterplots showing mean activity to objects and noise patterns for all neurons recorded in each experiment are shown in Fig. 8. The large symbols depict the single neuron examples shown in Fig. 7, demonstrating that this is a representative subset of the entire population. These plots summarize the activity of all recorded neurons and demonstrate directly the main findings of this study. During the sensory period, many neurons showed transient visual responses in both experiments. While there was no consistent preference for familiar objects over noise patterns, neurons did tend to prefer novel objects relative to noise. During the intermediate period by contrast, there was no consistent trend for novel objects, but familiar objects led to markedly less activity than noise patterns. During reactivation, neurons in both experiments again showed preference for objects.

Using these population scatterplots, we assessed directly how relative preference between objects and noise changed between the three task periods. We first identified neurons that showed shifts in preference between objects and noise patterns [e.g. from the sensory (S) to the intermediate (I) period] greater than an arbitrary threshold of 7 Hz. We did this to eliminate neurons that showed little or no preference shift. The vector shifts for these neurons are shown in Fig. 9a for familiar objects. The mean vector points to the left and slightly downward, consistent with a general decrease in activity from S to I, and a shift towards a preference for noise patterns over objects. Indeed, the majority (77%) of neurons shifted towards noise preference. Interestingly, the same was true in the novel object experiment (Fig. 9c), both in terms of the mean vector shift and the proportion of neurons shifting towards preference for noise. Analysis of the shift between the intermediate (I) and the reactivation (R) periods revealed a strikingly different result. For familiar objects (Fig. 9b), all (100%) of the shifting neurons shifted towards object preference, and the direction of the mean vector suggests that there was little change in mean firing rate. The results were similar for novel objects (Fig. 9d), although shifts towards object preference were apparent for 74% of neurons and the mean shift vector revealed a modest decrease in mean activity from I to R.

Discussion

In this study, we examine the timecourse of the activity of an ensemble of PF neurons in a delayed-matching-to-sample (DMS) task. We compare population activity evoked by objects, which needed to be retained during a short delay, to activity evoked by noise patterns that contained no task-relevant information and did not need to be retained. To assess effects of experience on this timecourse, we conducted the experiment with novel and highly familiar objects.

Three distinct periods were evident in the average population timecourse. During an initial visual sensory period, novel objects elicited greater activity than noise patterns while there was no systematic difference between activity to familiar objects and noise patterns. This finding is not surprising, as familiarity tends to cause a decrease in neural activity in many primate brain regions including the inferior temporal (Li *et al*., 1993), the perirhinal (Fahy *et al*., 1993) and the prefrontal (Asaad *et al*., 1998) cortices, as well as the hippocampus (Cahusac *et al*., 1993) and amygdala (Wilson & Rolls, 1993). Indeed, results from a selective lesion disconnecting the frontal from the temporal lobe suggest that the preference for novel objects in PF neurons may be a consequence of feed-forward activity from temporal cortical areas (Parker & Gaffan, 1998; Parker *et al*., 1998).

During an intermediate period, there was elevated activity for both noise patterns and novel objects, but not for familiar objects. In fact, even PF neurons that preferred familiar objects vs. noise during the sensory period did not maintain this preference in the form of elevated activity during the intermediate period (Fig. 6a). Intermediate period activity could not be ascribed to the offset of the visual stimulus, as it began well before the stimulus was turned off (see Fig. 4a). Neither was it a result of spike-frequency dependent adaptation of neural activity, as novel objects elicited the largest transient visual response but also showed robust activity during the intermediate period (Fig. 4b). Object information needed to be maintained through the intermediate period for both familiar and novel objects, yet we found sustained elevated activity through this period only for novel objects and noise patterns that did not need to be maintained (Fig. 7h), but not for familiar objects. Thus, maintenance of sensory information as elevated activity does not characterize the intermediate period well. This suggests that some other type of processing might occur during the intermediate period; but what might it be? One possibility is that intermediate period activity provides a signal that is instrumental in learning and causes changes in prefrontal or related cortical networks. This signal may be absent for familiar objects because no further leaning is required, whereas it may be evoked both by novel objects, which have not been overlearned, as well as noise patterns. Perhaps experience leads to the formation of inhibitory circuits within the PF cortex, which silence learning-related processing when a highly familiar input pattern is detected. Another possibility is that a dopaminergic or cholinergic signal up-regulates the excitability of PF neurons and thus produces more spiking during the intermediate period for novel stimuli or noise patterns but not for highly familiar over-learned patterns. dopamine, by causing an elevation in NMDA-current, and acetylcholine by causing in increase in the voltage activated calcium current can both lead to transient increases in spike generation and could thus underlie the additional activity seen in the intermediate period for novel stimuli or for the ambiguous noise patterns. Consistent with this hypothesis, robust learning-related differences have been described in the dopamine neurons which project to wide cortical target regions including the prefrontal cortex (Schultz *et al*., 1993; Schultz *et al*., 1997).

A reactivation period occurred in the late delay during which activity was elevated for both novel and familiar objects relative to noise patterns. It is activity during this reactivation period that resembles what is classically known as delay or persistent activity (Goldman-Rakic, 1990). For example, information about the sample object was maintained as elevated activity, and in many neurons activity also showed an increasing trend as the end of the delay approached. Previous work has implicated such PF climbing activity in anticipatory coding for objects (Rainer *et al*., 1999), reward (Watanabe, 1996) as well as motor preparation (Bruce & Goldberg, 1985). As in the present study, over 90% of trials with object samples were rewarded, compared to about 50% for trials with noise pattern samples (Fig. 2), it is possible that differential anticipatory coding for reward played a role during the reactivation period. Note that reward asymmetry between objects and noise patterns cannot explain results during the intermediate period, however, as familiar and novel objects were rewarded equally. The example neuron shown in Fig. 7g exhibited reactivation period activity consistent with reward expectancy, in that it was systematically more active on object-sample trials than on noise-sample trials. More generally though, activity during the reactivation period was modulated differentially according to the identity of the sample object, consistent with a role in short-term memory for objects, as in the example neurons shown in Fig. 7a, b, f and h. Motor preparation is unlikely to have played much of a role in the reactivation period, as monkeys could decide whether to release the lever only when presented with the test object after the delay.

The shift analyses presented in Fig. 9 demonstrate most directly the dynamics in preference for objects vs. noise patterns during the three task periods. From the sensory to the intermediate period, there was a similar shift for the two neural populations towards noise-preference (Fig. 9a and b). For familiar objects, this shift resulted in a marked and highly significant preference for noise during the intermediate period (Fig. 8b). For novel objects, however, preference for objects vs. noise patterns was distributed evenly during the intermediate period (Fig. 8e), despite this similar vector shift. The reason for this is the difference in the sensory period distributions in the two experiments. In the novel object experiment, the vector shift towards noise-preference abolishes the ensemble preference for objects evident during the sensory period. For familiar objects, evenly distributed ensemble preference during the sensory period is shifted towards noise-preference. The shifts from the intermediate to the reactivation period were, again, quite similar for the two experiments (Fig. 9b and d), yielding distributions that were biased significantly towards object preference. Thus, the population dynamics were actually quite similar between the two experiments, the major differences being a result for neural population preference for objects during the novel object experiment.

Taken together, the present results indicate that prefrontal delay activity – at least as assessed in the present study – is more complex than simple maintenance, and is subject to experience-dependent dynamics. Computational models of delay activity need to be extended to capture such dynamics, and provide more realistic accounts of activity profiles in different tasks to gain further insight into how the prefrontal cortex contributes to memory storage and manipulation.

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Abbreviations

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; PF, prefrontal; NMDA, *N*-methyl D-aspartate.

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