Neuronal activity related to elapsed time in prefrontal cortex

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Running head: Delay-dependent signals in prefrontal cortex

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ABSTRACT

We studied prefrontal cortex activity during a saccade task. On each trial, one of three delay periods elapsed between the onset of a visual stimulus and its offset, which triggered a saccade. After stimulus offset, many neurons showed phasic increases in activity that depended on the duration of the preceding delay period. This delay-dependent activity varied only weakly with reaction time and instead appeared to reflect a more-general aspect of elapsed time.

INTRODUCTION

Temporal information contributes to many neural functions, including coordination, interval estimation and episodic memory (Buonomano and Karmarkar 2002; Ivry and Spencer 2004). Neuronal correlates of elapsed time occur in posterior parietal cortex (Leon and Shadlen 2003; Janssen and Shadlen 2005) and premotor cortex (Lucchetti et al., 2005), and the cerebellum plays a key role in timing functions (Ivry and Spencer 2004). Evidence also supports a role for prefrontal cortex (PF). Neuroimaging data implicate PF in time-discrimination tasks (Lewis and Miall 2003; Onoe et al. 2001; Rao et al. 2001), and damage or stimulation there causes deficits in time estimation (Jones et al. 2004; Koch et al. 2002, 2003).

Despite this evidence, time relationships remain little studied in PF. Previous neurophysiological reports show, for example, that PF activity reflects the time until reward (Roesch and Olson 2005; Tsujimoto and Sawaguchi 2005a). A reward that occurs sooner has more value than one that comes later, however, and this factor could account for reward-delay activity, rather than timing per se. One previous report describes PF activity for two durations of visual stimuli in a temporal matching-to-sample task (Sakurai et al. 2004) and another one deals with the timing of a motor response (Niki and Watanabe 1979). Neither of these studies, however, show whether PF neurons reflect time relationships per se or reaction time (RT) instead. This distinction is important because the elapse of time often affects RT (Luce 1986).

In a study of abstract response strategies (Genovesio et al. 2005), we observed a delay-dependent signal.
The design of our study allowed us to test whether that signal reflected RT, reinforcement value, or the passage of time *per se*.

**METHODS**

Two rhesus monkeys (*Macaca mulatta*, 8.8 and 7.7 kg) performed a saccade task (Fig. 1A), called the strategy task in a previous report (Genovesio et al. 2005). After the monkeys fixated a central light spot (0.7°), three potential eye-movement targets (2.2° squares) appeared on a video monitor: 14° up, right and left from the center. Following a 1.0-s fixation period, a visual instruction stimulus (IS) appeared at the fixation point for 1.0, 1.5 or 2.0 s (selected pseudorandomly). The IS consisted of two superimposed ASCII characters, and its disappearance triggered a saccadic eye movement to one of the three targets. The correct target for a given trial depended on the sequence of ISs from trial to trial. When the IS repeated from the previous trial, the correct choice was the same target as was rewarded on that previous trial. When the IS differed from that on the previous trial, one of the other two targets was correct. After the monkeys fixated a target for 1.0 s, a fixed quantity of fluid reward followed contingently 0.5 s later.

Figure 1 near here

Single-neuron activity was recorded from PF, using up to 16 platinum-iridium electrodes (0.5–1.5 MΩ at 1 KHz) inserted into the cortex with a multielectrode drive (Thomas Recording, Giessen, Germany). Single-unit potentials were isolated offline using a cluster cutting technique (Off Line Sorter, Plexon, Dallas, Texas). Standard histological methods (Genovesio et al. 2005) showed that the lateral recordings came primarily from area 46 and that the medial recordings came from areas 8, 9, and rostral 6 (Fig. 1B), with no regional specializations relevant to this report.

To examine delay-dependent signals, we measured neuronal discharge rates during a 300-ms period beginning with the offset of IS, termed the *post-delay period*. We used a two-factor ANOVA (α = 0.05), with factors delay and target. In a separate test, we did the same analysis for the pre-reward and post-reward periods. *Post-hoc* tests contrasted delay levels (LSD method). We ran the ANOVA both with and without
balancing for the number of trials for each saccade target, with comparable results. For multiple regression analysis, delay duration (short, intermediate, long) and RT were used to predict discharge rates.

RESULTS

We analyzed only correctly performed trials, which exceeded 95% of the trials in both monkeys. The RTs showed a delay-duration effect, with longer delays associated with faster RTs (one-way ANOVA, $F_{2,4420} = 42.4, F_{2,2749} = 313.8$ for monkeys 1 and 2, respectively, $p < 0.001$). For monkey 1, mean RTs ($\pm$ SD) were 284 ± 49, 271 ± 70 and 261 ± 79 ms for short, intermediate and long delays, respectively. For monkey 2, the analogous RTs were 321 ± 39, 296 ± 41, and 275 ± 39 ms. Post-hoc tests revealed that all three pair-wise comparisons were significant for both monkeys ($p < 0.01$). Neither peak velocity nor saccade amplitude varied significantly with delay duration ($F_{2,4420} = 1.7, F_{2,2749} = 0.4$ for velocity in monkeys 1 and 2, respectively, $F_{2,4420} = 0.5, F_{2,2749} = 0.7$ for amplitude, $p >> 0.1$).

Of 1,454 PF neurons recorded, 132 (9%) showed post-delay activity levels that depended on the preceding delay duration (ANOVA, $p < 0.05$). According to post-hoc tests, 125 (95%) had significant activity differences between short and long delays, 71% differed for short versus intermediate delays, and 66% did so for long versus intermediate delays. Most cells had a preference (i.e., their highest activity) for either short delays (Fig. 2A, C left) or long delays (Fig. 2B, C right), with fewer preferring intermediate delays. Of the 132 delay-dependent cells, 41 (31%) showed significant differences among the three saccade directions, but the delay effect remained evident (Fig. 2D). On a cell-by-cell, rank-order basis (Fig. 3A), the activity of 75% of delay-dependent cells varied monotonically with the length of delay, with comparable proportions preferring long and short delays. Only 12% had a preference for intermediate delays.

Because both RT and delay duration could have affected post-delay activity, we used multiple linear regressions to evaluate the relative contributions. Delay duration had much greater effect on post-delay activity than did RT (Fig. 3B). A greater number of cells showed significant regression coefficients for
delay duration than for RT (49% versus 19% for cells preferring short delays, 62% versus 14% for those preferring long delays), which was a significant difference ($\chi^2 = 10.6$ for the former, $\chi^2 = 28.8$ for the latter, $p < 0.01$ for both). Fig. 2C shows the relation between activity, delay duration and RT for two neurons. In an additional analysis, we computed two regression models, one for activity vs. RT, the other for activity vs. both RT and delay duration. The result showed that $|R|$ significantly increased with the addition of delay duration to a model using RT alone (for monkey 1: 0.30 vs. 0.14, paired-t test, $t = 4.51$, $p < 0.001$; for monkey 2: 0.33 vs. 0.20, $t = 8.21$, $p < 0.001$, both averaged as in Fig. 3B). Similarly, $|R|$ significantly increased with the addition of delay duration to models using either saccade amplitude (for monkey 1: 0.27 vs. 0.10, $t = 9.4$, $p < 0.001$; for monkey 2: 0.22 vs. 0.10, $t = 12.7$, $p < 0.001$) or peak saccade velocity (for monkey 1: 0.28 vs. 0.12, $t = 9.4$, $p < 0.001$; for monkey 2: 0.26 vs. 0.15, $t = 11.4$, $p < 0.001$).

Population activity averages showed that phasic modulation reached a peak ~200 ms after IS offset, both for cells preferring short delays (Fig. 4A) and long delays (Fig. 4B). As with the single-cell data (Fig. 3A), these population averages showed a monotonic relationship of peak activity with delay duration. Post-delay population activity differed in delay-dependent cells by ~3.9–6.6 spikes/s between short and long delays (Fig. 4C, D, preferred saccade direction), which was statistically significant (repeated measures ANOVA, $F_{2,66} = 41.4$ and $F_{2,56} = 41.8$ for cells preferring short delays, monkeys 1 and 2, respectively; $F_{2,52} = 14.4$ and $F_{2,70} = 59.7$ for cells preferring long delays, all $p < 0.001$).

Activity before IS offset, at the end of the delay period, was also of interest. For cells preferring short delays, population activity appeared to increase at the end of short-delay intervals, but not longer ones, at least in monkey 2 (Fig. 4A, right). (The average shown in Fig. 4A, left, was dominated by two unusual cells and was not considered further.) For cells preferring long delays, activity prior to IS offset varied according to the delay interval (Fig. 4B, arrows), with activity increasing most for long delays. To examine this relationship on a cell-by-cell basis, we computed a delay-effect index \( \left( R_S - R_L \right) / \left( R_S + R_L \right) \) for both...
the final 300 ms of the delay period and post-delay activity, where $R_s$ and $R_l$ are mean discharge rates for short- and long-delay trials, respectively. The correlation observed in the population averages (e.g., Fig. 4B), where higher activity just before IS offset was associated with higher post-delay activity, was not seen for this cell-by-cell delay-effect index, either for cells preferring short delays ($r = 0.13$ and $r = -0.21$, monkeys 1 and 2, respectively) or for those preferring long delays ($r = 0.20$ and $r = 0.09$, all $p > 0.1$). The absolute magnitude of the delay-effect index reflected a three- to four-fold difference in post-delay activity ($0.54 \pm 0.28$ and $0.55 \pm 0.28$ for monkeys 1 and 2, respectively) and a much smaller difference at the end of the delay period ($0.30 \pm 0.32$ and $0.06 \pm 0.38$, SD).

**DISCUSSION**

We found PF neurons with phasic, post-delay activity modulation that varied with the duration of the preceding delay period, but this variation did not correlate strongly or consistently with RT. Although all three variables—post-delay activity, delay duration, and RT—correlated with each other, the correlation between delay duration and activity was much stronger than the correlation with RT, and only the former attained statistical significance. The weak correlation between neuronal activity and RT in PF contrasts with oculomotor areas such as the frontal eye fields (FEF) and the superior colliculus (SC), which show strong negative correlations between neuronal activity and RT (Dorris et al. 1997; Everling and Munoz 2000). These results suggest that the delay-dependent signal in PF does not contribute to the precise timing of an eye movement, as would be expected for FEF and SC, but plays a role in other processes related to time. Note that although these PF cells showed post-delay activity rates that varied with delay duration, there is no indication that they signaled temporal information at other times during the trial. Instead, they did so during a particular part of each trial, and likely played other roles at other times, as commonly observed for PF neurons (Isomura et al. 2003; Kim and Shadlen 1999; Rao et al. 1997; Takeda and Funahashi 2002). Because all task events were identical for the three delay intervals, including the stimulus, its foveal location, the probability and quantity of reward, and the eye movement that followed, these factors did not
contribute to the delay-dependency described here. Remaining possibilities include a role in estimating the preceding time interval, encoding its rank-order (shortest, longest, intermediate), or contributing to temporal aspects of event memories.

The finding of monotonic variation of post-delay activity with elapsed time, which occurred in 75% of the delay-dependent cells (Fig. 3A) and appeared prominently in the population averages (Fig. 4), provides some additional insight. It suggests a parametric, rather than categorical, encoding of temporal information because categorical encoding would likely involve all three durations equally. Whether delay-dependent activity has a parametric or categorical nature might depend on whether the task requires categorization (see Duncan 2001), and the present task had no such requirement. Alternatively, the post-delay activity of these cells might have signaled the degree of expectation of the “go” signal (see Janssen and Shadlen 2005) that had been attained during the preceding delay period (see below). This property also would account for the paucity of cells with an intermediate-delay preference in the post-delay period. Expectation-related signals in PF could be important for monitoring events expected at specific times during a trial (Tsujimoto and Sawaguchi 2004, 2005b), perhaps for monitoring goals and intentions (Lau et al. 2004; Owen et al. 1996; Petrides et al. 2002).

It is important to distinguish activity during the delay period from the post-delay activity that forms the basis for most of the present report. Nevertheless, the finding that delay-dependent cells began to increase activity toward the end of the delay period, especially for cells preferring long delays (Fig. 4B), raises some additional issues. Such “anticipatory” or “climbing” activity has been reported in several cortical areas, including PF (Bruce and Goldberg 1985; Moody and Wise, 2000). One modeling study suggested that climbing activity might lead to a phasic activity increase in neurons postsynaptic to these cells, which could read out accumulated temporal information (Durstewitz 2004). The post-delay, delay-dependent signal reported here could correspond to this read-out signal, and the increased activity during the delay period accords with human neuroimaging studies showing PF activation during a comparison of time intervals.
(Rao et al. 2001). Alternatively, the anticipatory or climbing activity could play a role in suppressing a response, with progressively stronger suppression required as the delay interval progresses and the probability of IS offset—the “go” or trigger signal—increases. One modeling study showed that hidden units with anticipatory activity played this role and their removal led to premature outputs (Moody and Wise 2000). Finally, anticipatory activity during the delay period might reflect the conditional probability of the “go” cue, as has been reported for posterior parietal cortex (Janssen and Shadlen 2005). This probability increases as the delay period progresses, but examination of population averages from PF, either aligned on IS onset or IS offset, yielded no evidence of such a signal for the population reported here (i.e., cells with delay-dependent, post-delay activity). We obtained the same result from two other, overlapping populations: cells with delay-dependent activity during the final 300 ms of the delay period and cells with statistically significant delay-period activity relative to the fixation period.

Finally, the delay-dependent signal observed here seems unlikely to code fine or precise time intervals or to play a role in timing per se: only a few spikes per second distinguish delay intervals that vary by 1 s, a sensitivity of only ~0.005 spikes/ms. Instead, this post-delay signal seems best suited to index event durations relevant to the present task, as suggested by Duncan’s (2001) adaptive coding model of PF function.

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Figure legends

Fig. 1. **A.** Sequence of events and video screens (gray rectangles). As monkeys fixated (dashed lines) the central spot (white dot), a visual instruction stimulus (IS)—a ‘?’ character superimposed on an ‘S’—appeared for a variable interval. Squares depict saccade targets. IS offset served as the trigger (“go”) stimulus. **B.** Penetration sites, a composite of the two monkeys. AS, arcuate sulcus; PS, principal sulcus.

Fig. 2. **A** and **B.** Two delay-dependent PF neurons. Activity (raster dots) is aligned on the end of the delay period (vertical lines), sorted by time to saccade onset (square marks). Background shading shows the post-delay period. Fix, onset of fixation; IS on, onset of IS; IS off, offset of IS; Sac on, onset of saccade. **C.** Discharge rates during the post-delay period for each trial plotted against RT, from the cells in **A** (C, left) and **B** (C, right), respectively. Symbols in the box near the ordinate axis represent mean (±SEM) activity for each delay interval. Partial regression coefficients in C left: \( \beta = -0.62, \ p<0.001 \) for delay, \( \beta = -0.02, \ p>0.1 \) for RT; in C right: \( \beta = 0.54, \ p<0.001 \) for delay, \( \beta = 0.20, \ p<0.05 \) for RT. **D.** Delay-dependent activity for the cells in **A** (D, left) and **B** (D, right), showing activity (± SEM) for the left (L), up (U), and right (R) saccade directions. Sp, spikes.

Fig. 3. **A.** Classification of post-delay activity by rank order of activity levels for short (S), intermediate (I) and long (L) delays. **B.** Multiple regression analysis for post-delay activity. Mean (±SEM) of the standardized partial regression coefficients of the cells preferring short delays (dark gray) and long delays (light gray). RT, saccadic reaction time.
Fig. 4. Post-delay population activity. A and B. Average activity levels ± SEM for total populations in each class for each monkey. The population sizes are given in the corresponding panels of C and D. Insets: population averages constructed from normalized z-scores for 10 cells with the greatest differential post-delay activity. A. short delay preference, full scale z=2.0 for left inset, z=1.2 for right inset. B. long-delay preference, full scale z=1.2 for left inset, z=1.0 for right inset. C and D. Mean (± SEM) firing rates for all delay-dependent cells, for each cell’s preferred saccade target (Best, black bars) and for the other two targets, the one with the lowest activity (Worst, light gray) and the one with intermediate activity levels (Middle, dark gray). C, short delay-, D, long-delay preference. Sp, spikes.
A Pre-reward 0.5 s Fixation 1 s Response and Target hold 1 s Trigger stimulus (IS off) Reward or not Intertrial interval 2.5 s Fixation 1 s Instruction stimulus (IS) Short delay 1 s Intermediate delay 1.5 s Long delay 2 s Trigger stimulus (IS off) Response and Target hold 1 s Pre-reward 0.5 s

B Fig. 1

AS PS

Fig. 1
Fig. 2

Activity (sp/s)

Reaction time (ms)

Saccade direction

A

B

C

D

- Short (1 s)
- Intermediate (1.5 s)
- Long (2 s)

- 10sp/s
- 1 s
- Fix
- IS on
- IS off
- Sac on
- Rew or not

- Short
- Intermediate
- Long

- Activity (sp/s)
- Reaction time (ms)
- Saccade direction

Fig. 2
Fig. 3

A

Monkey 2

- \( I > S > L \) (40%)
- \( L > S > I \) (3%)
- \( S > L > I \) (4%)
- \( S > I > L \) (38%)
- \( I > S > L \) (6%)
- \( L > S > I \) (3%)
- \( S > L > I \) (6%)
- \( I > L > S \) (42%)

B

Monkey 1

- \( I > L > S \) (8%)
- \( I > S > L \) (8%)
- \( L > S > I \) (6%)
- \( S > L > I \) (6%)
- \( S > I > L \) (38%)
- \( I > S > L \) (30%)
- \( I > L > S \) (9%)
- \( L > S > I \) (6%)

Monkey 2

- \( I > L > S \) (38%)
- \( I > S > L \) (6%)
- \( L > S > I \) (9%)
- \( S > L > I \) (4%)
- \( S > I > L \) (42%)
- \( I > S > L \) (38%)
- \( I > L > S \) (6%)
- \( L > S > I \) (3%)

Standardized partial coefficient

- Short-delay preference
- Long-delay preference

Monkey 1:

- \( n = 27 \)

Monkey 2:

- \( n = 36 \)

Delay and RT
**Fig. 4**

**A**

Comparative analysis of mean activity (sp/s) across different conditions for Monkey 1 (left) and Monkey 2 (right).

**B**

Detailed comparison of mean activity (sp/s) with specific annotations for IS off and 0.5 s intervals.

**C**

Bar graph depicting mean activity (sp/s) with error bars for Monkey 1, showing n=33.

**D**

Bar graph illustrating mean activity (sp/s) with error bars for Monkey 2, showing n=29.

**Legend**

- Short
- Intermediate
- Long

Best, Middle, Worst categories are represented with different colors and error bars for Short, Intermediate, and Long conditions.