Delay activity in rodent frontal cortex during a simple reaction time task

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Keywords: top-down, foreperiod, persistent activity, principal component analysis, reaction time

Running Head: Delay activity in rodent frontal cortex

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Figures: 10, Tables: 0
ABSTRACT

To understand how different parts of the frontal cortex control the timing of action, we characterized the firing patterns of single neurons in two areas of rodent frontal cortex, dorsomedial prefrontal cortex (dmPFC) and motor cortex, during a simple reaction time task. Principal component analysis was used to identify major patterns of delay-related activity in frontal cortex: ramping activity and sustained delay activity. These patterns were similar in dmPFC and motor cortex, and did not change as animals learned to respond at novel delays. Many neurons in both areas were modulated early in the delay period. Other neurons were modulated in a persistent manner over the duration of the delay period. Delay-related modulations started earlier in motor cortex than in dmPFC and terminated around different task events (at the time of release in dmPFC, just before release of the lever in motor cortex). A subpopulation of neurons was found in dmPFC, but not motor cortex, that fired in response to the trigger stimulus. These results suggest that populations of neurons in rodent frontal cortex are coordinated during delay periods to enable proactive inhibitory control of action.
INTRODUCTION

Medial regions of frontal cortex in rats, monkeys, and human beings have been implicated in controlling the timing of action (Naito et al., 2000; Botvinick et al., 2004; Rushworth et al., 2004; Li et al., 2006). These cortical regions are densely interconnected (Fisk and Wyss, 1999) and send massive projections to neuromodulatory systems in the brainstem (Sesack et al., 1989; Gabbott et al., 2005). Through these connections, medial frontal areas may form a brain system that exerts top-down control over the motor system to suppress responding during delay periods and update the frontal cortex about the consequences of actions (Brunia, 1999; Schall et al., 2002; Narayanan and Laubach, 2006; Picton et al., 2006b; Picton et al., 2006a; Stuphorn and Schall, 2006; Churchland and Shenoy, 2007; Boulinguez et al., 2008; Hanakawa et al., 2008).

Disruptions of neural activity in the medial frontal cortex lead to lasting deficits in the temporal control of action (Muir et al., 1996; Broersen and Uylings, 1999; Risterucci et al., 2003; Narayananan et al., 2006; Narayanan and Laubach, 2006). By contrast, inactivations of motor cortex do not increase premature responding (Matsumura et al., 1991; Martin et al., 1993; Narayanan et al., 2006). In a recent study, we found that inactivation of the dorsomedial part of the rat prefrontal cortex (or dmPFC; this region is comprised of the pregenual anterior cingulate area and the dorsal part of the prelimbic area) led to reduced delay period firing in the motor cortex (Narayanan and Laubach, 2006). This finding suggests that medial frontal neurons exert proactive control over the motor system to inhibit responding until the right time or right stimulus has occurred (Boulinguez et al., 2008).

Neurons in the medial frontal cortex show persistent firing during delay periods in primates (Niki and Watanabe, 1976b, a, 1979) and rodents (Batuev et al., 1990; Baeg et al.,
example, Narayanan and Laubach (2006) found that one-third of dmPFC neurons are modulated (i.e., fire at increased or decreased rates) during the delay period of a simple reaction time task. The firing rates of these neurons were predictive of successful waiting behavior and were significantly correlated with firing rates of neurons in the motor cortex (Narayanan and Laubach, 2006). We also found that many delay-related neurons in dmPFC and motor cortex are sensitive to errors in the task (Laubach et al., 2000; Narayanan et al., 2005; Narayanan and Laubach, 2006) and, most recently, we reported that neurons in dmPFC, but not in motor cortex, fire persistently through the inter-trial interval following an error until the beginning of the next trial (Narayanan and Laubach, 2008).

In the present study, we describe how neuronal activity in dmPFC and motor cortex is modulated during delay periods. As in a recent study by Paz et al. (2005), we used principal component analysis (PCA) (Reyment and Joreskog, 1996) to characterize major patterns of firing in populations of neurons from dmPFC and the motor cortex during the delay period. This analysis enabled us to measure potential differences in the timing of task-related activity in the two cortical areas, which is a prerequisite for top-down influences of dmPFC on motor cortex (Narayanan and Laubach, 2006). Our results suggest that the time-courses of neuronal activation in dmPFC and motor cortex are mutually dependent, and that persistent firing during the delay period may arise from task-related activity associated with lever pressing at the start of the trial. A second goal of this study was to assess the role of delay period activity in dmPFC and the motor cortex in preparing for the forthcoming stimulus and response. Neurons could be involved in maintaining the motor response during the delay period (Ollman and Billington, 1972; Narayanan et al., 2006) or could be sensitive to the expected timing of the stimulus (Naatanen,
To address this issue, we compared task-related activity on trials with 1.0 sec delays in sessions with one (1.0 sec) and two (0.4 or 1.0 sec) delay periods. If frontal neurons are involved in proactively inhibiting responding during the delay period, then there should be no major differences in delay period activity in sessions with one and two delay periods. On the other hand, if frontal neurons are involved in anticipating the timing of the forthcoming stimulus and the subsequent response, then there should be modulations of neural activity around the time of the short delay period (i.e., 0.4 sec) even when the stimulus does not occur at that time. If these effects reflect changes in preparatory activity, then they should be apparent at the population level (Brunia, 1999). We found no evidence for modulation of neuronal activity at the time of the short delay period, and suggest that single neuron activity in rodent frontal cortex is involved in proactive inhibitory control of responding.

MATERIALS AND METHODS

Subjects: Twelve male Long-Evans rats (3-4 months old; Harlan) were trained to perform a simple reaction time task and were then implanted with arrays of recording electrodes in dmPFC (N=8), motor cortex (N=3), or both cortical areas (N=1). Rats were motivated by water restriction, while food was available ad libitum. Rats consumed 10-15 ml of water during each behavioral session and additional water (5-10 ml) was provided 1-3 hours after each behavioral session in the home cage. Rats were maintained at ~90% of their free-access body weights during these experiments, and received one day of free access to water per week. The Animal Care and Use Committee at the John B. Pierce Laboratory approved all procedures.
Simple reaction time task: Rats were trained to perform a simple reaction time task using standard operant procedures (Figure 1A). Trials were initiated when rats pressed a lever. Response force was measured using a thin-film load cell (S100, Strain Measurement Devices, Meriden, CT), rated to 1 N, which was mounted on the lever. The lever press was maintained for a 1.0 sec delay period. A tone (frequency: 8 kHz, intensity: 72 dBA, duration: 100 ms) was presented at the end of the delay period. To receive liquid rewards, animals had to release the lever with a reaction time less than 0.6 sec (Correct responses). If the lever was released before the trigger stimulus (Premature error responses) or after the response window (Late error responses), there was a timeout period. Animals typically made premature responses on 25-35% of trials and late responses on less than 10% of trials. Neuronal activity from these error trials was excluded from all analyses in this manuscript.

Rats reached criterion (>60% correct) in 9.75 ± 1.5 sessions (1748 ± 343 trials) and performed the task with one delay period (1.0 sec) for a total of 24 ± 1.9 sessions (5776 ± 564 trials). They were then implanted with recording electrodes. Neuronal data was collected in three sessions with one delay period. Then, three sessions (567 ± 98 trials) were run with two randomly interleaved and equally likely delays of 0.4 sec (short) or 1.0 sec (long). To make the data sets comparable between the one and two delay sessions, all analyses in this manuscript were restricted to trials with delays of 1.0 sec (all trials in the one-delay sessions and trials with long delays in the two-delay sessions).

Prior to these experiments, nine of these rats (6 with recording electrodes in dmPFC, 3 with recording electrodes in motor cortex, and 1 with both) performed a modified version of the task, based on a study by Kornblum (1973). In these sessions, all trials had delays of 1.0 sec and trigger stimuli were presented on one-half of the trials (with pseudo-randomly interleaved types
of trials). On the other half of trials (called time production trials), no trigger stimulus was presented, and rats were rewarded for releasing the trigger stimulus. Animal behavior was not different on these two trials (Narayanan et al., 2006; Narayanan and Laubach, 2006). This task was used to assess tone-related firing in dmPFC and motor cortex.

*Microwire electrode arrays:* Twelve animals were implanted with 4x4 or 3x3x2 arrays of 50 μm stainless steel wires (250 μm between wires; impedance measured in vitro at 100-300 kΩ; Neurolinc: New York, NY) into the dorsal prelimbic region of rat frontal cortex (coordinates from bregma: AP: +3.2, ML: ± 1.4, DV -3.6 @ 10° in the frontal plane) targeting coordinates of previous inactivation (Narayanan et al., 2006) or into rat motor cortex (coordinates from bregma: AP: -0.5, ML: ± 2.5-3.5, DV: -1.5 @ -25° in the frontal plane) according to methods described in detail previously (Laubach et al., 2000; Narayanan et al., 2005; Narayanan et al., 2006). Electrodes were implanted unilaterally into dmPFC and/or motor cortex contralateral to the rats’ dominant paw (i.e., the paw used to press the lever) or bilaterally into dmPFC (Figure 2).

*Surgery:* Aseptic stereotaxic methods were used to implant microwire electrodes arrays into dmPFC or into motor cortex (Figure 2). Anesthesia was initiated with ~4% halothane and maintained with intraperitoneal injections of ketamine (100 mg/kg) and diazepam (10 mg/kg). A surgical level of anesthesia was maintained over the course of surgery with supplements (30 mg/kg) of ketamine every 45 mins-1 hour. Under aseptic conditions, the scalp was retracted, and the skull was leveled between bregma and lambda. A single craniotomy was drilled over the area above dmPFC cortex that spanned both hemispheres, or over motor cortex. Microwire arrays were then inserted in dmPFC and/or motor cortex, with electrophysiological signals used to
target placement into the deep layers of the cortex. Implants were then sealed with cyanoacrylate ('SloZap', Pacer Technologies, Rancho Cucamonga, CA) and an accelerator ('ZipKicker', Pacer Technologies), and methyl methacrylate (AM Systems, Port Angeles, WA). Animals were allowed to recover for one week and then acclimated to recording procedures over several sessions before recordings commenced in the simple reaction time task.

**Electrophysiological recordings:** Neuronal ensemble recordings were made using a Many Neuron Acquisition Program (Plexon, Dallas, TX). Putative single units were identified on-line using an oscilloscope and audio monitor. The Plexon off-line sorter was used to analyze the signals off-line and to remove artifacts due to cable noise and behavioral devices (pump that delivered fluid to the rats, click stimulus generated by a mechanical relay at the time of fluid delivery). Principal component analysis and waveform shape were used for spike sorting. Single units were identified as having 1) consistent waveform shape, 2) separable clusters defined by waveform parameters and analysis with principal component analysis (using the first two PCs), 3) average amplitude estimated at least three times larger than background activity, 4) a consistent refractory period of at least 2 ms in interspike interval histograms, and 5) consistent firing rates around behavioral events (i.e., ‘stationarity’, as measured by a runs test of firing rates around behavioral events; neurons with $|z|$ scores $> 4$ were excluded). Using these techniques, a total of 414 single units were included in this study. A quantitative analysis of these spike trains was carried out using NeuroExplorer (Nex Technologies, Littleton, MA) and custom routines for MATLAB (The Mathworks, Natick, MA) and R (http://www.r-project.org/).
Analysis of task-related modulations in firing rates: Only data from trials with correct responses (sustained until stimulus and RT less than 0.6 sec) and long delay periods (1.0 sec) were analyzed in both one-delay and two-delay sessions. Peri-event histograms were created for the epoch from 0.5 sec before to 1.5 sec after the lever press using bins of 1 ms. Spike density functions were created as follows. Spike counts for each trial were stored in a vector. The vector was padded with 25 zeros at each end (zero padding). Each trial was then convolved with a 25-point Gaussian window, decimated 25 times (using the “decimate” function in Matlab), and the first and last points in the resulting vector were cut off (to eliminate the padding). This gave an estimate of spike density with an effective time resolution of 25 ms. Spike density functions were then converted to a measure of firing rate by dividing the vector by the effective bin size. Population averages were calculated by simply averaging the raw firing rates of all neurons and by averaging together average firing rates that were normalized using z-scores. For these analyses, and those below, we only included neurons in the dataset if they fired at an average rate that was greater than 1 Hz over the peri-event epoch (from 1 sec before the lever press to 2 sec after the lever press). All analyses were also confirmed with 10 ms bins; bin size did not influence the results in this manuscript.

Correlations were performed between neural firing rate in the reaction time epoch (1.0 – 1.6 after lever press) and reaction times. Partial correlation was also performed between simultaneously recorded neurons controlling for reaction time using built in functions in Matlab (partialcorr.m).

We used principal component analysis (PCA) (Reyment and Joreskog, 1996; Paz et al., 2005) to capture major patterns of firing within the neuronal populations. For this analysis, we calculated average spike density functions (effective bin size of 25 ms) for all neurons in
sessions with one and two delay periods, arranged the neurons in a matrix with neurons as rows and bins as columns, normalized the rows of the matrix as z-scores, and then performed PCA using singular value decomposition. For each area, neurons were combined into the same matrix for this analysis, and potential differences between sessions with one and two delay periods was evaluated based on the scores for the PCs over neurons from each type of session. Importantly, neurons from dmPFC and motor cortex were analyzed using separate analyses. The covariance explained by each resulting principal component (PC) was explored by plotting the eigenvalues as scree plots. We found that each area (dmPFC and motor cortex) contained only a few components with large eigenvalues, each explaining >10% of covariance. The number of large components was further confirmed using functions in the Matlab Toolbox for Dimensionality Reduction (http://ticc.uvt.nl/~lvdrmaaten/).

*Analysis of stimulus-related activity:* Exploratory analysis suggested that a subset of neurons in dmPFC, but not in motor cortex, fired in response to the trigger stimulus. Such neurons had sharp modulations in firing rate immediately after stimulus onset and well before the rats released the lever. To evaluate the fractions of neurons in the two cortical areas with such activity, we analyzed data collected during a modified task in which stimuli occurred on half of the trials and at a delay of 1.0 sec. Stimulus-related modulations in these sessions were defined using a Wilcoxon rank-sum test (criterion of p<0.05) that compared spike rates in a 200 ms epoch at 1.0 sec into the delay period on trials with and without a trigger stimulus.

*Histology:* Once experiments were complete, rats were sacrificed via overdose with 100 mg/kg sodium pentobarbital and then transcardially perfused with either 10% formalin or 4%
paraformaldehyde. Lesions were made at select recording sites by passing a unipolar current (100 $\mu$A) for 10s. Brains were sectioned on a freezing microtome, mounted, and stained with thionin. Electrode locations were visualized using custom written 3-dimensional reconstruction software (Eyal Kimchi, Laubach Lab) based on an atlas of coronal sections by Swanson (1999). Software for electrode reconstruction (written by E.Y. Kimchi) is available at http://spikelab.jbpierce.org/Resources.

RESULTS

Behavioral data

Twelve rats were trained to perform a simple reaction time task (Figure 1A). Standard methods for operant training were used in all studies (see Narayanan et al., 2006). Arrays of microwire electrodes were implanted into dmPFC (N=8), motor cortex (N=3) or both areas (N=1) (Figure 2). Recordings were made in nine animals (6 animals in dmPFC, 3 animals in motor cortex, 1 animal with both) using the modified task.

In the standard reaction time task, animals performed on average 135±40 correct trials, 38±17 premature errors, and 28±23 late errors. Animals made equivalent numbers of correct responses in one-delay and two-delay sessions (one-delay sessions: 70±3%; two-delay sessions: 71±2%; paired $T_{(1,11)} = 0.6$, $p < 0.56$). However, on trials with long delays, reaction times were faster in two-delay sessions (0.201±0.12 sec) compared to one-delay sessions (0.257±0.19 sec; paired $T_{(1,11)} = 2.99$, $p < 0.01$) (Figure 1B).

In six animals, we measured the force applied to the lever in one-delay and two-delay sessions. On trials with long delays, animals achieved the same maximal lever force in one-delay sessions (0.39±0.02) compared to two-delay sessions (0.37±0.02 N; paired $T_{(1,5)} = 0.72$, $p < 0.50$).
These data indicate that animals’ movements were similar in one-delay and two-delay sessions, and that lever pressing was a stable habit.

**Neuronal database**

During one-delay sessions, we recorded 144 dmPFC neurons (in 9 animals, with an average of 16±2.5 neurons per animal). In the same nine animals, we recorded 135 dmPFC neurons (15±1.5 neurons per animal) after animals learned the two-delay task (i.e., after 3 days of training). We also investigated neural activity in motor cortex in four animals. We recorded from 65 motor cortex neurons (16.5±1.3 neurons per animal) during one-delay sessions. In the same animals, we recorded 70 motor cortex neurons (17.5±2.3 neurons per animal) in the third session with the two-delay design. Additional recordings of 88 neurons in dmPFC in six animals (17.3±7.4 neurons per animal) and 50 neurons in motor cortex in four animals (12.5±3.4 neurons per animal) were acquired during the modified reaction time task with time production trials (i.e., with stimuli presented on half of the trials).

**Population averages were strongly modulated in motor cortex, but not in dmPFC**

Neuronal activity was diverse across neurons in dmPFC. As a result, population averages of trial-averaged firing rates, synchronized to the time of the lever press, were not modulated around the task events (Figure 3A). However, by normalizing firing rates using z-scores prior to averaging, we were able to detect modulations in dmPFC activity, especially around the time of lever pressing. By contrast, activity in motor cortex was more consistently modulated around lever pressing (0 sec), during the delay period (between 0 and 1 sec after lever press), and during the reaction time epoch (1.0 to 1.6 sec after lever press) (Figure 3B). As in dmPFC, averages of
normalized firing rates also revealed modulation around these events, and exposed a significant
difference (based on comparisons of 95% confidence intervals, estimated using bootstrapping) in
motor cortex population activity during the reaction time period in sessions with one and two
delay periods.

Firing rates of rodent frontal cortex neurons could be correlated with behavioral reaction
times during the reaction time window (1.0 to 1.6 sec after lever press). In dmPFC, similar
fractions of neurons had significant (p < 0.05) firing rate correlations in one-delay sessions and in
two-delay sessions, (35 of 144 neurons in one-delay sessions, or 23%, 29 of 135 neurons in two-
delay sessions, or 22%, \( X_2 = 0.31, \) df=1, \( p<0.58 \), although correlations among these neurons
were stronger in two-delay sessions (\( R = |0.24|\pm0.9 \) in one-delay sessions, \( R = |0.33|\pm0.14 \) in
two-delay sessions, Wilcoxon rank-sum \( p < 0.002 \)). In motor cortex, although fewer neurons had
significant correlations in two-delay sessions than in one-delay sessions (43 of 65 neurons in
one-delay sessions, or 66%, 32 of 70 neurons in two-delay sessions, or 45%, \( X_2 = 5.7, \) df=1,
\( p<0.02 \), correlations among these neurons were stronger in two-delay sessions than in one-delay
sessions (\( R = |0.31|\pm0.14 \) in one-delay sessions, \( R = |0.42|\pm0.16 \) in two-delay sessions, Wilcoxon
rank-sum \( p < 0.002 \)).

Furthermore, simultaneously recorded rodent frontal cortex neurons could be correlated
with each other (Narayanan et al., 2005; Narayanan and Laubach, 2009). We investigated this
issue using the partial correlation method to control for correlation with reaction times (discussed
above) and identified significantly correlated (\( p < 0.05 \) via a partial correlation) pairs of neurons.
In dmPFC, similar numbers of simultaneously recorded pairs were correlated in one-delay (1161
of 17966 pairs, or 6.5%, \( R = |0.45| \)) and two delay sessions (1039 of 14205, or 7.3%, \( R = |0.45| ; \)
\( X_2= 0.78, \) df = 1, p-value = 0.3786). However, in motor cortex, more neurons had pairwise
correlations in two-delay sessions (876 or 6495, or 13.1%, $R = |0.47|$) compared to one-delay sessions (549 of 6051, or 9.1%, $R = |0.45|$, $X^2 = 5.63$, df = 1, $p < 0.02$). Strengthening correlations in motor cortex may underlie firing rate differences observed in Figure 3D.

**Slow modulations of population activity during the trial**

Several distinct firing patterns were revealed through application of principal component analysis (PCA) to matrices of neuronal firing rates during the delay period (from -0.5 sec before to 1.5 sec after the time of lever pressing). Two major components, each accounting for more than 10% of variance, were found in activity from dmPFC (Figure 4). The first component (PC1), which accounted for 20.3% of variance, was a steadily decreasing function that spanned from the time of the lever press until the time of pump activation (upper plot in Figure 4A). More neurons had positive loadings on PC1 (i.e., their activity resembled this negatively decelerating function) than had negative loadings (upper plot in Figure 4B; proportions test: $X^2 = 13.1$, df=1, $p<0.00001$). There was no difference in loadings on this function in sessions with one and two delay periods (left plot in Figure 4C). Examples of neurons with a large loading on PC1 (i.e., the coefficient of the neuron for the first eigenvector) are shown in Figure 5A,B. These neurons showed very slow rates of change in firing rate, and fired in a reciprocal manner during the course of the trial.

The second component (PC2) accounted for 14.8% of variance. This function changed at the time of the lever press, and then began to change in the opposite direction during the delay period until the time of the stimulus at 1.0 sec (lower plot in Figure 4A). This component resembled the activity of many dmPFC neurons with sustained delay activity (Narayanan and Laubach, 2006). There were equal numbers of neurons with positive and negative loadings on
this component (lower plot in Figure 4B; proportions test: p>0.05). As for PC1, the pattern of firing associated with PC2 was found in both the one-delay and two-delay sessions (right plot in Figure 4C). Examples of neurons with a large loading on PC2 are shown in Figure 5C,D. As above, these neurons showed very slow rates of change in firing rate, and fired in a reciprocal manner during the course of the trial.

In the motor cortex, there were three major components, each accounting for more than 10% of variance (Figure 6). The first component (PC1), which accounted for 29.5% of variance, was a similar to PC1 from dmPFC and had a steadily decreasing form spanning from the lever press until just before the end of the delay period (upper plot in Figure 6A). Equal numbers of neurons had positive and negative loadings on PC1 (upper plot in Figure 6B; proportions test: p>0.05) and there was no difference in loadings on this function in sessions with one and two delay periods (left plot in Figure 6C). Examples of neurons with a large loading on PC1 (i.e., the coefficient of the neuron for the first eigenvector) are shown in Figure 7A,B. These neurons showed much sharper modulations around lever pressing than the neurons that were related to PC1 from dmPFC. However, as in dmPFC, we found neurons with strong reciprocal patterns of firing that were related to PC1.

The second component (PC2) from the motor cortex accounted for 25.4% of variance. As in PC2 from dmPFC, this function changed at the time of the lever press, and began to change in the opposite direction during the delay period (middle plot in Figure 6A). The rate of change for PC2 from motor cortex was more gradual than the rate of change for PC2 from dmPFC and the change in rate continued through the end of the delay period. This component resembled the activity of many motor cortical neurons with sustained delay activity (Narayanan and Laubach, 2006). As for PC1, there were equal numbers of neurons with positive and negative loadings on
this component (middle plot in Figure 6B; proportions test: p>0.05) and the same range of
loadings were found in sessions with one and two delay periods (right plot in Figure 4C).
Examples of neurons with a large loading on PC2 are shown in Figure 7C,D. As for PC1, the
neurons were more sharply modulated than those found to weigh on PC2 in dmPFC.

The third component (PC3) from the motor cortex accounted for 15.1% of variance. This
function was modulated around both lever events, i.e., press and release (lower plot in Figure
6A). This component resembled the activity of many movement-related neurons in the motor
cortex (Narayanan and Laubach, 2006). As for PC1 and PC2, there were equal numbers of
neurons with positive and negative loadings on this component (lower plot in Figure 6B;
proportions test: p>0.05). However, this was the only component that showed a significant
difference in loadings from neurons in sessions with one and two delay periods (Wilcoxon rank-
sum p<0.00001). Examples of neurons with a large loading on PC3 are shown in Figure 7E,F.
Both of these neurons were modulated around lever pressing; however, other neurons were found
that modulated around lever release (not shown). These data correspond to differences in firing
rate modulation observed in motor cortex around lever events in Figure 3B,D.

Comparisons of the time-course of the PCs derived from activity in dmPFC and motor
cortex revealed that population activity in the two cortical regions changed at approximately the
same times during the trial (Figure 8). Moreover, the rates at which the PCs in each area changed
over time were highly related. In both areas, plots of the cumulative sum of PC1 were highly
similar to the time-course of PC2 (correlation coefficient >0.9; Figure 8A,B). For all PCs,
modulations occurred earlier in motor cortex compared to dmPFC (Figure 8C,D).

**Stimulus-related activity in dmPFC**
A subpopulation of dmPFC neurons was modulated around the trigger stimulus (Figure 9A,B). To determine if these neurons fired to the stimulus or were modulated by other factors, such as response preparation, we recorded from 88 dmPFC in 6 rats performing a modified reaction time task (based on Kornblum, 1973), in which stimuli occurred on half of the trials (on the other half of the trials, called time production trials, no stimulus occurred) and all delays were 1.0 sec in duration. Rats were rewarded for responding at a latency of 1-1.6 sec in these sessions regardless of whether a stimulus was presented. In these sessions, stimulus-driven firing was apparent for neurons that fired immediately after the onset of the stimulus and that did not fire on time production trials (i.e., when no stimulus was presented). Twelve such neurons (of 88, or 14%) were observed with significant stimulus-responsive activity (Wilcoxon rank-sum test p<0.05 comparing activity on trials with trigger stimuli to trials without for the epoch of 1.0 to 1.2 sec after lever press). Each animal had at least 1 stimulus-responsive neuron (range: 1 to 6 neurons per animal). As in our previous study (Narayanan and Laubach 2006), we did not observe stimulus-responsive firing in the motor cortex.

**DISCUSSION**

In the present study, we described how neuronal activity in rodent frontal cortex is modulated during delay periods. We used principal component analysis to identify two prominent patterns of firing during the delay period, ramping activity and sustained delay activity. These patterns were common to both dmPFC and motor cortex, and did not change as animals learned to respond at new delays. We also investigated whether delay period activity in rodent frontal cortex reflected proactive inhibition of responding or anticipation of forthcoming trigger stimuli. Neurons in dmPFC and motor cortex did not change after animals learned to
respond at the novel delay, suggesting that frontal processing is involved in proactively inhibiting delay-related behavior and is not sensitive to the timing of stimuli.

Delay activity has been reported throughout the frontal cortex of primates, especially in premotor and prefrontal regions (Niki and Watanabe, 1976b; Tanji and Evarts, 1976; Niki and Watanabe, 1979; Evarts et al., 1984; Mauritz and Wise, 1986; di Pellegrino and Wise, 1991; Brody et al., 2003; Churchland et al., 2006; Churchland and Shenoy, 2007). The present study, together with other recent reports (Batuev et al., 1990; Baeg et al., 2001; Baeg et al., 2003; Narayanan and Laubach, 2006; Cowen and McNaughton, 2007), shows that non-mnemonic delay activity is also found in the rodent prefrontal cortex. These data support the hypothesis that across species, prefrontal regions are critical to the temporal organization of behavior (Fuster, 1997) and establishes that the capacity for frontal cortical neurons to fire in a persistent manner during delay periods is not a unique property of the primate prefrontal cortex.

Our results support previous lesion and inactivation studies (Broersen and Uylings, 1999; Risterucci et al., 2003; Narayanan et al., 2006; Narayanan and Laubach, 2006) that demonstrate that rodent medial prefrontal regions are critical to inhibiting premature responses and to maintaining delay-related behavior. Together, these studies suggest that dmPFC neural activity is involved in inhibiting temporally inappropriate responses until the end of the delay period (Ollman and Billington, 1972; Narayanan et al., 2006; Narayanan and Laubach, 2006). Our data are also convergent with neuroimaging studies in human subjects that have implicated medial frontal regions in the prepotent inhibition of impending actions (Onoe et al., 2001; Rao et al., 2001; Lewis and Miall, 2006; Vallesi et al., 2007).

We found no evidence that dmPFC or the motor cortex is sensitive to the expected timing of the trigger stimulus. This kind of processing is assumed in many models of simple reaction
time performance (Los et al., 2001; Los and Van Den Heuvel, 2001; Los, 2004). One reason for our not finding such “orienting” activity might be that our animals had limited experience with stimuli presented at short delays (they only experienced stimuli at that time in two behavioral session prior to our recordings), although three days of two-delay task performance was sufficient to speed reaction times. Another possibility is that we are limited by insufficient statistical power, although we did observe differences in motor cortex activity as animals learn to respond new delays. Alternatively, it may be that temporal processing is represented by some other measure of activity in frontal cortex (e.g., through correlations between neurons or through correlations between spike trains and field potentials). Although anticipatory activity has been previously reported in rodent frontal cortex in a classical conditioning paradigm (Baeg et al., 2001), our data suggests that in an operant context, such activity does not change as animals learn to anticipate novel delays.

Analysis of neuronal population activity revealed two prominent patterns of firing during the delay period, ramping activity and sustained delay activity, which were found in both dmPFC and motor cortex. Ramping activity has been shown in several theoretical studies to be capable of representing the passage of time (Durstewitz, 2003; Reutimann et al., 2004). For example, Durstewitz (2003) proposed that temporal control could be achieved through interactions between a population of neurons with ramping neural activity and a population of stimulus-modulated neurons. Ollman and Billington (1972) and Kornblum (1973) made similar suggestions based on data from human subjects performing simple reaction time tasks. It is possible that dynamic changes in network activity (Haider et al., 2006), and not in the activity of individual neurons, are critical to temporal preparation. Evidence for such interactions might be found through analysis of noise correlations (Averbeck and Lee, 2003) between groups of
simultaneously recorded neurons or through studies of interactions between spike and field activity (Donoghue et al., 1998).

A second major pattern among dmPFC neurons was sustained, persistent delay-related activity. It is possible that this pattern of activity is directly responsible for inhibiting lever-response motor programs during the delay period (Narayanan and Laubach 2006) via top-down control over responding according to task rules (Miller, 2000), such as inhibiting responses until the longest delay at which a stimulus has been presented. Indeed, our study establishes that the medial frontal cortex has the necessary elements for implementing a “deadline model” (Ollman and Billington, 1972) for controlling responding during the delay period.

We previously proposed a model for the top-down control of action (Narayanan and Laubach, 2006), which was based on classic work on human simple reaction time performance by Ollman and Billington (1972) and Kornblum (1973). The model proposed that dmPFC serves to temporally inhibit responding during the delay period, and does so by having access to information about temporal and sensory events in the task. Inhibitory control was proposed to be due to a decaying inhibition over action during the delay period. This inhibition would be reduced by “excitatory” signals related to the prepotent tendency to release the lever (PC3 from the motor cortex, Figure 6) to obtain a reward and the presentation of the trigger stimulus (stimulus-responsive cells in Figure 9). Based on neuronal data in the present manuscript, on our previous recordings from rodent frontal cortex (Narayanan et al., 2005; Narayanan and Laubach, 2006, 2008), and on recent studies in human subjects (Boulinguez et al., 2008; Jaffard et al., 2008; van Elswijk et al., 2008), we are now able to revise this model as shown in Figure 10. Using terms from Jaffard et al.(2008), we propose that lever pressing triggers two competing processes, represented by two pools of neurons, that prepare for the forthcoming action (“the
prepotent response”) and that inhibit action during the delay period (“the proactive inhibition”). These two pools of neurons would excite themselves and inhibit the other pool. Based on theoretical work by Wang et al., (2002), Reutimann et al., (2004), Machens et al. (2005), and others, such mutually inhibitory neuronal pools would allow for the accumulation of ongoing activity in the network. Such network interactions may arise through an accumulation of press-related activity (Figure 8) and would be manifest as maintained persistent firing that includes cells in both dmPFC and motor cortex.

Potential anatomical routes exist that could enable this kind of processing. Direct cortico-cortical connections have been reported between dmPFC and the motor cortex, although the connections are very sparse (Wang and Kurata, 1998). Indirect cortico-cortical connections between dmPFC and motor cortex are also known to exist by way of the rostral forelimb area (Rouiller et al., 1993). Finally, interactions could arise through a thalamic route, for example, involving connections between dmPFC and the rostral forelimb areas by way of the medial dorsal nucleus (Conde et al., 1990).

Based on the present study and on Narayanan and Laubach (2006), we propose that there are functional interactions between dmPFC and motor cortex that are crucial for persistent delay activity. The present study (Figure 8) suggests that population activity in both cortical areas is modulated at the lever press and that this change in network activity leads to lasting persistent activity during the delay period. In this view, persistent firing may be generated by the animal’s own actions in the task.

Crucially, neurons in dmPFC are highly sensitive to the outcome of the current trial (Narayanan and Laubach, 2006) and immediately preceding trial (Narayanan and Laubach, 2008). As such, we suggest that the slow modulation of dmPFC population activity reflects the
recent history of the animal's success in performing the simple reaction time task and, through integration with network activity in the motor cortex, delay-related activity in dmPFC allows animals to control their waiting behavior based on current goals and the recent history of task performance.
ACKNOWLEDGEMENTS

This work was supported by funds from the National Science Foundation, Kavli Institute at Yale, and the John B. Pierce Laboratory for ML and from the Army Research Office for NSN. We thank Gidon Felsen, Nicole Horst, and Jeff Schall, for helpful comments on an earlier version of this manuscript. We also thank Xiao-Jing Wang, Nate Smith, and two anonymous reviewers for comments on the present version of the manuscript. Finally, we thank the Instruments Shop at the John B. Pierce Laboratory for outstanding technical support.
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FIGURE LEGENDS

**Figure 1: Simple reaction time task.** A) Rats were trained to perform a simple reaction time task. Lever presses were maintained over a 1.0 sec delay period ("one-delay" sessions) until a tone (frequency: 8 kHz, intensity: 72 dBA, duration: 100 ms) was presented. To receive liquid rewards, animals released the lever within 0.6 sec of stimulus onset. After implantation of recording electrodes and testing in three sessions with one delay period, animals were run in three additional sessions with delays of 0.4 or 1.0 sec ("two-delay" sessions). B) Reaction times are shown for the one-delay and two-delay sessions. Rats responded faster at the long delay in sessions with two delays, suggesting that the addition of the early delay period led to "delay-dependent speeding" of reaction times. Asterisk indicates significance at p < 0.01 by a paired t-test (see text for details). C) Lever force in one-delay and two-delay sessions for trials with correct responses and long delays. Animals maintained similar lever force during the delay period in one-delay and two-delay sessions.

**Figure 2: Location of microwire arrays.** Recording sites are shown in dorsomedial prefrontal cortex (9 animals) as white dots and in motor cortex (4 animals) as black dots. Reconstructions are shown for the horizontal (panel A) and frontal (panel B) planes. An example histological section is inset in panel A, as used to determine electrode location.

**Figure 3: Task-related modulations in neuronal activity in rodent frontal cortex.** Simple arithmetic averages of neuronal firing rates for dmPFC (A) and motor cortex (B) are shown as lines and the 95% confidence intervals around the average rates are shown as bands (sessions with one delay: gray lines, light gray bands; sessions with two delays: black lines, dark gray
bands). Average firing rates in dorsomedial prefrontal cortex were similar in sessions with one and two delay periods. However, motor cortical neurons were increasingly modulated during the reaction time epoch (from 1.0 to 1.6 sec) in sessions with two delay periods compared to sessions with one delay period. This effect may have been due to the faster reaction times that were found in the two-delay sessions (see Figure 1B). Averages of normalized firing rates, calculated as z-scores, are shown as in A-B for dmPFC (C) and motor cortex (D). Dashed lines depict the timing of delay periods (0.4 and 1.0 sec). Note that all analyses are restricted to correct trials with a long delay.

**Figure 4: Analysis of firing patterns in dorsomedial prefrontal cortex using PCA. A)**

Eigenvectors are shown for the first two principal components relative to the time of lever press. The eigenvectors describe patterns of firing in the neuronal population. PC1 accounted for 20.3% of variance in the neuronal population. Eigenvectors for PC1 increased slightly at the lever press and then “fell” to negative values during the delay period. PC2 accounted for 14.8% of variance in the neuronal population. Eigenvectors for PC2 increased around the lever press, were relatively constant during the delay period, and then were reduced during lever release. **B)** Histograms of the loadings of neurons onto the PCs are shown. The loadings reflect the correlation between the neuron's firing pattern and the firing pattern described by the PC. More neurons had positive loadings on PC1 than had negative loadings (proportions test, \(X^2=13.1\), df=1, \(p<0.0001\)); that is, more neurons “ramped down” than “ramped up” during the delay period (Figure 5A). Loadings on PC2 were equally positive and negative. **C)** Boxplots show that loadings on both PCs were equivalent for sessions with one and two delay periods. Note that all analyses are restricted to correct trials with a long delay.
**Figure 5: Examples of firing patterns in dorsomedial prefrontal cortex.** A) Spike rasters and average peri-event histograms (1 ms bins, smoothed with a 25-ms Gaussian) are shown for a neuron with one of the largest positive loadings (>3 standard units) on PC1. This neuron ”ramped up” during the delay period. B) A neuron with one of the largest negative loadings (<-3 standard units) on PC1. This neuron ”ramped down” during the delay period. C) A neuron with one of the largest positive loadings on PC2. This neuron fired persistently during the delay period. D) A neuron with one of the largest negative loadings on PC2. This neuron showed a persistent reduction in firing rate during the delay period. All rasters depict correct trials with a long delay.

**Figure 6: Analysis of firing patterns in motor cortex using PCA.** A) Eigenvectors are shown for the first two principal components relative to the time of lever press. PC1 accounted for 29.5% of variance and was modulated at the time of the lever press. PC2 accounted for 25.4% of variance and was modulated just before the lever press and then gradually was reduced in sign in the late delay period. PC3 accounted for 15.1% of variance and was sharply modulated around the press and release of the lever. B) Histograms of the loadings of neurons onto the PCs showed that all three PCs had equally positive and negative loadings. C) Boxplots show that loadings for PC1 and PC2 were equivalent for sessions with one and two delay periods. However, loadings for PC3 were significantly more positive in one-delay sessions compared to two-delay sessions (Wilcoxon rank-sum p<0.0001). Note that all analyses are restricted to correct trials with a long delay.
**Figure 7: Examples of firing patterns in motor cortex.** A) Spike rasters and average peri-event histograms (1 ms bins, smoothed with a 25-ms Gaussian) are shown for a neuron with one of the largest positive loadings (>3 standard units) on PC1. This neuron fired as the rat pressed the lever and then quickly came to fire at a lower rate. B) A neuron with one of the largest negative loadings (<-3 standard units) on PC1. This neuron fired at a reduced rate as the rat pressed the lever and then quickly came to fire at an elevated rate. C) A neuron with one of the largest positive loadings on PC2. This neuron fired at the time of the lever press and then fired persistently until just before the end of the delay period. D) A neuron with one of the largest negative loadings on PC2. This neuron fired at a reduced rate during the delay period, and also during the lever press during the trials near the end of the session (lower third of raster plot). D) A neuron with one of the largest positive loadings on PC3. This neuron fired at the time of the lever press and then at a lower rate during the rest of the trial. D) A neuron with one of the largest negative loadings on PC3. This neuron was strongly modulated at the time of the lever press and then again when the rat released the lever (after 1 sec). All rasters depict correct trials with a long delay.

**Figure 8: Comparison of firing patterns in motor and dorsomedial prefrontal cortices.** A) Eigenvectors for the first two principal components from motor cortex are shown as black and gray lines, respectively. In addition, the cumulative sum of PC1 is plotted as the dashed line and was highly similar to PC2, both in its time-course and extent of modulation. B) Eigenvectors for the first two principal components are shown from dorsomedial prefrontal cortex (dmPFC). As in motor cortex, the cumulative sum of PC1 is plotted as the dashed line and was highly similar to
PC2. In both areas, modulation of PC1 preceded modulation of PC2 and PC2 was subsequently “persistent”. C) A comparison of the time-course of modulation in PC1 from motor cortex and dmPFC shows that both areas modulated at about the same time, just before the lever press. The modulation in motor cortex was sharper than in dmPFC and decayed at a greater rate following the lever press. D) PC2 was similar from the two cortical areas, but the increase in PC2 from motor cortex preceded the increase in PC2 from dmPFC.

Figure 9: Stimulus-related activity in dorsomedial prefrontal cortex. A) Peri-stimulus rasters and histograms from a neuron with stimulus-related firing in dorsomedial prefrontal cortex (dmPFC). Activity is shown for trials with a stimulus presented at a delay of 1.0 sec (black) and for time production (or ‘catch’) trials (gray), on which the stimulus was not presented and rats were rewarded if they held the lever down for at least 1.0 sec. Approximately 14% of neurons in dmPFC showed this pattern of firing and no neurons like this were found in motor cortex. B) Activity from a dmPFC neuron in two-delay sessions that fired in response to the stimulus, similar to the neuron in A (0.4 sec delays: gray; 1.0 sec delays: black). This neuron also fired on some trials with long delays at the time of the short delay (black arrow), and these spikes are suggestive of stimulus-anticipatory activity. Note that all analyses are restricted to correct trials with a long delay.

Figure 10: Conceptual model for top-down control over action by rodent frontal cortex. A) Brain networks spanning rodent frontal cortex encode prepotent responses and proactive inhibition. This activity is terminated by the trigger stimulus, which facilitates rapid responding. When neurons mediating prepotent responding dominate, animals respond quickly and commit
more premature errors. When neurons mediating proactive inhibition dominate, animals respond more slowly and make fewer premature errors. B) Our data suggest a model of how neural activity within rodent frontal cortex interacts to control the timing of action. Ramping and sustained delay activity in rodent dorsomedial prefrontal cortex encodes the timing of rewards (i.e., Narayanan and Laubach 2008), whereas delay and pressing-related activity in motor cortex encodes the timing of actions (Laubach et al., 2000 and Narayanan et al, 2005). Delay-related networks in rodent prefrontal cortex are correlated with and control delay-related activity in motor cortex (Narayanan and Laubach 2006).