Correlated Discharges Among Putative Pyramidal Neurons and Interneurons in the Primate Prefrontal Cortex

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Constantinidis, Christos, and Patricia S. Goldman-Rakic. Correlated discharges among putative pyramidal neurons and interneurons in the primate prefrontal cortex. J Neurophysiol 88: 3487–3497, 2002; 10.1152/jn.00188.2002. Neurophysiological recordings have revealed that the discharges of nearby cortical cells are positively correlated in time scales that range from millisecond synchronization of action potentials to much slower firing rate co-variations, evident in rates averaged over hundreds of milliseconds. The presence of correlated firing can offer insights into the patterns of connectivity between neurons; however, few models of population coding have taken account of the neuronal diversity present in cerebral cortex, notably a distinction between inhibitory and excitatory cells. We addressed this question in the monkey dorsolateral prefrontal cortex by recording neuronal activity from multiple micro-electrodes, typically spaced 0.2–0.3 mm apart. Putative excitatory and inhibitory neurons were distinguished based on their action potential waveform and baseline discharge rate. We tested each pair of simultaneously recorded neurons for presence of significant cross-correlation peaks and measured the correlation of their averaged firing rates in successive trials. When observed, cross-correlation peaks were centered at time 0, indicating synchronous firing consistent with two neurons receiving common input. Discharges in pairs of putative inhibitory interneurons were found to be significantly more strongly correlated than in pairs of putative excitatory cells. The degree of correlated firing was also higher for neurons with similar spatial receptive fields and neurons active in the same epochs of the behavioral task. These factors were important in predicting the strength of both short time scale (<5 ms) correlations and of trial-to-trial discharge rate covariances. Correlated firing was only marginally accounted for by motor and behavioral variations between trials. Our findings suggest that nearby inhibitory neurons are more tightly synchronized than excitatory ones and account for much of the correlated discharges commonly observed in undifferentiated cortical networks. In contrast, the discharge of pyramidal neurons, the sole projection cells of the cerebral cortex, appears largely independent, suggesting that correlated firing may be a property confined within local circuits and only to a lesser degree propagated to distant cortical areas and modules.

INTRODUCTION

Simultaneous recording from multiple isolated neurons can reveal their patterns of connectivity and offer insights on the functional organization of the network that they comprise. Cells serially connected to each other or sharing common inputs are expected to display correlated discharges (Aertsen et al. 1989; Fetz et al. 1991; Perkel et al. 1967). The time scale of observed neuronal correlations varies considerably, from synchronization of action potentials in the millisecond scale to much slower common increases in firing probability (Bair et al. 2001; Brody 1998; Nowak et al. 1995). Discharge correlations are typically illustrated as peaks in cross-correlation histograms (CCH) of correspondingly variable width. Tight synchronization that produces narrow CCH peaks, in particular, has been investigated in depth as it can constitute evidence of synaptic connectivity (Das and Gilbert 1999; Reid and Alonso 1995).

Much slower discharge correlations between two neurons manifest themselves as trial-by-trial co-variations in the averaged firing rate elicited in response to a particular stimulus. Responses of adjacent cortical neurons, recorded extracellularly from a single electrode, have been shown to be positively but weakly correlated in that fashion (Gawne and Richmond 1993; Jung et al. 2000; Lee et al. 1998; Maynard et al. 1999; Zohary et al. 1994). These concurrent deviations from the neurons’ respective mean response rates have been termed correlated noise, and they are thought to represent direct or indirect shared inputs that vary randomly from trial to trial (Bair et al. 2001). The extent of such correlations among neurons with different functional properties has been used to constrain quantitative models of network architecture (Shadlen and Newsome 1998; Shadlen et al. 1996). Although the implications of pooling correlated and anti-correlated signals have been discussed on theoretical grounds (Abbott and Dayan 1999; Johnson 1980), little experimental evidence on the sources of correlated noise among the diverse classes of cortical neurons, e.g., excitatory and inhibitory neurons, has been available.

We addressed this issue in the dorsolateral prefrontal cortex (PFC). Neurons in this area exhibit broad spatial tuning and are thus fairly insensitive to small sensory and motor variations typically observed across behavioral trials, which can confound the interpretation of correlated firing in cortical areas with much smaller receptive fields (Gur and Snodderly 2001; Gur et al. 1997; Leopold and Logothetis 1998). Recent studies from this and other laboratories have exploited the action potential waveforms and discharge rate properties of cortical neurons to distinguish pyramidal cells and interneurons and to study their spatial and temporal interactions in vivo (Constantinidis et al. 2002; Csicsvari et al. 1998; Frank et al. 2001; Jung...
forms were sorted into separate units using a template-matching point, back-projected onto a tangent screen, while a 1 recorded extracellularly may vary considerably based on the (et al. 2001; Jung et al. 1998; Swadlow 1995). The width of spikes Wilson et al. 1994) and other laboratories (Csicsvari et al. 1998; Frank and inhibitory neurons, as previously done in our (Rao et al. 1999; 1969). We relied on the spike width and baseline regular spiking (RS) neurons recorded in vivo (Mountcastle et al. mick et al. 1985), generally corresponding to fast spiking (FS) and GABA-containing, inhibitory interneurons produce action potentials of much shorter duration than excitatory, pyramidal neurons (McCor- 2-tauotic task that required them to maintain fixation on a 0.2° central point, back-projected onto a tangent screen, while a 1° cue stimulus flashed for 500 ms at an eccentricity of 14°, followed by a delay period of 3 s (Fig. 1). At the end of this period, the fixation point was extinguished, and the monkeys were trained to make a saccade to the remembered target location in the absence of any visual cues. The cue could appear at one of eight possible locations randomly interleaved across trials. Eye position was recorded with a 10-ms resolution, and the trial was terminated immediately if it deviated by more than a predetermined distance (~2° for most recordings). Neuronal activity was monitored using varnish-coated tungsten electrodes (1–4 MΩ at 1 kHz). Up to four electrodes spaced 0.2–1 mm apart of each other were independently advanced into the cortex through a set of micro-motors (Alpha-Omega Engineering, Nazareth, Israel). Neuronal activity was amplified 1,000 times, band-pass filtered (400 Hz to 10 kHz), and digitally sampled with 30-μs resolution. Sampled waveforms were sorted into separate units using a template-matching algorithm (CED, Cambridge, UK).

**Spike classification**

Intracellular recordings in slice preparation have demonstrated that GABA-containing, inhibitory interneurons produce action potentials of much shorter duration than excitatory, pyramidal neurons (McCormick et al. 1985), generally corresponding to fast spiking (FS) and regular spiking (RS) neurons recorded in vivo (Mountcastle et al. 1969). We relied on the spike width and baseline firing rate of extracellularly recorded units to classify them into putative excitatory and inhibitory neurons, as previously done in our (Rao et al. 1999; Wilson et al. 1994) and other laboratories (Csicsvari et al. 1998; Frank et al. 2001; Jung et al. 1998; Swadlow 1995). The width of spikes recorded extracellularly may vary considerably based on the filtering parameters used in the experiment and electrode geometry (Henze et al. 2000). Our current recordings used a narrower filter range than previously used in our laboratory (Rao et al. 1999), and for this reason, we used a slightly different procedure for classifying units into FS and RS. In the present study, we computed spike width as the time distance between the two troughs of an action potential waveform (Fig. 2A), which is easier to assess than the first deflection from baseline, used previously. The frequency distribution of spike widths appeared bimodal (Fig. 3A) and was modeled as the sum of two Gaussians, centered at 465 and 637 μs. To test whether a single Gaussian fitted the data equally well, we performed an F test, which compares the ratio of the variance accounted for by the second Gaussian divided by the residual variance. The test indicated that the two-Gaussian fit accounted for a significant amount of additional variance (P < 10−5). Adding a third Gaussian in the model did not account for a significant further increase in variance (P > 0.2). We drew the same conclusions when we repeated this test for data collected separately from the two monkeys.

Because some degree of overlap existed between the two distributions, we additionally used the baseline fixation firing rate of the neurons to better resolve the two populations. Firing rates corresponding to the two distributions defined by spike width were significantly different from each other (t-test, P < 10−5). The percentage of neurons with baseline firing rate reaching or exceeding the average rate of our entire sample (9.5 spikes/s) is shown in Fig. 3B. A transition point in baseline firing rate was evident for spikes >540 μs. Two alternative methods were used to classify neurons. The first method, which we will refer to as the narrow classification criterion, assigned neurons as FS if they exhibited a spike width of ≤540 μs and baseline firing rate reaching or exceeding 9.5 spikes/s. Units were characterized as RS if their spike width exceeded 540 μs and baseline firing rate did not exceed 9.5 spikes/s. We classified 367/526 (70%) of our units as FS (89/367, 24%) or RS (278/367, 76%) in this fashion. To estimate the expected error rate of this classification scheme, we fitted Gaussian curves to the distributions of firing rates corresponding to the two populations of neurons. We then calculated the probability that a neuron belonging in the RS population would display a spike width less than our criterion level as well as the probability that its firing rate would exceed our corresponding rate criterion. The product of the two probabilities represents the expected percentage of excita- tory neurons falsely classified as FS. This was 1.3% for RS units and 2.8% for FS units (weighted average, 1.7%).

We used a second method to classify neurons, which we will refer to as the broader classification criterion, by performing a two-means cluster analysis. The method classifies each observation into one of two groups so as to maximize the between-group variation relative to the within-group variation regardless of the biological significance of the underlying dimensions. The analysis was performed with the statistical package SYSTAT (SPSS, Chicago, IL). All 367 neurons classified as RS or FS by the spike-width and firing rate criteria described in the preceding text were assigned to the same respective group by cluster analysis. The
remaining 159 neurons were classified as FS or RS, mostly based on spike width (Fig. 3, C and D). Overall, 34% of the neurons were classified as FS. This percentage is higher than the incidence of interneurons in the cortex revealed by anatomical studies, and it is likely to represent a substantial classification error and to dilute the contrast in physiological properties between excitatory and inhibitory neurons. However, all the systematic differences between RS and FS units we report in the following text, regarding the width of spatial tuning, correlated noise and incidence, and strength of cross-correlation peaks were statistically significant when we classified units based on either method. We will present results primarily based on the cluster analysis as it provides a more conservative estimate for the difference between the putative pyramidal and interneuron populations.

Data analysis

We computed the firing rate of each unit in five different time windows, during the fixation period (500 ms), cue presentation (500 ms), delay period (3,000 ms), presaccadic period (250 ms after fixation point turning off), and postsaccade period (500 ms following the end of the presaccade period). Only neurons that exhibited signifi-
cantly different firing rates in a task epoch compared with baseline fixation were included in the results (paired t-test, *P* < 0.05, adjusted for multiple comparisons).

A neuron’s spatial tuning in each task epoch was assessed with a vector algorithm, as described previously (Constantinidis et al. 2001). Briefly, the firing rate of each trial was represented as a vector whose direction was determined by the location of the cue in the eight-target ODR task, and its amplitude was proportional to the firing rate recorded. Statistical significance was assessed with a bootstrap test comparing the size of the resultant vector to that produced by randomizing distribution of trials across the eight target locations (Lurito et al. 1991). The test was evaluated at the 0.01 significance level. The difference in spatial tuning between neurons was estimated as the angular difference in direction between their corresponding resultant vectors. This measure was computed separately for each task epoch. If two neurons exhibited significant spatial tuning in more than one common epoch, the average distance was computed.

To evaluate the width of spatial tuning, Gaussian curves were fitted to responses of neurons with significant spatial tuning. Responses to the eight target locations in one task epoch were fitted according to the equation

\[
\mathbf{f}(\mathbf{d}) = B + R \cdot e^{-|\mathbf{d}|^2/2\sigma^2}
\]

where *B* is a measure of the neuron's baseline firing rate during the epoch and *R* is the maximum response rate. The peak of the Gaussian is represented as *D* and the *SD* of the Gaussian provides a measure of the tuning width. The fitting was performed with a computer program implementing the Levenberg-Marquardt algorithm (Press et al. 1992).

Each neuron's temporal pattern of activation across the different task epochs was characterized as follows. Responses were pooled from all spatial locations, and the average discharge rate was computed for each task epoch. For the purposes of this analysis, the delay epoch was further divided into three periods, each 1-s long (although results were similar when we averaged firing rates from the entire delay period). The Pearson correlation coefficient between the averaged responses of two neurons across corresponding task epochs provided a measure of the similarity in temporal profile of activation for each pair.

To determine correlated noise, we computed the average and *SD* of firing rate for each task epoch and target location. We subtracted the mean value of the corresponding task condition from the rate recorded in each trial and divided it by the *SD* to provide a normalized estimate independent of condition (Zohary et al. 1994). For simultaneously recorded pairs, we then computed the Pearson correlation coefficient between their normalized values. Correlation values were computed both separately for each task epoch and for all task epochs combined. To ensure uniformity in our data set, we only computed correlated noise in pairs of neurons tested with the eight-target ODR task and at least eight correct trials per stimulus location (typically 10).

Cross-correlation histograms (CCHs) were constructed for all pairs of simultaneously recorded neurons (Perkel et al. 1967). For each CCH, a shift predictor was calculated to help identify potential correlated firing, time-locked to the stimulus. The statistical significance of CCH peaks was evaluated as described previously (Constantinidis et al. 2001). Briefly, we first computed the baseline of the raw correlogram defined as the average of half the bins in the flanks of the CCH. We then identified peaks that exceeded the baseline by a number of *SD*s corresponding to a probability value of 0.001. We identified “narrow” CCH peaks, centered within 5 ms of the center bin and exhibiting a width at half-peak height of ≤5 ms (Kruger and Aiple 1988; Michalski et al. 1983). The strength of correlated firing between two units was computed as the number of spikes under the CCH peak that exceeded the baseline (computed in a 5-ms window centered at 0), divided by the total number of spikes from each neuron.

### RESULTS

#### Single-neuron response properties

Neuronal activity was sampled from the dorsolateral prefrontal cortex (areas 8 and 46) of two awake, behaving monkeys performing the ODR task (Fig. 1). Analysis was based on 526 neurons exhibiting firing rates significantly modulated by the task. We classified these neurons as FS or RS based on a cluster analysis, as described in METHODS. We also used a narrower criterion to classify with greater certainty a subset of 367 neurons. The laminar position of each unit could not be reliably established, but our recordings focused on the supragranular layers; 91% (477/526) of responsive units were recorded no deeper than 1 mm from the initial appearance of neuronal activity. There was no significant difference between the distributions of recording depths for FS and RS units (mean values: 492 and 480 μm, respectively, *t*-test, *P* > 0.5).

FS and RS units were found to differ in their distributions of inter-spike intervals, particularly for brief intervals that, in some cases, reflected firing of bursts. For the purposes of this analysis, we processed the last second of the fixation period before the onset of the cue, and used the ratio of inter-spike intervals <5 ms divided by all intervals ≤100 ms as an index of bursting. Because this ratio is dependent on firing rate, we normalized it by the same ratio that would be expected by a Poisson process of equal mean rate. We refer to this normalized bursting index as B1. The median value of B1 across our entire sample of neurons was 0.90, very close to the expected value of 1 for a Poisson process. However, B1 varied widely across neurons, with 10% (50/526) exhibiting values of ≥4, suggestive of a high incidence of bursts. These bursting neurons were almost exclusively classified as RS (98% based on our narrow and 84% on our broader classification criterion). On the other hand, the inter-spike interval distributions of many FS neurons were characterized by a relative scarcity of short (<5 ms) inter-spike intervals (Fig. 2C).

Variability of responses was very much in line with that recorded in other cortical areas. The variance of responses was generally higher than the mean both for RS and FS units. Variance could be expressed as a power law function of the mean firing rate (M). The best fits for activity during the 3-s delay period were very similar for FS and RS units

\[
\text{RS units: } \sigma^2 = 0.72 \cdot M^{0.36}
\]
\[
\text{FS units: } \sigma^2 = 0.88 \cdot M^{1.19}
\]

We analyzed the spatial tuning of neurons using vector-averaging and Gaussian fitting techniques (see METHODS). Agreement between the peak of the Gaussian fit and best location of the vector algorithm was excellent; the median value of the absolute difference between the two estimates was 5.6°. Approximately equal percentages of FS and RS units were spatially tuned in at least one task epoch (79 and 71%, respectively). We observed that FS neurons were more broadly tuned than RS neurons, as exemplified in Fig. 4. The average tuning width, as judged by the SD of the Gaussian curve, was 45° for RS and 51° for FS neurons. The difference was statistically significant (*t*-test, *P* < 0.005) and consistent across all task epochs (Fig. 4C). A two-way ANOVA test confirmed that the effect of cell type on tuning width was significant (*P* < 0.005) but that the effect of task epoch and the interaction of
for FS units, respectively, for the 4 epochs. Curve represents best for each of the 8 cue locations for an RS and FS neuron, respectively. Curve fi neurons (1’s preferred location (opponent memory fi sition period. Neuron in A had a narrower spatial tuning width (41°) than neuron in B (60°), representative of our population of RS and FS neurons. C: bars represent average spatial tuning widths for RS (●) and FS neurons (□), computed separately in each task epoch. Error bars represent SE. The tuning of FS neurons was significantly more narrow (2-way ANOVA test, P < 0.001). Sample sizes were 122, 125, 105, 111 for RS and 74, 81, 80, 56 for FS units, respectively, for the 4 epochs.

Factors were not (P > 0.1). The tuning difference was more pronounced when we identified units using our narrower classification criterion (average tuning width was 43° for RS and 53° for FS). Interestingly, a recent model of spatial tuning in prefrontal neurons has indicated that broader tuning of FS neurons is required for the stability of the working memory system (Compte et al. 2000).

Previous experimental work has indicated that prefrontal neurons exhibit opponency, i.e., discharge at higher rates than baseline for preferred targets in the visual field and at lower than baseline for orthogonal targets (Funahashi et al. 1989; Goldman-Rakic et al. 1990). In keeping with the empirical data, several recent models have simulated spatial tuning in prefrontal neurons by assigning a net inhibitory input to units with memory fields away from the remembered location (Compte et al. 2000; Tanaka 1999). We confirmed this observation for both FS and RS neurons by comparing baseline firing rate to delay period activity after the target diametric to the neuron’s preferred location (opponent memory field). This was usually, but not necessarily, the location with the lowest firing rate during the delay period. The average delay-period rate after presentation of the target at this location away from the memory field was 12.4 spikes/s for FS and 4.0 spikes/s for RS units (see neurons in Fig. 4 for examples). This was lower than the response during fixation (15.4 and 4.6 spikes/s, respectively), and the difference was statistically significant for both FS and RS units (paired t-test, P < 0.005). This finding provides statistical confirmation that PFC neurons receive a higher proportion of inhibitory inputs during memory maintenance of a stimulus appearing away from the receptive field.

Cross-correlation analysis

We quantified correlated firing in a total of 423 simultaneously recorded pairs of neurons, tested with the eight-target ODR task. Of those, 80 pairs were recorded from the same electrode, 198 pairs from separate electrodes 200 μm apart, 97 pairs 300 μm apart, 6 pairs 500 μm apart, and 42 pairs 1,000 μm apart. For each pair of these neurons, we constructed CCHs and identified significant, narrow (<5 ms wide) peaks, as described in METHODS. The incidence and strength of correlated firing was highest for neurons recorded from the same electrode and declined as a function of distance. However, results obtained from the same electrode are not directly comparable to those from separate electrodes as synchronous spikes (within 1–2 ms of each other) cannot be resolved from the same electrode and the correlation in firing rates due to these spikes cannot be determined. We therefore focused our analysis on neurons recorded from electrodes 200–300 μm apart. Significant, narrow CCH peaks in this sample were almost always centered at zero lag time, suggesting that the two neurons fired a larger proportion of synchronous action potentials than would be predicted by chance. This pattern is consistent with the two neurons sharing common input.

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We next examined whether the frequency and strength of CCH peaks differed for FS and RS pairs. This was indeed the case. Only 8% of RS-RS pairs recorded 200–300 μm apart exhibited significant narrow cross-correlation peaks, compared with 14% of RS-FS pairs and 37% of FS-FS pairs (Fig. 5A). The distribution between the three groups was significantly different from uniform (χ² test, \( P < 0.005 \)). The same conclusions were reached when we examined the CCH strength for all pairs of neurons, whether they displayed a significant peak or not. The cross-correlation strength, as defined here, represents the ratio of spike counts under the center 5 ms of the CCH that exceed the baseline, divided by the total number of spikes of each unit. CCH strength provides a measure of the proportion of common inputs shared by the two neurons. The results, shown in Fig. 5B, revealed that correlation strength was highest among pairs of FS units (average correlation strength, 2.0 ± 0.2%) and lowest among pairs of RS units (average correlation strength, 0.5 ± 0.1%). The difference between the three groups was statistically significant (ANOVA test, \( P < 0.005 \)). Correlation strength among RS-RS pairs was even lower for neurons classified based on our narrower criterion (average value, 0.3 ± 0.1%).

**Trial-to-trial correlations**

Correlated noise was computed as the amount of correlation in the trial-to-trial variations of average firing rate around the mean discharge rate (Zohary et al. 1994). Similar to cross-correlation peaks, correlated noise depended on distance between the recording electrodes (Fig. 6A). Additionally, the estimate of noise correlation varied as a function of integration window in our data (Fig. 6B). When we divided the delay period (the longest epoch in our task) in successive 50- to 1,000-ms windows and computed the average noise correlation among normalized rates in these intervals, we observed a logarithmic relationship between the duration of the integration interval and noise correlation computed based on that interval. No systematic differences were observed in noise correlation during different task epochs (Fig. 6C). The average correlation computed in a 250-ms interval for neurons recorded 200–300 μm apart was not significantly different between epochs (ANOVA test, \( P > 0.5 \)).

Similar to our cross-correlation results (Constantinidis et al., 2001), noise correlation was higher among neurons with more similar spatial tuning (Fig. 7A). A regression analysis of correlated noise on tuning difference revealed that there was a significant dependence between the two variables (\( P < 0.005 \)). We also evaluated the similarity between the neurons’ patterns of activation across different task epochs. Correlated noise was higher among neurons co-active in the same task epoch as evaluated by the \( r \) value of their averaged firing rates in corresponding epochs (Fig. 7B). A regression analysis again showed a significant dependence between the two factors (\( P < 0.005 \)). The measure of noise correlation shown here is closely related to that obtained with cross-correlation analysis as both measures reflect shared input between two neurons that can often be identified as a peak in the cross-correlation histogram (Bair et al. 2001). This was the case for our data set, as well. Neurons that exhibited significant CCH peaks also exhibited higher correlated noise on average (Fig. 7C).

After analyzing the factors influencing correlated discharges, we quantified noise correlation between different cell types. The average values for pairs recorded 200–300 μm apart was \( r = 0.035 ± 0.01 \) for RS-RS pairs, \( r = 0.08 ± 0.01 \) for RS-FS pairs, and \( r = 0.11 ± 0.02 \) for FS-FS pairs (Fig. 7D). The difference among the three groups was statistically significant (ANOVA test, \( P < 0.005 \)). The correlation among RS-RS units was even lower when we classified neurons based on our narrower criterion (average value \( r = 0.02 ± 0.01 \)).
Noise correlation was higher among neurons with more similar spatial tuning and temporal profile of activation (Fig. 7, B and C); however, in every case, the correlation was lowest among RS-RS pairs ($r = 0.06 \pm 0.03$) and highest among FS-FS pairs ($r = 0.18 \pm 0.05$) as shown in Fig. 7D.

A caveat in this analysis is that the estimate of correlated noise may be biased toward lower values in neurons with low firing rate because they are more likely to produce spike counts of zero due to random measurement errors. Because cell types were partly defined based on firing rate, we questioned whether differences in correlated noise between different cell types were accounted purely by firing rate. Therefore we tested the hypothesis that correlated noise was significantly dependent on the neurons’ firing rate. This analysis was performed separately among each group of neurons (FS-FS, RS-RS, and RS-FS) as defined with the narrow classification criterion. No significant dependence of correlated noise on firing rate was observed for either group (regression analysis, $P > 0.1$ in each case), and firing rate accounted for 1.2–6.3% of the variance in correlated noise between different pairs. The result indicates that the difference in correlated discharge between the different cell types was not simply an artifact of firing rate differences between them.

**Behavioral sources of correlated discharge**

We have assumed so far that trial-to-trial correlations in the discharges of cortical neurons reflect shared anatomical inputs; however, in principle, the variability of firing rates may solely arise due to subtle variations in sensory or motor parameters of individual trials, and the correlation of such variations between RS and FS neurons may simply reflect a similarity in their coding properties. To evaluate the relative impact of shared input in generating the differences in discharge correlation between different types of neurons, we next examined the correlations determined by sensory and motor variations that may occur in each trial. Although the cue in our paradigm was identical in each trial, small variations in the animal’s behavior cannot be excluded. For example, eye movements around the fixation point may displace the position of a stimulus in or out of the receptive field; this could result in common deviations of firing rate around the mean. This sort of correlated discharge would reflect similar receptive field positions for two neurons but not necessarily shared inputs between them (Gur and Snodderly 2001; Gur et al. 1997; Leopold and Logothetis 1998).

We first analyzed the eye position records of each trial and identified micro-saccades of $\geq 0.4^\circ$ during the delay period when no stimulus was present at the screen. The animals made 1.15 such eye movements per second (1.2 and 0.9 for the 2 animals, respectively), with a median amplitude of 0.55° (0.57 and 0.49°, respectively). We reasoned that if eye movements toward or away from the overlapping receptive fields of two neurons are solely responsible for the co-variations of firing rate we observed, correlations should be restricted or at least be substantial higher around these saccadic events. This seemed unlikely from the outset, given that all the neurons analyzed in this study were selected on the basis of their responsiveness to $14^\circ$ eccentricity stimuli and typically displayed broad tuning for two or more targets. Nevertheless, we estimated discharge rates in a 250-ms period centered around each micro-saccade, the time period most likely to include either pre- or postsaccadic modulations, and computed the correlation coefficient...
between the normalized discharge rates of each pair of neurons. The average value of noise correlation during microsaccades was $r = 0.047$, which was not significantly different from the $r = 0.048$ value we recorded in 250 windows sampling the entire delay period (paired t-test, $P > 0.5$). No significant difference ($P > 0.5$) was observed when we used shorter windows of 100 or 200 ms.

Correlated discharges may still be related to common variations of firing rate relating to internal factors, such as the locus of the animal’s attention or degree of arousal and motivation, in otherwise identical trials. We therefore wished to discount an artificial inflation of the value of correlated noise due to common modulation of firing rate related to possible variability in attention location. Although we had no way of monitoring the locus of the animal’s attention over the extended time interval of a behavioral trial, we were able to do so by measuring correlated noise during the interval over which attention was least likely to vary from trial to trial: the time period following the onset of the cue. The size of the cue itself was fairly small (1°) making variations of the locus of attention across its surface negligible across trials. Abrupt onset of stimuli appearing at random locations is thought to capture attention automatically (Egeth and Yantis 1997; Theeuwes 1994), even when identifying the location of the cue is not critical for correct performance of the task, as in fact it was in our case. The value of correlated noise in a 200-ms window offset from the onset of the cue by 100 ms, to adjust for neuronal latency, was on average $r = 0.042$. That value was significantly higher than zero (1-sample t-test, $P < 10^{-5}$) but not significantly different from the average value computed across 200-ms windows spanning the entire length of the trial, $r = 0.046$ (t-test, $P > 0.3$). No significant difference was observed when we repeated this analysis using 100-ms windows, offset from the cue appearance by 100 or 200 ms. These results indicate that possible variations in attention location from trial to trial could only account for a minor degree of the noise correlation we observed, if at all.

Finally, we sought to evaluate the effect of motivation or variation in motor performance based on the animal’s response characteristics in each trial. We examined the effect of reaction time (RT), saccade duration (DUR), and accuracy (ACC) by using them as independent variables in a linear regression model. We also included in the model the serial presentation of each trial (SER) to account for the possible effect of satiation in consecutive trials that might reduce neuronal excitability. The dependent variable of the model was the product of firing rates of the two neurons in each trial, which were normalized by subtracting the mean and dividing by the SD of each ($z_i z_j$).

This product of standardized rates is directly related to the correlation coefficient, which is typically defined as

$$r = \frac{\Sigma (x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma (x - \bar{x})^2 \Sigma (y - \bar{y})^2}}$$

This definition is equivalent to

$$r = \frac{\Sigma z_i z_j}{n}$$

Our linear model was therefore defined as

$$z_i z_j = a_0 + a_1RT + a_2DUR + a_3ACC + a_4SER$$

We performed a regression analysis independently for each task epoch and pair of neurons and tested the null hypothesis that all coefficients were zero, and therefore no variable relating to performance or serial presentation had a significant effect on discharge correlation between two neurons. Among all pairs recorded at 200–300 μm apart, the null hypothesis was rejected at the 0.05 significance level for 14 (5%) neuron pairs, during the cue period. The percentage of pairs that rejected the null hypothesis was slightly higher during the postsaccadic period, when 21 pairs (7%) exceeded statistical significance. These results suggest that although variables relating to behavioral performance had some influence on the degree of firing rate covariation in our task, this accounted for a relative minor component of correlated discharge in a small percentage of neurons.

**DISCUSSION**

Our results demonstrate that fast spiking, putative inhibitory cells exhibit substantially correlated discharges and indeed more so than regular spiking, putative excitatory neurons. Both short time-scale (<5 ms) synchronization of action potentials as well as trial-by-trial covariations of averaged firing rate were found to be significantly higher among putative interneurons. It is important to point out that the neuronal type of cells recorded extracellularly cannot be determined with certainty and that our classification of units into just two categories of RS and FS neurons is an unavoidable simplification. Recent anatomical and in vitro physiological studies have revealed a diversity of neuronal types. While our FS group most likely consists of inhibitory interneurons, it is possible that our RS group contains nonpyramidal neurons, either excitatory (e.g., spiny stellate, such as those with low firing rate and high incidence of bursting) or inhibitory (Cauli et al. 1997; Connors and Gutnick 1990; Kawaguchi 1995; Kawaguchi and Kubota 1997). Several classes of inhibitory interneurons have been recently identified that could be potentially misclassified as RS; however, these comprise a small group compared with excitatory neurons and their physiological properties are still distinct from pyramidal cells (Krimer and Goldman-Rakic 2001). Furthermore, all of our main conclusions in this study were found to be true when we classified neurons based on either a narrow classification criterion (unlikely to misclassify neurons, but excluding 30% of neurons from our sample) or a broader classification criterion (most likely misclassifying some neurons but including our entire sample).

Our present results confirmed earlier studies demonstrating that both FS and RS units possess spatially tuned memory fields (Rao et al. 1999; Wilson et al. 1994). Similar proportions of FS and RS neurons were spatially tuned in our behavioral task and similar power functions described the variability of their responses. We additionally demonstrated that FS units were more broadly tuned than RS ones. This systematic difference is consistent with the idea that a range of inhibition broader than excitation serves to shape the spatial extent of memory fields in prefrontal cortex (Compte et al. 2000; Tanaka 1999). Our results suggest that dorsolateral prefrontal neurons receive a net inhibitory input at spatial locations diametric to the peak of the memory field during the delay period, as evidenced by discharge rates below baseline fixation, confirming observations from earlier experiments (Funahashi et al. J Neurophysiol • VOL 88 • DECEMBER 2002 • www.jn.org
The role of inhibition in shaping the memory fields of PFC neurons has been directly demonstrated by experiments using iontophoretic application of GABA antagonists that cause expansion of PFC memory fields (Rao et al. 2000).

**Short scale synchronization**

Functional classes of neurons have been previously defined based on their firing rate properties (Taira and Georgopoulos 1993), and differences in terms of their correlated discharges have been identified; however, these differences involved the relationship between signal and noise correlation rather than the actual value of noise correlation that we report here (Lee et al. 1998). The higher discharge synchronization between FS units that we report in this study is consistent with intracellular recordings suggesting that nearby interneurons form a tight network of electrotonic synapses and synchronize their firing with millisecond precision (Beierlein et al. 2000; Gibson et al. 1999). Our results are also in agreement with recent extra-cellular recordings from the rodent hippocampus and entorhinal cortex, similarly showing elevated discharge correlation among FS units (Frank et al. 2001). Our data indicate that firing among RS units recorded at distances 200–300 µm apart is only marginally correlated. That may appear unexpected, as the dendritic fields of pyramidal neurons integrate inputs over hundreds of micrometers, and PFC neurons are known to possess dendritic structures at least as large as in any other cortical area, which would be expected to overlap if the somata of two neurons were located more than 300 µm apart (Elston et al. 2001; Lund et al. 1993). PFC neurons indeed receive horizontal connections from clusters of cells arranged in stripe-like structures, 200–800 µm wide (Goldman-Rakic 1984; Kritzer and Goldman-Rakic 1995; Levitt et al. 1993; Lund and Lewis 1993; Pucak et al. 1996). The physiological correspondence of such stripes is not known, but neurons in interconnected stripes can be presumed to share functional properties (Goldman-Rakic 1995). However, recent anatomical studies indicate that cortical neurons at even closer distances receive quite distinct sets of inputs (Sawatari and Callaway 2000). Even in the case of FS-FS pairs recorded 200–300 µm apart, which exhibited the highest degree of correlated firing, direct common input producing tightly synchronized firing represented on average only 2.0% of their total number of spikes (Fig. 5). Taken together, these findings suggest that discharges driving neurons at distances approximating the dimensions of a cortical column are largely independent.

**Trial-to-trial covariations**

Our study verified that discharges of cortical neurons are correlated on a trial-by-trial basis. We detected an overall positive correlation, which was to a large degree independent of sensory and motor parameters and variables relating to the animal’s performance and motivation, providing an instance of residual noise correlation in the cortex that cannot be accounted by behavioral factors. Correlated noise between neurons recorded from the same electrode was in the same broad range as in other cortical areas of the monkey (Gawne and Richmond 1993; Lee et al. 1998; Maynard et al. 1999; Zohary et al. 1994) as well as the frontal cortex of the rat (Jung et al. 2000). It is difficult however to directly compare these values as sensory and motor variations in each trial may have a higher impact in other cortical areas. Trial-by-trial variation of motor factors may in fact account for the relatively higher levels of correlated noise recorded in the motor cortex (Maynard et al. 1999).

Noise correlation in our experiment decayed as a function of lateral distance, did not vary significantly among task epochs, and increased as a function of the integration window over which it was computed. Similar effects of integration time have been reported in various other areas, especially when the neuronal responses analyzed are not stationary (Jung et al. 2000; Oram et al. 2001; Reich et al. 2001). Maximal activation of two neurons, for example, at the onset and offset of the delay period respectively, would result in increased correlated firing at time lags approximating the duration of the task period. This would also produce broad cross-correlation peaks, and we indeed observed such peaks in our population. It is notable that the study reporting the lowest noise correlation values among cortical neurons \( r = 0.02 \) was based on a relatively short (<200 ms) interval (Ericksen et al. 2000). These latter experiments may have also relied disproportionately on pyramidal neurons as analysis was restricted to a subset of units with the largest-amplitude action potentials. In any case, the correlation values we report here, computed over the entire task epoch, tend to overestimate correlated noise over shorter intervals during which a subject is able to pool neural activity to assess sensory information, often in the range of 50 ms (Oram and Perrett 1992; Werner and Mountcastle 1965).

Theoretical studies have postulated that correlated noise can limit the ability of a neuron to improve the reliability of a signal by averaging a large number of afferent inputs. The signal-to-noise ratio of a pooled input has been shown to reach an asymptotic limit for pool sizes of 50–100 neurons, two orders of magnitude lower than the number of synaptic inputs integrated by pyramidal neurons (Shadlen et al. 1996; Zohary et al. 1994). This has important implications on population coding schemes as the behavior of the cortical network may be radically constrained by correlated discharges, and this limitation may, in fact, explain why the performance of an animal in a behavioral task is no better than what could in principle be achieved by the output of a single neuron (Shadlen and Newsome 1998; Shadlen et al. 1996). The postulated limitation to signal reliability by correlated noise, however, depends on a large extent on the particular pooling mechanism employed by cortical neurons. Noise correlation becomes critical if a neuron averages correlated inputs as would be the case for excitatory inputs sharing the same preference for stimulus properties (Shadlen et al. 1996; Zohary et al. 1994). However, correlated noise could be reduced or canceled if a cell pooled inputs from neurons with opposite stimulus preference (Johnson 1980) or with appropriate combination of excitatory and inhibitory inputs. It has been formally demonstrated that the information carried by populations of neurons of increasing size does not necessarily reach a fixed limit, provided that the cortex implements an appropriate extraction mechanism (Abbott and Dayan 1999).

The present results offer experimental data necessary for the refinement of neuronal population models. The findings suggest that correlation among excitatory cortical neurons, whose outputs are transmitted from one cortical area to another, are significantly lower than among interneurons embedded in local
circuits, which only have local effects. This property does not appear unique to the primate prefrontal cortex but is most likely shared across diverse areas and species (Frank et al., 2001). It therefore has important implications for the construction of biological plausible network models of cortical architecture as it suggests that tightly correlated firing may be a property confined within local circuits and only to a lesser degree propagated by pyramidal neurons to distant cortical areas and modules.

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