Isodirectional Tuning of Adjacent Interneurons and Pyramidal Cells During Working Memory: Evidence for Microcolumnar Organization in PFC

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Rao, Srinivas, G., Graham V. Williams, and Patricia S. Goldman-Rakic. Isodirectional tuning of adjacent interneurons and pyramidal cells during working memory: evidence for microcolumnar organization in PFC. J. Neurophysiol. 81: 1903–1916, 1999. Studies on the cellular mechanisms of working memory demonstrated that neurons in dorsolateral prefrontal cortex (dPFC) exhibit directionally tuned activity during an oculomotor delayed response. To determine the particular contributions of pyramidal cells and interneurons to spatial tuning in dPFC, we examined both individually and in pairs the tuning properties of regular-spiking (RS) and fast-spiking (FS) units that represent putative pyramidal cells and interneurons, respectively. Our main finding is that FS units possess spatially tuned sensory, motor, and delay activity (i.e., “memory fields”) similar to those found in RS units. Furthermore, when recorded simultaneously at the same site, the majority of neighboring neurons, whether FS or RS, displayed isodirectional tuning, i.e., they shared very similar tuning angles for the sensory and delay phases of the task. As the trial entered the response phase of the task, many FS units shifted their direction of tuning and became cross-directional to adjacent RS units by the end of the trial. These results establish that a large part of inhibition in prefrontal cortex is spatially oriented rather than being untuned and simply regulating the threshold response of pyramidal cell output. Moreover, the isodirectional tuning between adjacent neurons supports a functional microcolumnar organization in dPFC for spatial memory fields similar to that found in other areas of cortex for sensory receptive fields.

INTRODUCTION

Investigations of spatially tuned neuronal activity promise to elucidate the respective roles of afferent organization and local cortical circuitry in the processes underlying regional cortical function. Directionally selective activity has been observed in many areas of the brain, from the visual (Hubel and Wiesel 1962) to the prefrontal cortices (Funahashi et al. 1989). Orientation selectivity for bars or edges of neurons in the primary visual cortex (V1) was originally hypothesized to be the result of structured excitatory thalamocortical input to this area (Hubel and Wiesel 1962). However, subsequent work suggested that GABA-mediated inhibition is also involved in the generation of tuning, although the determination of its precise role was elusive (Crook et al. 1997; Ferster et al. 1996; Pfleger and Bonds 1995; Sillito 1984; Somers et al. 1995). One mechanism that was proposed is that of isoorientation inhibition (Bonds 1989; Ferster 1986; Martin 1988). This is conceptually similar to lateral inhibition and refers to the suppression of the tuned response of a pyramidal neuron by inhibitory interneurons possessing orientation tuning similar but not identical to that of the pyramidal cell, a process that increases the latter’s spatial selectivity (Ringach et al. 1997). Another mechanism, cross-orientation inhibition, refers to the suppression of a pyramidal neuron’s tuned output by inhibitory interneurons possessing directionally opposite tuning (Sillito 1984), thereby increasing the pyramidal neuron’s signal-to-noise ratio (Wörgötter and Koch 1991). Both mechanisms may operate collectively in the construction of spatial tuning by local circuits in cerebral cortex (Martin 1988; Wörgötter and Koch 1991).

Spatial tuning was also observed in the dorsolateral prefrontal cortex (dPFC), where it takes the form of the directionally selective neuronal responses that are observed during tasks that test spatial working memory. The latter refers to that domain of memory that transiently holds information “on-line” about the location of a stimulus relative to retinotopic coordinates until an appropriate behavioral response is made (Goldman-Rakic 1995). In the oculomotor delay-response (ODR) task, spatially tuned neuronal activity in dPFC occurs during the sensory (cue), mnemonic (delay), and motor (response) phases with a bias for the contralateral visual field (Funahashi et al. 1989–1991). Spatially selective delay-period activity, referred to as the “memory field” of the cell, is thought to represent the neural substrate for spatial working memory (Funahashi et al. 1989; Goldman-Rakic 1995).

The existence of spatially tuned activity in the prefrontal cortex during the ODR task raises the question of whether inhibitory processes play a role in shaping this tuning. If this is the case, do iso- or cross-directional inhibitory mechanisms (analogous to iso- and cross-orientation inhibition in V1) play a role in this process? Further, given the temporal nature of prefrontal processing, do the roles of these inhibitory mechanisms vary with respect to the different phases of the ODR task? To address these questions, one must be able to discriminate the response properties of inhibitory interneurons from pyramidal cells in vivo. Mountcastle et al. (1969) demonstrated the existence of “regular” and “thin” spikes in extracellular recording studies of primate cortical neurons and suggested that these different waveforms corresponded to pyramidal and “stellate” neuronal morphologies, respectively. Subsequent intracellular work in vitro confirmed the existence of two classes of action potential waveforms, “regular spiking” (RS) and “fast spiking” (FS), that differ in their widths and that correspond to neurons with pyramidal and sparsely spiny stellate morpholo-
gies, respectively (McCormick et al. 1985). FS neurons have been shown to represent the parvalbumin positive group of GABAergic interneurons. This class of neuron consists primarily of basket and chandelier cells (Kawaguchi 1995), which have been shown to provide a major source of inhibitory input to pyramidal neurons (Gabbott and Bacon 1996; Jones 1993; Martin et al. 1983; Williams et al. 1992). A number of in vivo studies utilized the physiological distinction between FS and RS units recorded extracellularly to discern the functional properties of putative interneurons and pyramidal cells (Brumberg et al. 1996; Kyriazi et al. 1996; Simons 1978; Swadlow 1995; Swadlow et al. 1998). In the earlier studies by Funahashi et al. (1989–1991), the neuronal population was not segregated into FS and RS units. However, a more recent study in this laboratory demonstrated inverted patterns of activity between nearby putative pyramidal cells and interneurons while monkeys engaged in a variety of sensory-guided tasks (Wilson et al. 1994).

The goal of this study was to explore the contributions that interneurons and pyramidal cells make to spatial mnemonic processing in dPFC. To attain this goal, the tuning characteristics of individual neurons within both the FS and RS populations were characterized during the cue, delay, and response phases of the task. Further, the responses of neurons recorded simultaneously at the same site (and hence closely adjacent) were compared to assess the tuning relationships between FS–RS and RS–RS pairs in mnemonic processing.

METHODS

Experimental methods

Extracellular recordings were made in the left dPFC of two rhesus monkeys (Macaca mulata) in accordance with the Yale University Animal Care and Use Committee. These animals had undergone previous surgery under barbiturate anesthesia for placement of both a chronic recording cylinder for acute daily recordings and a scleral eye coil for detecting eye position. The recording cylinders (19- and 25-mm diam) were positioned over the caudal principal sulcus of the dPFC by using both X-ray imaging and stereotaxic coordinates (17 mm lateral and 29 mm anterior to ear-bar zero). A schematic of the location of the recording cylinders is provided in Fig. 1A (based on the X-ray photographs and stereotaxic coordinates that were then matched with the magnetic resonance images of another adult monkey). All recordings were within 5 mm of the center of the cylinder. Further anatomic data are not available at this time as the two monkeys are currently involved in ongoing iontophoretic studies. The cells described subsequently are characteristic of dPFC neurons in that their functional properties identify them as being engaged in spatial processing, including mnemonic function.
The animals were trained in the spatial ODR task shown in Fig. 1B. The monkey commences each trial by fixating on the central light-emitting diode (LED) for 0.5 s, whereupon a cue LED is illuminated at one of eight locations with 13° eccentricity and 45° separation for a period of 0.5 s. After the cue LED extinguishes, a 2.5- or 3.0-s delay period begins. During both the cue presentation and the delay period, the monkey is required to maintain central fixation. At the end of the delay, the central LED extinguishes, and the monkey is required to make a saccade to the location of the cue shown previously. If the monkey’s response is completed within 0.5 s of the offset of the fixation LED and is within 2° of correct location, the trial is considered successful, and the monkey is rewarded with fruit juice. The position of the cue stimulus is randomly selected for each trial of the task such that it must be remembered on a trial-by-trial basis. The monkey’s eye position was monitored with an eye-coil system (CNC-Engineering, Seattle, WA), and the ODR task was generated by a TEMPO system (Reflective Computing, St. Louis, MO).

Neuronal activity was recorded with a carbon-fiber microelectrode, produced by placing a 20-µm (ELSI, San Diego CA) or 33-µm (AVCO, Lowell MA) diam carbon fiber in the central barrel of seven-barrel capillary glass (WPI, Sarasota, FL). This assembly was then pulled into a microelectrode 60–62 mm in length on a custom-built, computer-controlled microelectrode puller. The tip of the electrode was further fashioned to a length of 20–50 µm by a combination of spark etching and beveling on a diamond wheel (Stoelting, Wood Dale, IL). The low impedance (100–500 kΩ) of carbon-fiber electrodes gives them distinct advantages over those made with tungsten, including exceptionally low-noise (<5 µV pk-pk), improved ability to record multiple units, and increased sensitivity to smaller neurons (Armstrong-James and Millar 1979; Fox et al. 1980). Recordings were made through a custom low-noise amplifier and were band-pass filtered between −150 Hz and 12 kHz (4-pole Tchebychev Krohn-Hite). The six barrels of the microelectrode were normally filled with up to three different receptor-specific pharmacological agents that varied on a monthly basis. However, all data presented in this paper were acquired only within the drug-free, control condition.

The dura was punctured with a 25-gauge guide tube, and the electrode was advanced into the brain with a Narishige (Japan) MD-2 digital microdrive. At each recording depth, we generally tested 8–12 trials of the task at each of the eight cue locations. Neuronal data were acquired by a micro1401/Spike2 system (Cambridge Electronic Design, Cambridge, UK), which can sort up to eight units at a single site (we successfully isolated as many as 5) based on a waveform-matching algorithm. To ensure that units were well isolated, interspike interval (ISI) histograms were generated for all units that were recorded simultaneously with at least three other units. Given that cortical neurons can rarely fire at instantaneous rates of >500 Hz (primarily because of their absolute refractory period), ISI histograms should not show a significant frequency of counts at ≤2 ms (Armstrong-James and Fox, 1983). Thus a significant percentage of counts with such short ISIs is indicative of false-positive errors, i.e., inclusion of spikes arising from another cell close by. A total of 66 units were thus analyzed, and we found that the average percentage of spikes that occurred within 2 ms of the previous spikes was only 0.3% (maximum for any unit was 2.2%). The average percentage of spikes that occurred within 2.5 ms of a previous spike was only 0.8% (maximum was 4.0%). Thus even with four or five units being recorded simultaneously all units were well isolated.

In extracellular recordings, the voltage deflections produced by an action potential approximate to the first-order derivative of the membrane potential (McCormick et al. 1985; Mountcastle 1980), a fact that was experimentally verified in a study involving simultaneous intra- and extracellular recording (Matthews and Lee 1991). Therefore to estimate the intracellularly measured parameter of spike-base width we measured the elapsed time from the inflection point marking the beginning of the initial negativity to the return to baseline after the first positive going deflection (Fig. 2A). Units were categorized as being FS based on their short spike-base width (<0.90 ms), relatively low amplitude, relatively high firing rates, and characteristic sound on an audio monitor, (McCormick et al. 1985; Mountcastle et al. 1969; Wilson et al. 1994). Finally, although some RS neurons could be tracked for several hundred microns, FS cells were rarely tracked for more than 20 µm, presumably reflecting the large principal apical dendrites of the former and the smaller soma size and dendritic field of the latter.

The values that we obtained for the spike-base widths for FS neurons compared quite well with those measured intracellularly by McCormick et al. (1985) (0.66 ms ± 0.19 SD vs. 0.67 ms ± 0.16, see results). However, the value for the spike-base width that we obtained for RS neurons was considerably shorter than that noted in the previous study (1.11 ms ± 0.29 vs. 1.74 ms ± 0.41), which may be due to several reasons. First, our definition of spike-base width represents an approximation to the actual spike-base width as defined intracellular recordings of membrane potential. Second, in many cases, it was difficult to define the return to baseline of the positive deflection for RS neurons because the slope gradually approaches zero and blends into the baseline noise. A third issue may be signal filtration, as we used a four-pole Tchebychev high-pass filter set at ~150 Hz to reduce fine noise. This attenuates the low frequencies of a waveform that are important for defining spike width, an effect that would tend to be less important for FS neurons because of the inherently higher frequencies contributing to these waveforms. A final issue may relate to differences between in vivo versus in vitro physiology relating to PO₂, temperature, etc.

**Analysis**

For purposes of data analysis, each trial in the ODR was divided into four epochs (Cue, Delay, Pre, and Post), and the average spike rate across each epoch in a trial was used in subsequent analysis (see Fig. 1B). The Cue epoch lasts for 0.5 s and corresponds to the stimulus presentation phase of the task. Delay lasts for 2.5 or 3.0 s and reflects the mnemonic component of the task. The presaccadic response epoch Pre starts immediately after the Delay epoch and lasts 0.25 s. As the name implies, the bulk of the neuronal activity during the Pre epoch is associated with saccade initiation (Funahashi et al. 1989). Post (the postsaccadic epoch) starts 0.5 s after the end of Delay and lasts for 0.5 s. By definition, a successful saccade is completed by the time Post begins. The animal receives its reward 0.50–0.55 s after Delay at the onset of the Post epoch. A given unit can show spatial tuning in any number of epochs (Funahashi et al. 1989, 1990, 1991), and in this study all combinations were noted in both the FS and RS populations.

The directionality of a unit’s response in each of the four epochs was assessed statistically with a vector-algorithm technique that provides a value for the overall strength and direction of tuning. A brief description follows. As stated earlier, 8–12 trials were recorded at each cue location for each neuron. From these data, the individual trials were arranged into "loops," where the ith loop was defined as consisting of the ith trial at each cue location. A loop-vector was then generated for each of the 8–12 loops thus created. The magnitudes of these vectors ranged from 0 to 1 and were defined mathematically with a measure of circular dispersion (Fisher 1993) to represent the strength of directionality of the unit’s response within a single loop. A value of 0 represents a response with no inherent directionality, whereas a value of 1 represents a highly directional response (i.e., firing at only one cue location). The direction of a given loop-vector indicated the overall preferred firing direction of the unit within that loop. All the loop-vectors for the cell (within an epoch) were then added together, and the magnitude was normalized to range from 0 to 1, thus creating a mean resultant vector. Dot products were then computed between the individual loop vectors and the mean resultant vector. These dot products provide scalar values that reflect the contribution each individual loop vector made to the resultant. In a highly tuned cell all dot products will have a value close to one.
whereas in a poorly tuned cell the dot products will have values that are much closer to zero. Hence to assess if a unit is tuned we statistically compared the dot product values with a threshold. This comparison was effected nonparametrically with the Wilcoxon signed-rank test (Conover 1971). The values of the dot products were compared with 10 increasing thresholds ranging from 0 to 0.45, with increments of 0.05. We then defined a tuning factor (TF) that corresponds to the threshold and that ranges from 0 to 10. A value of 0 indicates that a unit is untuned, e.g., the dot-product values were not statistically significantly greater than 0. A value of 10, on the other hand, corresponds to a unit whose dot-product values were significantly greater than a threshold of 0.45. In this way, TF provides a direct indication of the strength of directionality of a cell during each epoch, with increasing values of TF corresponding to better tuning.

This vector algorithm was implemented as a C++ program, which takes as input intermediate files created by a Spike2 script and creates an output file, which is then imported directly into a database created in Filemaker from Claris (Santa Clara, CA). Other analyses and graphs were generated with Statview (Abacus Concepts, Berkeley, CA) and Deltagraph (DeltaPoint, Monterey, CA), respectively.

We first examined the visual field biases (proportions tuned in the 2 halves of the visual field) and TFs of the FS and RS neuronal populations during the four task epochs. The next step in the analysis involved looking for within-cell differences in directional tuning between epochs for individual FS and RS neurons. This analysis was performed by first finding neurons that showed statistically significant tuning in at least two epochs, e.g., Cue and Post. The tuning angle of one epoch is then subtracted from the other, and the resulting value is normalized between 0 and 180°, thus making irrelevant the order of subtraction. This manipulation was carried out in a pairwise manner for all epochs: Cue, Delay, Pre, and Post. Depending on the number of epochs in which a unit was tuned, as many as six groups of difference data were generated for each unit: Cue versus Delay, Cue versus Pre, Cue versus Post, Delay versus Pre, Delay versus Post, and Pre versus Post. This procedure effectively renders the data linear and thus allows for the subsequent use of statistical analysis techniques that are otherwise inappropriate for circular data (Fisher 1993). The distributions of these within-cell tuning differences were visualized with histograms with three discrete intervals corresponding to the angle ranges 0° . . . <60°, 60° . . . <120°, 120° . . . 180°.

The final step in the analysis was to look for across-pair differences in directional tuning for each epoch, examining both FS–RS and RS–RS pairs. (Pairs of FS neurons are a relatively uncommon occurrence, a fact that prevented us from doing any statistical analysis on them.) Pair analysis was performed in a manner analogous to that used for determining between-epoch differences in tuning described previously. A pair of simultaneously recorded neurons showing tuning in the same epoch (e.g., Cue) is first found, and then the tuning angle of one unit is subtracted from the other. This value is then normalized to the range of 0–180°. Again because of normalization the order of
subtraction is not important. This technique was used to compare on an epoch-by-epoch basis the responses of multiple neurons, taken pairwise, recorded simultaneously at the same site. Once again, to visualize the differences in tuning across neurons, histograms were generated with angle ranges 0°...<60°, 60°...<120°, 120°...180°. We defined the 0°...<60° range as reflecting isodirectional activation or inhibition, depending on whether we were referring to RS–RS or FS–RS pairs, respectively. Likewise, the 120°...180° range was defined as reflecting cross-directional activation or inhibition. Finally, the 60°...<120° range represents a region of intermediate directionality.

To analyze the nature of any connectivity that was shared or occurred between units in a pair we examined the cross-correlation histograms (obtained from Spike2 analysis) of the two cells within the pair for signs of (1) feedforward excitation (a peak occurring with short latency after a spike in the other cell, reflecting the rise time of an excitatory postsynaptic potential) (Cope et al. 1987; Matsumara et al. 1996), (2) feedforward inhibition (a dip with a short latency, reflecting the duration of an inhibitory postsynaptic potential) (Aertsen and Gersten 1985; Aertsen et al. 1992), and (3) shared afferent input (a peak centered on the time of origin of a spike in the other cell) (Bryant et al. 1973; Moore et al. 1970). The cross-correlograms were obtained by analyzing the firing patterns of the two cells across an entire experiment without regard for task epochs or stimulus presentation. Hence commonly used techniques to assess temporal coherence (such as the shuffle predictor method) were not applicable (Nowak and Bullier 1998). Further, a detailed cross-correlation analysis was beyond the scope of this study and was limited by the spike detection process used (while a template match is in process for 1 spike waveform, another spike waveform cannot be detected on the same channel). See Nowak and Bullier (1998) for a complete review of this technique.

RESULTS

RS and FS populations

A total 144 FS and 382 RS neurons were recorded from, including 82 FS–RS pairs and 86 RS-RS pairs, in the two monkeys. The waveforms of representative RS and FS neurons are shown in the Fig. 2A. The spike-base widths for 19 FS and 17 RS neurons selected randomly are shown in Fig. 2B. As described in METHODS, the mean and SD values for spike-base width were 0.67 ms ± 0.16 and 1.11 ms ± 0.29 for FS and RS neurons, respectively. This difference in the mean values of the spike-base width was significant by unpaired t-test (P < 0.00001).

Seventy-one percent (270/382) of RS neurons and 69% (99/144) of FS neurons were tuned (i.e., TF > 0, see METHODS) in at least one epoch. Figure 3 shows the percentages of RS and FS neurons that were tuned in each epoch as well as the visual field bias (i.e., the relative proportions being tuned in the ipsilateral and contralateral visual fields) and TFmean of each population. The proportions of the RS and FS neurons tuned in each epoch were remarkably consistent across the two populations. Further, the visual field bias of the two populations did not differ significantly from each other within any one epoch [test of 2 proportions (TP) (Welkowitz et al. 1982): maximum z = 1.826 in Pre] nor were differences found in the magnitude of TF between FS and RS neurons by factorial analysis of variance (ANOVA), either taken overall (overall TFmean: FS = 1.85, RS = 2.03; P = 0.292) or on an epoch-by-epoch basis (minimum P = 0.1503 for Pre). Also for both RS and FS neuronal populations taken individually there were no statistically significant differences by factorial ANOVA in the values of TFmean between epochs (P = 0.260).

An example of an FS neuron showing spatially tuned activity throughout the ODR task is shown in the rastergrams and summed histograms of Fig. 4A. This neuron was consistently activated in the contralateral (right) visual field at 45° and 90° during the Cue, Delay, and Pre epochs. However, typical of the FS population shown in Fig. 3, the cell then underwent a complete inversion of tuning (by 167°), exhibiting robust post-saccadic activation at 225° and 270° in the ipsilateral visual field. Further, in all epochs one can appreciate the suppression of activity in directions opposite to those in which the unit showed its maximal response. Figure 4B shows the same data in mean and SE format, with the results of tuning analysis by the vector algorithm for each epoch superimposed. The results of the analysis confirm the observations noted above.

Analysis of FS–RS and RS–RS pairs

A major goal of this study was to determine the functional relationships between adjacent neurons with respect to the
**FIG. 4.** A: example of an FS neuron that showed spatially tuned responses in all task phases, presented in rastergram and histogram format. Activity in the Pre and Post epochs is indicated by the shaded and solid bars, respectively, in the overlying trial bar. Note the consistency in tuning direction present during the Cue, Delay, and Pre epochs as well as the inversion of tuning that occurs between the Pre and Post epochs. B: same unit shown in mean and SE in polar plots with superimposed tuning analysis results. The change in tuning angles between successive epochs is indicated by the value shown between them. Scale for the radial direction for spike rate is indicated on plot. Scale for the tuning analysis vectors is 10. The spontaneous activity for this cell was $3.4 \pm 0.7$ spikes/s.
development of spatial tuning in dPFC. Accordingly, we investigated the differences in spatial tuning between neurons recorded simultaneously at the same site. We found an overwhelming tendency for isodirectional relationships in tuning between members of such pairs, whether they were FS–RS or RS–RS. As shown in Fig. 5, during the Cue, Delay, and Pre epochs, >70% of units in FS–RS pairs and >60% of units in RS–RS pairs showed directions of tuning within 60° of one another, indicating a predominant, isodirectional relationship between these cells. Note that FS–FS pairs also showed isodirectionality in five cases but never exhibited a cross-directional relationship in a total of nine pairs where both units were tuned during the same epoch. To determine whether such isodirectional processing arose from feedforward connectivity between adjacent cells or from sharing spatially specific inputs arising from the same network, simple cross-correlations were performed on 56 pairs, focusing on those showing tuning during the Cue epoch (when presumably the cells might be sharing similar inputs). Remarkably, no cross-correlations indicative of feedforward inhibition were found within any pair of cells, whereas feedforward excitation was evident between two RS–RS pairs and one FS–RS pair (from RS to FS). The cross-correlations indicative of feedforward excitation in the RS–RS pair are shown in Fig. 6A, and those in an FS–RS pair are shown in Fig. 6B. It can be seen in both histograms that a sharp peak arises with a latency of <2 ms after the occurrence of a spike in the other member of the pair. Cross-correlations indicative of shared excitatory afferents were found in 15 pairs (1 FS–FS, 3 FS–RS, and 11 RS–RS). A good example of this effect in RS–RS pairs was found at one site where four units were recorded simultaneously and only one of which was untuned during the Cue period. As shown in Fig. 6C, positive cross-correlations showing coincidence of long-lasting rise in spiking were found among the three tuned neurons but did not include the untuned RS cell (RS1). Thus, at least in this simple cross-correlation analysis, a considerable number of cells recorded at the same site appears to share their afferent input, and in rare cases RS units provide feedforward excitation to their immediate neighbors, whether RS or FS.

The isodirectional relationship between units in FS–RS pairs was found to dissociate at the end of the task. Thus the differences in tuning angles between FS and RS neurons during the Post epoch distributed almost evenly between the 0–60° range (47%) and the 120–180° range (41%), reflecting both iso- and cross-directional relationships, respectively (Fig. 5). The differences in distribution between the iso- and cross-directional data for the Pre epoch data versus Post data were significant ($\chi^2$, $P < 0.05$). Further analysis of the units constituting the FS–RS pairs in Post revealed that the FS units in such pairs show predominantly ipsilateral visual field tuning regardless of whether they belonged to an iso- or cross-directional pair (Fig. 7). RS neurons in an isodirectional pair also show a predominantly ipsilateral visual field bias; however, RS units in a cross-directional pair tended to show a contralateral visual field bias. A comparison of the visual field preferences of the RS neurons in the iso- and cross-directional pairs was significant ($\chi^2$, $P = 0.0187$). These results indicate that a subset of RS neurons that remains contralaterally tuned in Post is responsible for the cross-directional relationship with their FS.
neighbors. An example of an FS–RS pair showing an isodirectional relationship in the Pre epoch (54°) and a cross-directional relationship in the Post epoch (143°) is shown in Fig. 8 in mean and SE format. The FS neuron in this pair shows a tuning shift of 94° between Pre and Post, whereas the tuning of the RS neuron only shifts by 5° between these two epochs. The FS neuron becomes ipsilateral in its tuning during Post, whereas the RS neuron remains contralaterally tuned. In contrast to the FS–RS pairs, RS–RS pairs maintained their isodirectional relationship in tuning throughout all epochs of the trial. Accordingly, no significant difference between the distributions of the Post epoch and the Pre epoch was found in these pairs ($\chi^2 P > 0.05$).
Tuning shifts across task epochs

A previous study by Funahashi et al. (1991) observed that the prefrontal neuron’s preferred direction often shifted between the sensory and responses epochs of the task. We examined this tendency in the FS and RS populations and found that both classes of neurons exhibited similar shifts in visual field preference as the task progressed. During stimulus presentation (Cue epoch), a significantly larger proportion (by a test of proportional significance) (Welkowitz et al. 1982) of both the RS (71%) and especially the FS (81%) populations was tuned toward the visual field contralateral to the hemisphere of our recording sites. This contralateral visual field bias continued throughout the mnemonic component of the task (Delay epoch) in both populations; however, the bias was not as pronounced as that observed in Cue (67% of FS units and 61% of RS units). The results of the within-cell analysis are shown in Table 1, and the distribution of Cue versus Delay (as well as Cue vs. Pre and Cue vs. Post) is plotted in histogram format for both FS and RS neurons in Fig. 9. It is apparent from these analyses that there was a high degree of similarity (i.e., remaining $\pm 0°$–$60°$) in the direction of tuning between the Cue and Delay epochs for both FS (68%) units and RS (79%) units.

Both of the response-associated epochs Pre and Post were different from the sensorimnemonic epochs in several respects. First, a higher percentage of FS and RS neurons exhibited tuning in Post compared with the preceding epochs (54% of RS units and 49% of FS units). Second, although RS neurons continued to display a contralateral visual field bias during Pre, FS neurons lost their bias and underwent a significant shift in their tuning angles from that observed in the delay period ($\chi^2 P = 0.0457$). Therefore this shift in tuning in the FS population occurred before the saccadic response at the end of the Pre epoch. Finally, by the Post epoch the RS units lost their visual field bias, a result that was consistent with a significant shift in visual field bias observed between Pre and Post ($\chi^2 P = 0.0457$).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>n</th>
<th>RS, $\Delta\theta$</th>
<th>n</th>
<th>FS, $\Delta\theta$</th>
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</thead>
<tbody>
<tr>
<td>Cue vs. Delay</td>
<td>53</td>
<td>41.6 ± 5.9°</td>
<td>19</td>
<td>41.7 ± 10.0°</td>
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<tr>
<td>Cue vs. Pre</td>
<td>41</td>
<td>56.7 ± 6.9°</td>
<td>15</td>
<td>77.5 ± 13.6°</td>
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<tr>
<td>Cue vs. Post</td>
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<td>18</td>
<td>111.2 ± 12.3°</td>
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<tr>
<td>Delay vs. Pre</td>
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<td>40.7 ± 6.1°</td>
<td>10</td>
<td>56.4 ± 15.8°</td>
</tr>
<tr>
<td>Delay vs. Post</td>
<td>49</td>
<td>100.4 ± 7.0°</td>
<td>18</td>
<td>118.4 ± 11.9°</td>
</tr>
<tr>
<td>Pre vs. Post</td>
<td>58</td>
<td>88.3 ± 7.5°</td>
<td>20</td>
<td>94.9 ± 13.9°</td>
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</tbody>
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Values are means ± SE.

FIG. 7. An examination of the visual field preferences of the FS–RS pairs showing iso- and cross-directional inhibition in the Post epoch previously in Fig. 6. No difference was found between the FS neurons in the two groups; however, the RS neurons that were in the isodirectional group were ipsilaterally tuned, whereas those in the cross-directional group were contralaterally tuned.

FIG. 8. An example of an FS–RS pair showing an isodirectional tuning relationship in the Pre epoch and a cross-directional tuning relationship in the Post epoch. The data are presented as mean and SE data in polar plots with superimposed tuning analysis results. The differences in tuning directions in the Pre and Post epochs between the FS and RS neuron is shown between the polar plots for the corresponding epochs. Scale for the radial direction for spike rate is indicated on plot. Scale for the tuning analysis vectors is 5. Spontaneous activity was $3.5 ± 0.4$ spikes/s for unit1 and $4.7 ± 0.8$ spikes/s for unit4.
0.0044) for these units. However, FS units now showed a significant tuning bias toward the ipsilateral visual field, despite the fact that in the Post epoch the eye position is uncontrolled as the trial is complete.

These shifts in visual field bias noted at the population level were confirmed by analysis of the within-cell differences in tuning between epochs shown in Table 1 and Fig. 9. For both FS and RS units, the disparity in tuning direction between the Cue and Post epochs was significantly greater than that between the Cue and Delay epochs (posthoc Scheffe: RS, $P < 0.0001$; FS, $P = 0.0095$). It is apparent from the posthoc results of the ANOVA and from Fig. 9 that a shift in visual field bias occurred within individual FS and RS neurons as the trial progressed. A significantly higher proportion of FS units (27%) showed inversion of tuning (i.e., a shift in angle by 120–180°) between Cue and Pre than did RS units (7%). By Post, however, a large proportion of both RS and FS units showed an inversion of their tuning compared with that in Cue (41 and 61%, respectively).

**DISCUSSION**

Inhibitory mechanisms in working memory

The role of inhibition in the construction of receptive field properties has been intensively studied in the visual system, particularly with regard to orientation selectivity. However, evidence ranges from inhibition having “no role in the creation of orientation selectivity” to inhibition “being the architect of orientation selectivity” (Ferster 1986; Sillito 1984). Consensus appears, not surprisingly, to be in between (Hata et al. 1988; Martin 1988). Several modeling studies confirmed the importance of inhibition in the creation of tuning. For example, Wörgötter and Koch (1991) hypothesized a combination of iso- and cross-orientation inhibition for the optimal construction of spatial tuning.

The prefrontal cortex has been shown to have structural organization not unlike that of the primary visual cortex, including a columnar organization of inputs such as those from posterior parietal cortex (Goldman-Rakic and Schwartz 1982) and the contralateral hemisphere (Goldman and Nauta 1977). A number of anatomic studies have described the types of interneurons present in primate prefrontal cortex as well as their connectivity to pyramidal and spiny-stellate interneurons (Gabbott and Bacon 1996a,b; Jones 1993; Thomson et al. 1996). In this study, the interneurons thought to be represented by FS units were clearly distinguished from RS units that are considered to represent pyramidal or spiny-stellate cells. The FS waveform has been shown in vitro to be highly characteristic of parvalbumin-positive basket and chandelier cells (Kawaguchi 1995). Although these neurons are thought to represent ~25% of the total interneuron pool, they can have a profound effect on pyramidal neuron output caused by the pattern of their connectivity with pyramidal neurons (Gabbott and Bacon 1996a,b; Martin et al. 1983; Thomson et al. 1996).

A major finding of this study is that putative basket and chandelier cells in the primate prefrontal cortex show tuned sensory, mnemonic, and response-related activity very similar to that of putative pyramidal cells. Moreover, these FS cells show a great propensity to be isodirectional in tuning with closely neighboring RS cells before the completion of a mnemonically guided saccadic response. This evidence suggests that at least one subset of interneurons plays an integral and dynamic role in the local circuits engaged by working memory in prefrontal cortex and is spatially specific in regulation of pyramidal cell firing. Therefore inhibitory processes may be an important component of the functional organization of the underlying circuitry that shapes spatially tuned activity in dPFC.

**FS and RS neuronal populations**

Both FS and RS neurons showed a contralateral visual field bias at the start of the trial during the Cue epoch, and both...
populations showed a progressive shift in tuning from the contralateral toward the ipsilateral visual field during the course of the trial. At both the beginning and end of the trial (the Cue and Post epochs, respectively), the FS population showed a greater degree of lateralization than did RS neurons. By examining the differences in the tuning among successive task epochs of individual neurons, we found that the visual field shift noted at the population levels appears to be a result of individual neurons progressively shifting their tuning preferences during the course of a trial. Such an effect would have important consequences for the population vector encoded by the output of the pyramidal cells. Within a given epoch of the ODR task, shifts in the direction of the population vector are primarily the result of different dPFC neurons being activated for the different stimulus locations, as is the case in the primate motor cortex (Georgopoulos et al. 1982, 1986). However, in contrast to the motor cortex (Georgopoulos et al. 1989), shifts in the direction of the population vector occurring as a trial progresses appears to be the result of individual neurons shifting direction.

Our finding of a contralateral field bias for RS neurons during Cue, Delay, and Pre is consistent with work of Funahashi et al. (1989–1991), which revealed a progressively smaller contralateral visual field bias at the population level as the trial progressed. This suggests that the findings of Funahashi et al. were primarily representative of the pyramidal cell population, a result that is not surprising considering that pyramidal cells constitute a large majority of prefrontal cortical neurons. Funahashi et al. also noted that postsaccadic activity often showed tuning in the opposite direction to that of preceding epochs. In particular, in cells with memory fields, postsaccadic activity often occurred in the opposite direction and coincided with a return of delay activity to baseline (Funahashi et al. 1989, 1991). This inversion of tuning was confirmed by this study for a subset of RS neurons. Moreover, we also found this inversion to be present for the majority of FS neurons. Note that eye position is not controlled in the postsaccadic epoch as the trial is complete. However, regardless of eye position, the previously predominantly isodirectional tuning relationship changes, indicating that FS and RS neurons respond differentially before and after the goal-directed cascade. The changes in tuning noted in the Post epoch indicate that the nature of postsaccadic activity varies within the RS population. One subset of RS neurons was found to invert their tuning in Post and become ipsilaterally tuned. This subset may be involved in the generation of eye movements opposite to that encoded in the presaccadic epoch, i.e., returning fixation to the center. Alternatively, they may have a function more intrinsic to the working memory process, e.g., serving to extinguish the encoding of the current target in memory. The other subset of RS cells did not show inversion and instead remained tuned contralaterally. This subset may serve to help maintain attention on a target that is associated with reward and thus may be part of a process that supports the working memory function (Funahashi et al. 1993). That pairs of RS cells do not show cross-directional relationships may be because they are functionally coupled in local pyramidal cell clusters without inhibitory interneurons (Kritzer and Goldman-Rakic 1995). These issues require further investigation.

The increased role of cross-directional inhibition seen in FS–RS pairs in Post point to a dynamic role of inhibitory mechanisms in the dPFC, shifting with real-time events occurring during cognition. Part of this shift takes place during the task, when interneurons show a significant rotation of their tuning angle between the delay and presaccadic epoch. This phenomenon may be the result of a new source of afferent input to dPFC, possibly related to the GO signal provided by the offset of the fixation spot. One source of such input that was previously suggested is the thalamus (Funahashi et al. 1991; Goldman-Rakic 1995). Alternatively, the cross-directional input may also arise from the contralateral hemisphere, as a great deal of evidence points to a hemispheric organization of prefrontal cortical function (Goldman and Nauta 1977; Hughes and Peters 1992; McGuire et al. 1991a,b; Sawaguchi and Goldman-Rakic 1994).

**Iso- and cross-directional mechanisms**

Our findings that neurons recorded at the same site tended to share very similar angles of tuning regardless of the direction of the cue stimulus may appear to contradict those of an earlier study in this laboratory (Wilson et al. 1994), which showed inverted patterns of stimulus-specific responses between FS and RS cells in the inferior convexity. However, an important methodological difference between these two studies arises from the distance between the units constituting the pairs being analyzed. The previous work considered as pairs any two neurons located ≤400 μm of one another, a distance that may in fact cross functional columnar boundaries within the prefrontal cortex (Goldman and Nauta 1977; Leichnetz 1980; Schwartz et al. 1988). Such a columnar effect was indicated by a combined single unit recording and optical imaging study in inferotemporal cortex (Wang et al. 1996), which demonstrated the regional clustering of cells responding to similar features in “spots” of ~500 μm in diameter. Thus from the results of the previous study of FS and RS units it was inferred that “distal” (i.e., hundreds of micrometers away) cross-directional inhibition between functionally opponent columns may exist in dPFC (Goldman-Rakic 1995). We considered only neurons simultaneously recorded at a single site and hence closely adjacent to one another to constitute pairs. Considering the size of our recording tips and the fact that previous anatomic studies suggested a size of 50–60 μm on center for microcolumns (Peters and Kara 1987), it is likely that we recorded from within one or between two adjacent microcolumns within the same functional column. Thus we submit that isodirectional tuning is an important phenomenon for closely adjacent units, and this has important implications for local lateral inhibitory mechanisms.

In the light of these hypotheses, we propose a model of inhibitory local circuits in dPFC in which the processes involved in spatial tuning are dependent on the distance between interneurons and their target cells, particularly pyramidal neurons. Specifically, we propose that isodirectional inhibition prevails within a functional column, whereas cross-directional inhibition is exerted primarily between functional columns in primate prefrontal cortex. As depicted in Fig. 10 (top left), a functional organization of closely spaced microcolumns provides an ideal framework for isodirectional relationships among neurons, whether they reside within or between the microcolumns inside a single functional column. The probable anatomic substrate for this circuitry is illustrated (Fig. 10, top...
in the form of regularly dispersed dendritic bundles arising from clusters of cells representing microcolumns in the dPFC of rhesus monkeys similar to those in rat visual cortex first described by Peters and Kara (1987). The isodirectionality observed between members of FS–RS and RS–RS pairs during much of the task can be interpreted as implying the existence of a local cortical unit of spatial processing, with RS and FS neurons becoming synchronously entrained as a result of connectivity (DeFelipe et al. 1990; Mountcastle 1997). Our cross-correlation analysis of paired units supports the interpretation that closely adjacent neurons within the same microcolumn may share a high proportion of their spatially tuned afferents (Matsumura et al. 1996; Shadlen and Newsome 1998; Swadlow et al. 1998) and thus form a strong consensus in tuning preferences (a form of neural averaging) (see Zohary et al. 1994). Indeed, no evidence was found of feedforward inhibition in cross-correlations between simultaneously recorded FS and RS units, indicating a lack of functional inhibition between closely adjacent cells. However, isodirectional inhibition could occur between microcolumns, consistent with the findings of Thomson and coworkers (1996, 1997), who reported considerable inhibition between FS interneurons and pyramidal cells at distances of ~50–250 μm. The differences in consensus of tuning between neighboring microcolumns may therefore provide the circuit basis for isodirectional inhibition, which serves to sharpen spatial tuning in a mechanism similar to that proposed for visual cortex (Crook et al. 1997; Kisvarday et al. 1994; Peres and Hochstein 1994; Ringach et al. 1997; Somers et al. 1995; Worgotter and Koch 1991).

In the model illustrated in Fig. 10 (middle) cross-directional inhibition may predominate between functional columns that contain microcolumns possessing considerably different directional preferences, a situation similar to ocular dominance and orientation columns in visual cortex described by Hubel and Wiesel (1977). The anatomic basis for the columnar organization of dPFC is clearly illustrated (Fig. 10, bottom) by the spatial distribution of afferents to the principal sulcus from the contralateral hemisphere. In keeping with this model, it has been shown that basket cells project to nearby isooriented sites at 100–400 μm as well as to more distant cross-oriented sites 400–800 μm away (Kisvarday et al. 1994). These columnar dimensions are in good accord with those reported for interdigitating columnar projections to the principal sulcus from the contralateral hemisphere. In this model, it has been shown that basket cells project to nearby isooriented sites at 100–400 μm as well as to more distant cross-oriented sites 400–800 μm away (Kisvarday et al. 1994). These columnar dimensions are in good accord with those reported for interdigitating columnar projections to the principal sulcus from the contralateral hemisphere. Taken as a whole, the evidence supports a microcolumnar organization of functionally grouped neurons in prefrontal cortex, which serve to enhance the resolution of stimulus encoding and/or retrieval in the working memory process.

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FIG. 10. A model of iso- and cross-directional inhibition in dPFC. Top left: hypothetical circuit demonstrating isodirectional tuning relationships between adjacent microcolumns with small differences (~5°) in their directional preferences and (bottom) cross-directional relationship between distant, functionally defined microcolumns with a wide difference (~120–180°) in their directional preference. The bulk of interactions is considered likely to take place in layer III, where a large proportion of horizontal connections and parvalbumin-positive interneurons can be found. Top right: section from dPFC showing 5-HT₁₅-like immunoreactivity in prefrontal cortex (courtesy of Robert Jakab) in which bundles of apical dendrites ascend from clusters of large pyramidal cells in layer V, previously described as the principal components microcolumns in visual cortex (Peters and Kara 1987). Bottom inset: autoradiographic visualization of the columnar organization of afferent input (corresponding to functional macrorcolumns) to the principal sulcus of the monkey (courtesy of Goldman-Rakic) after injection of tritiated leucine into the contralateral hemisphere.


