Neurons with inverted tuning during the delay periods of working memory tasks in the dorsal prefrontal and posterior parietal cortex

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Zhou X, Katsuki F, Qi XL, Constantinidis C. Neurons with inverted tuning during the delay periods of working memory tasks in the dorsal prefrontal and posterior parietal cortex. J Neurophysiol 108: 31–38, 2012. First published April 4, 2012; doi:10.1152/jn.01151.2011.—The dorsolateral prefrontal and posterior parietal cortices are two interconnected brain areas that are coactivated in tasks involving functions such as spatial attention and working memory. The response properties of neurons in the two areas are in many respects indistinguishable, yet only prefrontal neurons are able to resist interference by distracting stimuli when subjects are required to remember an initial stimulus. Several mechanisms have been proposed that could account for this functional difference, including the existence of specialized interneuron types, specific to the prefrontal cortex. Although such neurons with inverted tuning during the delay period of a working memory task have been described in the prefrontal cortex, no comparative data exist from other cortical areas that would establish a unique prefrontal role. To test this hypothesis, we analyzed a large database of recordings obtained in the dorsolateral prefrontal and posterior parietal cortex of the same monkeys as they performed working memory tasks. We found that in the prefrontal cortex, neurons with inverted tuning were more numerous and manifested unique properties. Our results give credence to the idea that a division of labor exists between separate neuron types in the prefrontal cortex and that this represents a functional specialization that is not present in its cortical afferents.

METHODS

Three male rhesus monkeys (Macaca mulatta), weighing 5–12 kg, were used in this study. All surgical and animal use procedures in this study were reviewed and approved by the Wake Forest University...
Institutional Animal Care and Use Committee following National Institutes of Health guidelines.

**Surgery and neurophysiology.** Experiments were performed as described previously in detail (Meyer et al. 2011; Qi et al. 2011). Briefly, 20 mm diameter recording cylinders were implanted over the prefrontal and the parietal cortex in each animal (Fig. 1). Penetrations analyzed here sampled areas 46 and 8a of the prefrontal cortex and areas 7a and lateral intraparietal cortex (LIP) of the posterior parietal cortex, determined based on structural MRI. Area LIP was determined anatomically, based on the depth of electrode penetration (>3 mm) from the surface of the cortex. Recordings were collected with either glass-coated Tungsten electrodes of 250 μm diameter and an impedance of 1 MΩ, measured at 1 kHz (Alpha Omega Engineering, Nazareth, Israel), or epoxylite-coated Tungsten electrodes with a diameter of 125 μm and an impedance of 4 MΩ at 1 kHz (FHC Bowdoin, ME). Epoxylite-coated electrodes were advanced into the brain by traversing the dura, glass coated through a dura-piercing guide tube. Electrical signals recorded from the brain were amplified, band-pass filtered between 50 Hz and 8 kHz, and stored via a modular data acquisition system at a temporal resolution of 25 μs (APM system, FHC).

**Behavioral tasks.** The monkeys performed tasks that required them to remember visual stimuli presented on a screen while maintaining fixation, monitored with an infrared eye-position tracking system (model RK-716; Iscan, Woburn, MA) and sampled at 240 Hz. One fixation, monitored with an infrared eye-position tracking system to remember visual stimuli presented on a screen while maintaining fixation (APM system, FHC).

**Fig. 1. Schematic diagram of the monkey brain.** The shaded areas indicate the recording sites in the dorsolateral prefrontal cortex (PFC) and posterior parietal cortex (PPC). AS, arcuate sulcus; IPS, intraparietal sulcus; LIP, lateral intraparietal area; PS, principal sulcus; STS, superior temporal sulcus.

To evaluate the spatial tuning of stimulus selectivity in the delay period, we rotated firing rates of each neuron for the eight peripheral presentations. A neuron’s spike width was determined by calculating the distance between the two troughs of the average waveform, as described previously (Qi et al. 2011). Based on that classification, units were classified as fast spiking (FS; putative interneurons) if their spike width was ≤550 μs and regular spiking (RS; putative pyramidal neurons) if they exhibited spike widths >550 μs.

Firing rate of units was determined by averaging spikes in each task epoch. We identified neurons that had a significantly increased delay-period activity by comparing the discharge rates with the baseline fixation interval. We refer to these neurons as excited in the delay period. We similarly identified neurons that exhibited significantly decreased activity in the delay period. For this analysis, we compared the last 0.5 s of the delay-period activity following presentation of the stimulus at each location with the baseline fixation activity recorded from the same trials, using a paired t-test. Neurons with a significant decrease ($P < 0.05$) in firing rate in the delay period for at least one spatial location and no significantly elevated responses in the delay period for any other location were identified. This comparison, in practice, required a minimum firing rate of two spikes/s in the fixation period, below which decreases could be detected. Additionally, we identified a subgroup of neurons inhibited in the delay period, which also exhibited spatial selectivity for the delay period following stimuli at the nine different spatial locations (ANOVA, $P < 0.05$). This latter subgroup constitutes the inverted tuning neurons. To estimate the experiment-wise error rate of inverted tuning neuron identification, we performed a bootstrap test. Trials were first randomized with respect to the location of the stimulus; then, the selection criteria were applied as above. The randomization procedure was repeated 10 times for each neuron in our sample (1,909 neurons in total, across all areas), yielding a total of 19,090 tests, over which the false-positive rate was estimated.

To evaluate the spatial tuning of stimulus selectivity in the delay period, we rotated firing rates of each neuron for the eight peripheral stimuli, so that its peak response rate would always be at the same location. We then averaged responses for each location, relative to the...
peak location across all neurons, separately by cortical area and excited or inverted tuning neuron type. All data were expressed in units of stimulus separation from each other (which was either 10° or 15° of visual angle). The averaged data were then fitted to a Gaussian curve of the form

\[ r(x) = B + A \cdot e^{-\frac{(x-\mu)^2}{2\sigma^2}} \]

Here, \( r \) represents average population firing rate at each location \( x \), \( B \) the baseline, \( A \) the amplitude, \( \mu \) the peak, and \( \sigma \) the SD of the Gaussian. We fitted Gaussians separately to the inverted tuning neurons from the prefrontal and parietal cortex and also to all neurons pooled from both areas. To obtain an estimate of fit, we calculated the absolute value of the residuals from the Gaussian on a neuron-by-neuron basis. We then compared the residuals from the single and pooled population models using a paired \( t \)-test.

RESULTS

**Database.** Our analysis involved two brain regions in the frontal and parietal lobe (Fig. 1), which are interconnected directly (Cavada and Goldman-Rakic 1989) and share very similar functional properties (Chafee and Goldman-Rakic 1998). A total of 1,227 neurons was analyzed in the posterior parietal cortex of three monkeys (monkey DA: 571, CA: 367, and EL: 289, respectively). Most of these neurons were collected in area 7a (\( n = 1,165 \)); a small sample was collected from area LIP (\( n = 62 \)). A total of 682 neurons was analyzed from the dorsolateral prefrontal cortex (DA: 230, CA: 2, and EL: 450, respectively). Most of these neurons were recorded in area 46 (\( n = 647 \)); a smaller sample was recorded posterior to the principal sulcus and anterior to the arcuate, corresponding to area 8a (\( n = 35 \)). Comparable quality of signals was obtained in the prefrontal and parietal cortex; there was no significant difference (\( t \)-test, \( P > 0.7 \)) in the signal-to-noise ratio of average spike waveforms collected from the two areas (mean and SE for prefrontal cortex: 7.62 ± 0.10; posterior parietal cortex: 7.67 ± 0.13). The monkeys were trained to perform spatial working memory tasks; one of the monkeys (EL) performed the match-nonmatch task (Fig. 2A), and two of the monkeys (DA and CA) performed a delayed match-to-sample task (Fig. 2B). A total of 558 posterior parietal neurons responded to the task, evidenced by a significantly elevated firing rate during any of the stimulus presentations and delay periods in the task. Similarly, 437 prefrontal neurons exhibited a significantly elevated firing rate in one or more task periods.

**Incidence of neurons with inverted tuning.** We identified neurons with inverted tuning during the delay period of the working memory tasks as those that exhibited three criteria, as defined previously (Wang et al. 2004): 1) average firing rates were significantly lower in the (first) delay period than the baseline fixation for at least one spatial stimulus, 2) no excited responses during the delay period following another stimulus, and 3) significant selectivity for the location of the preceding cue. Identical analysis methods were used for all areas and animals. We were thus able to compare the percentage of neurons with inverted tuning in the two areas. If inverted tuning is a characteristic of a special type of neurons (calbindin interneurons), as previously speculated (Wang et al. 2004), then a higher incidence of them would be expected in the prefrontal cortex.

We encountered neurons with inverted tuning during the delay period in both the posterior parietal and dorsolateral prefrontal cortex (including areas LIP and 8a). An example neuron recorded from the dorsolateral prefrontal cortex is shown in Fig. 3 and an example from the posterior parietal cortex in Fig. 4. Across our entire sample, a total of 24/682 (3.5%) neurons exhibited inverted tuning in the prefrontal cortex and 20/1,227 (1.6%) in the parietal cortex. The respective proportion of inverted tuning neurons was significantly higher in the prefrontal than the parietal cortex (\( \chi^2 \) test, \( P < 0.05 \)). No significant difference (\( \chi^2 \) test, \( P > 0.4 \)) was observed in the percentage of inverted tuning neurons obtained from sessions in which epoxylite-coated and glass-coated electrodes were used (2.6% and 2.1%, respectively; 8/126 in the prefrontal cortex and 16/813 in the parietal compared with 16/556 in the prefrontal and 4/414 in the parietal), and the majority of prefrontal recordings was obtained with glass-coated electrodes (which yielded the overall lower percentage), whereas most parietal recordings were obtained with epoxylite-coated electrodes. Since the total percentage of neurons with inverted tuning was fairly small, we sought to estimate the expected error rate of inverted tuning neuron incidence by performing a bootstrap test, applying the selection criteria to neuronal re-

![Image](http://www.jn.org)
responses after first randomizing trials with respect to stimulus location. The experiment-wise, false-positive rate was 0.6%. Essentially, identical, expected error rates were obtained for the sample of prefrontal (0.7%) and posterior parietal neurons (0.6%). The observed rate of inverted tuning neurons was significantly higher than the respective, expected error rates (χ² test, \( P < 10^{-3} \) for the prefrontal cortex; \( P < 0.01 \) for the posterior parietal cortex). Expressed as a percentage of the total number of neurons modulated by the task [sum of the neurons with excitatory responses and those with inverted tuning, as in Wang et al. (2004)], inverted tuning neurons represented 5.2% of the prefrontal neurons and 3.5% of the parietal ones. The use of a more stringent significance criterion of \( P = 0.005 \) for the decrease in firing rate between the delay and fixation period resulted in an even greater disparity between areas, yielding 4.5% of prefrontal neurons and 1.4% of parietal neurons with inverted tuning. For comparison, 23.5% of dorsolateral prefrontal and 28.6% of posterior parietal neurons exhibited tuned, excited responses during the delay period.

Properties of neurons with inverted tuning. We proceeded to examine whether the population of prefrontal neurons with inverted tuning displayed functionally unique properties vs. those identified in the parietal cortex. Neurons with inverted tuning, previously identified in the prefrontal cortex, were found to have average spike widths intermediate between neurons that were characterized as FS and RS (Wang et al. 2004). Spike width measured extracellularly is not a precise indicator of neuron type (Vigneswaran et al. 2011); however, it is notable that when we examined the spike widths of neurons identified as manifesting inverted tuning (Fig. 5), we observed a significant difference in average spike width between the prefrontal (692 μs) and parietal (820 μs) populations (Wilcoxon signed-rank test, \( P < 0.001 \)). No such difference was present for the neurons with excited delay-period activity.

Fig. 4. Rasters and peristimulus time histograms of a single inverted tuning neuron in the PPC. Conventions are the same as in Fig. 3.

Fig. 5. Distribution of spike widths of neurons with excited delay-period activity and inverted tuning in the dorsal PFC and PPC. Shaded area in excited neurons indicates the spike widths corresponding to fast spiking neurons (putative interneurons); unshaded area represents regular spiking (putative pyramidal) neurons. Dotted lines represent mean spike widths of inverted tuning neurons in the 2 areas.
(Wilcoxon signed-rank test, $P > 0.1$). Had we wished to classify the inverted tuning neurons into FS and RS categories based on spike width, 12.5% of prefrontal neurons and 5% of parietal neurons would have been classified as FS. Within the group of neurons with increased delay-period activity, similar percentages of neurons were classified as FS and RS (11% and 89% in prefrontal; 8% and 92% in parietal cortex, respectively); these percentages represented no significant difference between areas ($\chi^2$ test, $P > 0.4$). The results indicated that prefrontal neurons with an inverted tuning curve had unique biophysical properties, consistent with the idea that they corresponded to calbindin interneurons.

For neurons with inverted tuning to play a role in disinhibiting excitatory neurons, which have been activated by a remembered stimulus, the excited and inverted tuning populations must have comparable tuning widths. We therefore tested the tuning of neurons for the spatial stimuli and compared the posterior parietal and prefrontal populations. Overall, the population of prefrontal neurons displayed narrower tuning. The SD of the Gaussian curve that provided the best fit was 0.73 for the prefrontal population of inverted tuning neurons vs. 1.84 for the parietal population (in units of stimulus separation), more than twice as wide (Fig. 6). To test whether a single Gaussian curve fit the data equally well, we determined the optimal fit for the entire pool of neurons ($\sigma = 1.04$) and then compared the neuron-by-neuron residuals of the single- and two-Gaussian models. Fitting the data to two curves resulted in a significant decrease of the fitting error (paired $t$-test, $P < 0.05$). Importantly, the SDs, which provided the best fits for the excitatory populations, were 0.77 and 0.79 for the prefrontal and parietal cortex, respectively (Fig. 6). In this case too, only the prefrontal population of inverted tuning neurons exhibited similar tuning with the population of excited neurons, as hypothesized for calbindin interneurons.

DISCUSSION

Our results demonstrate that neurons with inverted tuning during the delay periods of working memory tasks are much more numerous in the dorsolateral prefrontal than the posterior parietal cortex of monkeys. Furthermore, prefrontal neurons with inverted tuning exhibit unique biophysical and response properties consistent with the idea that a specialized type of prefrontal interneurons plays a special role in the prefrontal network during working memory. Computational models predict considerable benefits in the stability of persistent activity with the addition of a small percentage of neurons with inverted tuning in the same order (~5%) of the neurons that we detected in the prefrontal cortex (Wang et al. 2004). Therefore, this population of neurons may be partly responsible for the ability of the prefrontal network to resist interference by distractors, although inverted tuning may not be the sole property that confers this functional specialization. Regardless of their ultimate functional implications, our current results add to a short list of subtle functional differences in the physiological properties of the prefrontal and posterior parietal cortex, two areas implicated in different cognitive functions but otherwise exhibiting very similar functional properties (Constantinidis and Procyk 2004).

Inverted tuning neurons. Our study relied on the analysis methods of an earlier report of inverted tuning neurons in the prefrontal cortex (Wang et al. 2004) using monkeys trained in different spatial working memory tasks. Our present results in the prefrontal cortex essentially replicated the earlier findings. We find that a small but consistent population of prefrontal neurons (5.2% in the present study compared with 4.5% in the earlier study) exhibited delay-period activity, which was significantly below the fixation baseline rate, and was tuned for the spatial location of the stimuli (the definition of inverted tuning). Calbindin interneurons are reported to make up ~5%
of neurons in the prefrontal cortex (Gabbott and Bacon 1996). Spike width, measured extracellularly, is not a precise indicator of neuron type, as large, pyramidal motor neurons have been shown to exhibit narrow spikes (Vigneswaran et al. 2011). Nonetheless, we observed systematic differences between the two areas, although recordings were performed in the same monkeys, executing the same task, with the same recording techniques. The average spike width of prefrontal neurons with inverted tuning in our study (692 μs) fell between the means of the FS and RS distributions of the data set, from where the current data were derived [418 and 775 μs, respectively, in Qi et al. (2011)], also consistent with the biophysical properties of calbindin interneurons identified anatomically (Kawaguchi and Kubota 1993; Zaitsev et al. 2005). The prefrontal-inverted tuning neurons finally exhibited average tuning width that was very similar to the tuning width of neurons with elevated delay-period activity. This would be a critical property for inverted tuning neurons if they were to play a role in disinhibiting excited neurons with similar tuning (Wang et al. 2004).

In contrast, a significantly smaller population of posterior parietal neurons exhibited inverted tuning during the delay periods of the tasks. Even those neurons that fit the statistical criterion exhibited average spike width (820 periods of the tasks). Even those neurons that fit the statistical criterion exhibited average spike width (820 μs), more consistent with RS neurons, differing significantly from that of prefrontal neurons. Furthermore, parietal neurons with inverted tuning were much more broadly tuned than parietal neurons with excited delay-period activity. For these reasons, they seem unsuitable to play a role in disinhibiting persistent delay-period activity in a manner that would decrease distractibility during the working memory task.

Neurophysiological specialization of the prefrontal cortex. The dorsolateral prefrontal and posterior parietal cortices share multiple functional properties; similar percentages of neurons are active during equivalent epochs of behavioral tasks and exhibit levels of activity and temporal envelopes of responses that are virtually indistinguishable (Chafee and Goldman-Rakic 1998, 2000). The result is not particularly surprising, considering that the two areas are directly connected with each other (Cavada and Goldman-Rakic 1989). In our study too, similar percentages of dorsolateral prefrontal and posterior parietal neurons were observed with tuned, excitatory activity in the delay period of the tasks.

Subtle differences in the properties of the dorsolateral prefrontal and posterior parietal cortex have been identified in tasks requiring monkeys to remember the spatial location of a sample stimulus and to ignore intervening stimuli (Rawley and Constantinidis 2008). Initial studies comparing activity in the ventral prefrontal cortex and the inferior temporal (IT) cortex in object working memory tasks revealed that whereas IT neurons track the most recent stimulus of a sequence (Miller et al. 1993), prefrontal neurons are able to represent the stimulus actively retained in memory (Miller et al. 1996). Subsequently, an equivalent resistance to interfering stimuli was documented in spatial working memory tasks. In such tasks, neurons in posterior parietal areas 7a and LIP represent the location of the most recent stimulus, whether it is the remembered sample or the behaviorally irrelevant distractor (Constantinidis and Steinmetz 1996; Powell and Goldberg 2000). In contrast, prefrontal neurons can maintain the representation of the actively remembered sample (di Pellegrino and Wise 1993; Qi et al. 2010). The prefrontal representation of behaviorally relevant stimuli has been documented further in human imaging experiments (Cornette et al. 2002; Sakai et al. 2002), and it appears to represent a fundamental difference in cortical processing between the prefrontal cortex and its afferent inputs.

Intrinsic circuit differences between prefrontal and parietal cortex. Computational models have been successful in replicating the properties of delay-period activity in the prefrontal cortex (Compé et al. 2000) and have been insightful in determining how resistance to interference may arise. The higher relative density of NMDA receptors compared with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and the role of dopamine have been identified as critical in this respect (Chen et al. 2004; Durstewitz et al. 2000; Seamans et al. 2001; Wang et al. 2008; Wang 2001; Yang and Seamans 1996). Other, more elemental differences between the prefrontal and parietal intrinsic network organization have also been suggested. It is notable, for example, that prefrontal neurons exhibit the most extensive dendritic trees and highest number of spines of any cortical neurons (Elston 2000, 2003), although the physiological implications of such anatomical specialization are yet unknown.

Differences in interneuron types in the prefrontal cortex provide another mechanistic explanation, which could contribute to the unique properties of the prefrontal cortex. Most interneurons in the cortex are parvalbumin-containing neurons (Fig. 7A), which physiologically make up the FS category of interneurons, and inhibit neurons with stimulus selectivity different than their own (Krimer et al. 2005; Zaitsev et al. 2005). In the prefrontal cortex, calbindin-containing interneu-
rons are more numerous than in other cortical areas (Elston and Gonzalez-Albo 2003), although a direct, anatomical comparison with the parietal cortex is not available. Calbindin interneurons are inhibitory to calretinin interneurons and in turn, inhibit the dendrites of pyramidal neurons in close vicinity, spatially restricted in vertical columns (Conde et al. 1994; Gabbott and Bacon 1996; Krimer et al. 2005; Zaitsev et al. 2005). The functional consequence of such a circuit in spatial working memory proposed by Wang et al. (2004) is illustrated in Fig. 7. After a stimulus has been presented and is maintained in memory, a population of pyramidal cells exhibits persistent activity. These neurons activate local parvalbumin interneurons, which in turn, inhibit pyramidal neurons with different stimulus preference. At the same time, the activated pyramidal neurons excite calretinin interneurons, which in turn, inhibit calbindin (inverted tuning) interneurons. The precisely localized axons of the calbindin interneurons then further disinhibit the pyramidal neurons that are already activated (Fig. 7B). Nonactivated, pyramidal neurons (selective for other stimuli) continue to be tonically inhibited by the combined action of parvalbumin interneurons and tonic inhibition of calbindin interneurons in their own microcolumns. As a result, once a stimulus is already held in memory in the circuit of Fig. 7B, a distracting stimulus appearing at a different location is less effective in suppressing the persistent activity of already-activated pyramidal neurons, which are both mutually excited and receive less inhibition by calbindin interneurons (Wang et al. 2004). Apart from resistance to the interference of actual distracting stimuli, the tonic action of calbindin interneurons during working memory suppresses the baseline activity of neurons tuned for stimuli away from the remembered stimulus, further sharpening the representation of the remembered stimulus in the population and essentially reducing the noise in the circuit.

Calbindin interneurons may play other unique roles in the prefrontal cortex. Impairments in GABA neurotransmission among non-FS interneurons have been implicated in schizophrenia, a condition that compromises prefrontal function among non-FS interneurons have been implicated in schizophrenia, a condition that compromises prefrontal function (Lewis et al. 2008). Anatomical studies also indicate that a balanced interneuron population and essentially reducing the noise in the circuit.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: C.C. conception and design of research; F.K., X-L.Q., and C.C. performed experiments; X.Z., F.K., X-L.Q., and C.C. analyzed data; X.Z. and C.C. interpreted results of experiments; X.Z. and C.C. prepared figures; X.Z. and C.C. drafted manuscript; X.Z., F.K., X-L.Q., and C.C. edited and revised manuscript; X.Z., F.K., X-L.Q., and C.C. approved final version of manuscript.

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