Differences in spectral profiles between rostral and caudal premotor cortex when hand-eye actions are decoupled

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Sayegh PF, Hawkins KM, Hoffman KL, Sergio LE. Differences in spectral profiles between rostral and caudal premotor cortex when hand-eye actions are decoupled. J Neurophysiol 110: 952–963, 2013. First published May 22, 2013; doi:10.1152/jn.00764.2012.—The aim of this research was to understand how the brain controls voluntary movement when not directly interacting with the object of interest. In the present study, we examined the role of premotor cortex in this behavior. The goal of this study was to characterize the oscillatory activity within the caudal and rostral subdivisions of dorsal premotor cortex (PMdc and PMdr) with a change from the most basic reaching movement to one that involves a simple dissociation between the actions of the eyes and hand. We were specifically interested in how PMdr and PMdc respond when the eyes and hand are decoupled by moving along different spatial planes. We recorded single-unit activity and local field potentials within PMdr and PMdc from two rhesus macaques during performance of two types of visually guided reaches. During the standard condition, a visually guided reach was performed whereby the visual stimulus guiding the movement was the target of the reach itself. During the nonstandard condition, the visual stimulus provided information about the direction of the required movement but was not the target of the motor output. We observed distinct task-related and topographical differences between PMdr and PMdc. Our results support functional differences between PMdr and PMdc during visually guided reaching. PMdr activity appears more involved in integrating the rule-based aspects of a visually guided reach, whereas PMdc is more involved in the online updating of the decoupled reach. More broadly, our results highlight the necessity of accounting for the nonstandard nature of a motor task when interpreting movement control research data.

Cognitive motor integration; local field potentials; neurophysiology; nonstandard reaching; premotor cortex

Movement control relies on a network of brain regions including the dorsal premotor cortex (PMd) and superior parietal lobule (SPL) (Battaglia-Mayer et al. 2001; Caminiti et al. 1999; Kalaska et al. 1998). It is not well understood how activity within this network is modulated during a reaching movement when there is a dissociation between the actions of the eyes and hand, termed a “nonstandard” movement (Wise et al. 1996). There is strong evidence that the actions of the eyes and hand are tightly linked (Gauthier and Mussa Ivaldi 1988; Gielen et al. 1984; Gorbet and Sergio 2009; Henriques et al. 1998; Morasso 1981; Neggers and Bekkering 2000; Prablanc et al. 1997; Sergio and Scott 1998; Terao et al. 2002; Vercher et al. 1994); hence, the brain must employ specific mechanisms to break this link during nonstandard movements (Murray et al. 2000; Sergio et al. 2009; Wise et al. 1996). Inhibition of this linkage likely depends on neural circuitry that is different from but interconnected with the circuitry important for controlling natural reaching movements (Clavagnier et al. 2007; Gail et al. 2009; Sergio et al. 2009). Support for this idea comes from the observation that decoupled eye-hand coordination develops only later in childhood (Bo et al. 2006; Piaget 1965; Sergio et al. 2009), that movement slows and accuracy declines when eye and hand movements are decoupled (Epelboim et al. 1997; Goodbody and Wolpert 1999; Gordon et al. 1994; Henriques et al. 1998; Messier and Kalaska 1997; Terao et al. 2002), and that patients with neurological disorders show impaired nonstandard reaching while standard reaching is largely unaffected (Ghilardi et al. 1999; Halsband and Passingham 1982, 1985; Jackson et al. 2005; Karnath and Perenin 2005; Tippett and Sergio 2006; Tippett et al. 2007).

Cell activity in PMd is modulated by gaze direction, wrist orientation, hand direction, and intended movement kinematics (Boussaoud 2001; Boussaoud and Wise 1993a, 1993b; Boussaoud et al. 1998; Caminiti et al. 1990; Cisek and Kalaska 2002; Raos et al. 2004). Recent findings suggest that the rostral and caudal subdivisions of PMd (PMdr and PMdc) have separate roles in the visuomotor transformation needed to plan an upcoming visually guided reach (Raos et al. 2004). These results are strengthened by anatomic studies demonstrating that PMdr and PMdc have separate cortical connections. PMdr has strong reciprocal connections with prefrontal regions (Lu et al. 1994), whereas PMdc has strong connections to the primary motor cortex and the spinal cord (Barbas and Pandya 1987; Geyer et al. 2000; Luppino et al. 1990); therefore, PMdc may be involved in coding limb movement parameters (Alexander and Crutcher 1990; Cisek et al. 2003). In contrast, PMdr may have a role in the context-dependent selection and planning of movements in conditions that involve nonstandard mappings (Boussaoud 2001; Cisek and Kalaska 2005; Raos et al. 2004), although this remains to be tested directly.

Oscillatory activity within the local field potential (LFP) has been used to measure many neuronal processes such as attention, memory, action, and perception (Baker et al. 1999; Brovelli et al. 2005; Cooper et al. 2003; Donoghue et al. 1998; Jensen et al. 2007; O’Leary and Hatsopoulos 2006). LFPs represent the activity within local cell assemblies (Scherberger et al. 2005) and are believed to represent the input into an area (Scherberger et al. 2005). Since spiking activity reflects suprathreshold inputs or outputs from pyramidal cells, it is very likely that spike and LFP recordings carry different sets of information and can therefore be complementary tools for brain analysis (Pesar et al. 2002; Sanes and Donoghue 1993). In fact, some researchers have concluded that LFP activity is...
more accurate than spike activity when decoding certain behavioral states (Mitzdorf 1985). Another benefit to LFP analysis is its strong relationship with blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) activity in humans (Goense and Logothetis 2008; Nir et al. 2007) and with spike activity in nonhuman primates (Fries et al. 2001). The relationship between LFP, spike, and BOLD activity can be used to bridge the gap between neurophysiological data in animals (single-cell recording) and human fMRI recordings.

In the present study, we examine how oscillatory and spike activity within PMd are modulated when gaze and hand motions are spatially incongruent, relative to more natural spatially congruent reaches. In addition to overall changes in the spectral profile as a consequence of nonstandard reaching, we predict that the rostral subdivision of PMd will show greater modulation relative to the caudal subdivision, particularly during the planning phases of the nonstandard task.

METHODS

Apparatus and Behavioral Task

Two rhesus monkeys (female Macaca mulatta, monkeys A and B, body weight 5.2 kg) were trained to perform visually instructed, delayed reaching tasks in standard and nonstandard conditions. All surgical and animal handling procedures were in accordance with Canadian Council on Animal Care guidelines on the use of laboratory animals and were preapproved by the York University Animal Care Committee.

During the experiment, the monkey was seated in a custom-built primate chair 40 cm in front of a 38.1-cm vertical screen, which was set at monkey eye level and centered with her midline. An additional 38.1-cm horizontal touch-sensitive screen (Touch Controls, San Diego, CA) was set in front of the animal, between the animal's waist and xiphoid process, so that she could reach over the entire surface of the screen comfortably (Fig. 1). The horizontal touch screen was designed to detect spatial displacements as small as 3 mm using infrared beams at a sampling rate of 100 Hz. Continuous tracking of the eye was performed using the ISCAN-ETL 200 Eye Tracking System (ISCAN, Burlington, MA) at a sampling rate of 60 Hz. Performance in both conditions required the animals to reach toward one of eight peripherally cued targets on the horizontal touch screen. The animals were trained to perform similar movements during both conditions, and the biomechanical features of the reach movements were monitored to ensure that the movement profiles were similar between conditions. In addition, to minimize any interference from the nonreaching limb, the animals were trained to maintain their nonreaching hand on a metal lever just beyond the lower corner of the horizontal touch screen. In this way it was ensured that the animals only used the appropriate arm without the unused limb having to be forcefully restrained.

The visual targets were identical across conditions, but the spatial plane of presentation was altered. At the start of each trial, a red circular target (70 mm in diameter) appeared at the center of the screen with an additional smaller white circular target (40 mm in diameter; 5.7° of visual angle) on top of it. The red target instructed where the monkey should touch, and the white target instructed where the animal should maintain eye fixation. After a baseline period of 500 ms, one of eight green-colored peripheral targets appeared (70 mm in diameter). All eight targets were equally spaced (45°) and appeared randomly, based on a randomized-block design. The peripheral target appeared 5 times at each location for a total of 40 trials per condition. After a variable instructed delay period (IDP; 2,000 ± 500 ms), the red central target extinguished and the white target jumped to the peripheral target. This served as the go signal (GO) instructing the animal to move the eyes and hand from the central target to the peripheral target (Fig. 1). The movements were made from the center of the central target to the center of the peripheral target (roughly 80 mm; see Fig. 2). Once the eyes and hand arrived at the peripheral target, the monkey was required to hold them there for 500 ms, after which a liquid reward was delivered to the animal. In addition, to reinforce similar hand paths between conditions, movement alleys were included to ensure that reaches were directed along a fairly straight trajectory. These alleys were set at ±40 mm from a straight line spanning from the central to the peripheral targets. If the monkey’s hand deviated beyond the set alley, the trial was stopped. To provide feedback on the current position of the hand, a crosshair representing the position of the finger on the touch screen was displayed.

In the standard condition, the actions of the eyes and hand remained congruent. The visual presentation of the task and the reaching movements were both made on the horizontal touch-sensitive screen placed in front of the animal (Fig. 1A). In the nonstandard condition, the actions of the eyes and hand were decoupled: the visual presentation of the task was on the vertical screen while the animal’s limb movement remained on the horizontal touch screen (Fig. 1B). Thus the animal was required to direct its gaze along the vertical monitor but move its finger along the horizontal monitor to displace the cursor from the central to the peripheral target. To ensure that the animal did not track its hand position extrafoveally, an opaque screen was placed 10 cm above the animal’s arm to block vision of the limb. For each condition, two epochs during the trial were considered, the instructed delay epoch (IDP) and the movement epoch (MOVE). IDP comprised the 500 ms baseline period and the first 1,000 ms of the instructed delay. MOVE comprised the last 500 ms of the instruction delay up until 500 ms after movement onset.

We also had a gaze-only condition, to determine if the oscillatory activity within the subregions of PMd was affected solely by the overall shift in gaze angle between conditions. Gaze-only data were collected for every recording. The visual display consisted of nine white circles (40 mm in diameter, 5.7° of visual angle) that appeared in the same locations as the white targets that appeared during the experimental conditions. The monkey was instructed to fixate on each of these white circles while maintaining both hands beside the hori-
zontal touch screen. The white circles appeared one at a time in each location 3 times for a total of 27 saccades for each plane.

Muscle activity was recorded from 13 proximal arm muscles in separate recording sessions. Pairs of Teflon-insulated 50-μm single-stranded stainless steel wires were implanted percutaneously. Implantations were verified by passing current through the wires to evoke focal muscular contractions (<1.0-mA, 30-Hz, 300-ms train; Sergio and Kalaska 2003). Multi-unit electromyography (EMG) activity was amplified, band-pass filtered (100–3,000 Hz), half-wave rectified, integrated (5-ms time bins), and digitized online at 200 Hz. The muscles studied included the anterior deltoid, medial deltoid, posterior deltid, dorsoepitrochlearis, infraspinatus, latissimus dorsi, pectoralis, supraspinatus, teres major, rostral trapezius, caudal trapezius, triceps lateralis, and triceps medialis. These recordings were performed to assess the general effects of the standard and nonstandard tasks on EMG activity and were not designed as a definitive biomechanical study of the muscle properties.

Behavioral and Muscle Data Analysis

Hand paths recorded from the touch-sensitive monitor were analyzed to confirm that the movements were biomechanically similar between conditions. The individual movement paths were first low-pass filtered at 10 Hz, and the movement onsets and endpoints were automatically scored as 8% peak velocity at the beginning and end of the velocity profile, respectively. The movements were then cut at the onsets and endpoints and divided into 21 equal segments. The five trials for each direction were then pooled, and the means and standard deviations were calculated at each segment along the path. An equality of variance test was performed between the two conditions on the mean x and y components of the trajectory for each target (Snedecor and Cochrane 1989). Since a given trial was only kept if the animal went from the central to the peripheral target within a somewhat narrow alleyway, this test determined if, despite this behavioral training, there were any systematic differences in movement variability between tasks. Mean reaction times (from the GO signal to 8% peak velocity) were also calculated for each condition, and paired-samples t-tests were performed to compare reaction times between the standard and nonstandard conditions. Repeated-measures ANOVAs were performed on the EMG data during the IDP and MOVE epochs for each muscle recorded to determine the effect of target (motion direction) and condition (standard and nonstandard) on maximum EMG amplitude. It was expected that reach direction would have an effect on EMG amplitude but that condition would not.

Neural Recordings

Monkeys were implanted with a recording cylinder under standard aseptic surgical techniques (Kalaska et al. 1989). Briefly, a Plexiglas cylinder (used to hold the electrode manipulandum) was positioned on the 19-mm craniotomy and fixed into place using cranioplastic acrylic and titanium neurosurgical screws. A small metal fixation pole used to stabilize the head during recording was also implanted into the acrylic. The stereotaxic coordinates for chamber placement over PMd (both monkeys: interaural anteroposterior, +16 mm; mediolateral, +11 mm) were determined using The Rhesus Monkey Brain in Stereotaxic Coordinates (Paxinos et al. 2000). The border between rostral and caudal PMd and primary motor cortex (M1) was drawn according to previously proposed physiological and cytoarchitectonic criteria (Fujii et al. 2000). The experiments began a week after surgery following a complete recovery.

LFPs and single units were collected from the extracellular recordings within PMd. A hydraulic multichannel driver (MCM-4; FHC, Bowdoin ME) mounted to the implanted chamber was used in conjunction with a multichannel processing system (MCP; Alpha-Omega Engineering, Nazareth, Israel). Standard tungsten microelectrodes (impedance 1–3 MΩ; FHC) were used for recording the neural activity within PMd. The multichannel provided simultaneous recordings from up to two penetration sites at a time. Neural activity from each electrode was preamplified (5,000 times), band-pass filtered (1 Hz–10 kHz), and split into lower (LFP) and higher (single units) frequency bands. Higher frequency signals were sampled at 12.5 kHz and passed through the multispike detector (Hawkins et al. 2013). The lower frequency signals (below 100 Hz) were sampled at 390.6 Hz.

Data Analyses

Directional tuning was determined on the basis of previously described methods (Georgopoulos et al. 1982; Hawkins et al. 2013). Briefly, a sinusoidal regression on the mean discharge rates for each target direction was performed, and the goodness of the regression fit was calculated. The regression equation was then reexpressed in terms of the peak of the sine wave, which is the direction for which the cell was most active (i.e., the “preferred direction”) (Georgopoulos et al. 1982). A bootstrap test was then performed using 1,000 shuffled activities to determine the significance of the tuning based on a 95% confidence interval (CI). The mean firing rates of each cell were then normalized to each cell’s individual mean baseline firing rate. The baseline firing rate was calculated as the mean firing rate during the first 300 ms of each trial when the animal was instructed to hold its hand at the central target. This generated a normalized firing rate for comparison with the LFP data, which were also normalized to the same baseline time period (see below). Significance was determined by performing a three-way mixed ANOVA with condition (standard vs. nonstandard) and time (early vs. late) as within-subject factors and location (PMdr vs. PMdc) as between-subject factor. All ANOVA results were reported with Greenhouse-Geisser-corrected P values, and post hoc comparisons were corrected for multiple comparisons (Bonferroni). All successfully recorded LFP sites were included in all analyses. The converted data were analyzed in MATLAB (The MathWorks, Natick, MA) using both custom-written and open-source (Chronux.org) programs. Chronux script files were used to analyze the spectral profiles and generate time-frequency spectrograms for all penetrations for both conditions (Jarvis and Mitra 2001; Pesaran et al. 2002). We used spectral analysis to categorize the power at different frequency bands. To estimate the frequency structure of the LFP activity, we used the multitaper spectrum analysis (previously described by Jarvis and Mitra 2001; Pesaran et al. 2002). The multitaper technique provides an optimal estimate of the spectrum by reducing spectral leakage and variance of the estimate by averaging the spectral estimates from several orthogonal tapers (Jarvis and Mitra 2001). The orthogonal tapers are Slepian np prolate functions (Jarvis and Mitra 2001). A Fourier transform was then applied to the tapered signal. The multitaper estimates of the spectrum Sx(f) for each recording were then calculated (Pesaran et al. 2002; Scherberger et al. 2005). The spectrum was z-transformed to the baseline period, which consisted of 300 ms at the start of each trial during which time the animal was maintaining eyes and hand at the central target. Normalizing to a baseline period was necessary to compare between conditions and locations. No significant differences were observed between baseline activity when comparisons were made between conditions within each region (P >0.05). Spectrograms were calculated using a 200-ms window shifted in 50-ms increments with a 3-Hz frequency resolution. Mean spectra reflect the spectra z-transformed from individual trials and then collapsed across target directions and electrode sites within the respective PMd region.

To determine the statistical significance of task-related differences (P <0.05), the normalized spectrum from each electrode site was divided into four frequency bands (0–10, 10–30, 30–45, and 45–70 Hz). Data from above 70 Hz were not presented because the pattern of activity within this range has been shown to be closely related to spiking activity (Ray and Maunsell 2011; Zanos S et al. 2012; Zanos TP et al. 2011). The average spectral value across time for each frequency band was determined for each condition (standard and

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Behavioral Results

To ensure that any oscillatory differences seen between conditions were not a result of differences in the movement of the hand, we compared hand trajectories between conditions. We wanted to ensure that the biomechanical features of the limb movements were identical throughout the experiment. This guarantees that the interpretations of the LFP data are not affected by a difference in limb movements. The use of alleys helped support the animal in maintaining similar hand trajectories during both conditions (see METHODS). Figure 2A shows the mean reach trajectories during both conditions for each animal. Except for a few segments, there were no significant differences in the reach trajectories between standard and nonstandard conditions. An analysis of the EMG data revealed that for 11 of 13 muscles there was no main effect of condition during the IDP and MOVE epochs \((P > 0.01)\). For two muscles, medial deltoid and teres major, there was a marginal effect of condition on EMG activity during the IDP epoch \((0.05 > P > 0.01)\). This may have been due to a slight alteration in the animals’ starting posture in reaction to the board placed over their arm in the nonstandard condition. There was, as expected, a main effect of target for all proximal arm muscles studied during the movement epoch \((P < 0.01)\). Finally, reaction times between the standard \((537.9 \pm 12.82,\)

![Mean Reach Trajectories](image-url)

**Fig. 2.** A: mean reach trajectories. Gray lines indicate mean movement trajectories; black tick marks indicate SD. Red asterisks denote trajectory segments that were significantly \((P < 0.05)\) more variable compared with the standard condition. B: penetration sites for monkey A (top) and monkey B (bottom). Larger circles indicate where recordings were obtained on 2 occasions. AS, arcuate sulcus; CS, central sulcus; LF, longitudinal fissure. Arrows show anterior (A), posterior (P), medial (M), and lateral (L) directions; dotted line denotes division between penetration sites classified as rostral (left of line) or caudal (right of line).
mean ± SE) and nonstandard (522 ± 9.89) conditions were also not significantly different \[ t(45) = 0.927, P = 0.359 \]. Taken collectively, these results strengthen the conclusion that any neural differences observed between conditions are not a direct result of changes in the biomechanics of the reaching movement, but rather the control of the movement.

**Neural Activity**

We obtained 66 (59 from monkey A, 7 from monkey B) LFP recordings and classified 36 of these recordings as coming from PMdr and 30 recordings as coming from PMdc, based on the stereotaxic coordinates of the recording chamber and the penetration location in the chamber (Fig. 2B). All successfully recorded LFP sites were included in the analysis. In addition, 52 single cells were recorded within PMd (28 PMdr and 24 PMdc) during both conditions, of which only 29 were found to be task-related (directionally tuned during either the IDP or MOVE epoch). In support of our hypothesis, we observed that oscillatory and single-unit activity in PMd were modulated by the type of eye-hand coordination: standard (direct object interaction) or nonstandard (decoupled effectors). In addition, we observed that the temporal and spectral profile of change depended on the region (rostral or caudal) of PMd sampled.

**Task-Related Differences Within PMd**

**Oscillatory activity.** Our first main finding was that we observed salient differences in the oscillatory activity during performance under the standard condition compared with the nonstandard condition in both subregions of PMd. We analyzed the neural activity within two epochs (see METHODS). During the IDP epoch, the animal received information about the location of the peripheral target but was required to maintain hand and eye position at the central location. Thus during this epoch the early stages of movement planning would be occurring. Figure 3 shows an example of the increase in power that occurs across each frequency band for a single PMdc recording site during both epochs. An example spectrum from each animal for each epoch is also shown in Fig. 3, C and D, to demonstrate the similarity in spectral profile between animals. Because of these similarities, all analyses were pooled between animals. Additionally, the second 500 ms of this epoch represents the first 500 ms of the reaching movement, which was the same
between conditions (i.e., only the eye movement coupling varied between conditions; Fig. 1).

During the IDP epoch, changes to the overall oscillatory activity occurred when the eyes and hand were decoupled vs. when they were congruent (Fig. 4, A–F, left). Shortly after peripheral cue onset (black vertical line), there was an increase in oscillatory activity that occurred in both regions of PMd during both conditions (Fig. 4, A–D, left). To show task-related differences more clearly, Fig. 4, E and F, display the main differences in power across conditions, masked for significance (see METHODS). We observed a significant increase in PMdr oscillatory power within the 10- to 70-Hz range during the nonstandard condition relative to the standard condition (Fig. 4E, left; P < 0.05). Contrary to this finding, PMdc showed a reduction in oscillatory activity between conditions within the 0- to 45-Hz range (Fig. 4F, left; P < 0.05). Figure 5 displays the z score for each frequency bin across time and allows for a more in-depth look at the pattern of change between conditions. As shown in Fig. 4B, the oscillatory activity within PMdr shows a greater increase in power during the nonstandard compared with the standard condition (Fig. 5, B–D, top left; P < 0.05). On the other hand, stronger oscillatory activity occurred for the standard compared with the nonstandard condition within PMdc (Fig. 5, A–C, bottom left; P < 0.05).

Our findings during the MOVE period showed more subtle differences in oscillatory activity when the eyes and hand were decoupled (Fig. 4, A–D, right). Task-related differences were only observed within the 0- to 10-Hz range before movement onset for both PMdr and PMdc (Fig. 4, E and F, right). Both regions showed a significant reduction in oscillatory activity during the nonstandard compared with the standard condition within this frequency range (Fig. 5A, right; P < 0.05). After movement onset, PMdc shows both a reduction of power within the 10- to 30-Hz range (Fig. 5B, bottom right; P < 0.05) and an enhancement of power within the 45- to 70-Hz range (Fig. 5D, bottom right; P < 0.05). These results demonstrate that just before movement, both PMdr and PMdc activity were modulated by decoupling the action of the eyes from the hand. By movement onset these conditional differences were less evident, with PMdc showing slight differences between reaching movements (Fig. 4, E and F).

Spiking activity. The task-related differences observed within the LFP data are also supported by single-cell findings (Fig. 6). In line with the observed increase in oscillatory activity, a significant increase in firing rate can also be observed within PMdr during the planning phase of a nonstandard compared with a standard reach (Figs. 6B and 7A; P < 0.01). Contrary to the observations in the oscillatory activity within PMdc, a significant increase in firing rate also occurred during decoupled reaching movements within this region (Fig. 7A; P < 0.01). During the MOVE period, we decided to separate the analysis into an early (before movement) and late period (after movement began) to address whether the spike activity follows the same pattern observed for the LFPs during this MOVE period. In this analysis, we observed that task-related differences in spiking activity occurred only after movement onset in both regions (Fig. 7B; P < 0.01). PMdc was most active during standard reaching movements, and firing rates decreased during eye-hand decoupling (Fig. 7B, late epoch; P < 0.01). PMdr, however, showed enhanced spiking activity only following movement onset (late epoch) during nonstandard reaches, whereas standard reaches showed a significant

![Figure 4. Population time-frequency spectrograms of oscillatory activity during the IDP and MOVE epochs. A and B: population spectrograms of power in rostral portion of dorsal premotor cortex (PMdr) during standard (A) and nonstandard conditions (B). C and D: population spectrograms of PMdc power for standard (C) and nonstandard conditions (D). E and F: population spectrogram showing only significant differences between standard and nonstandard conditions within PMdr (E) and PMdc (F). Each time-frequency bin was masked at a 95% confidence interval (CI) based on bootstrapped data (see METHODS). Power is color-coded on a log scale. For E and F, colors coded above 0 indicate greater power within the nonstandard condition, and colors coded below 0 indicate greater power within the standard condition. Black line indicates peripheral target onset during IDP epochs and movement onset during MOVE epochs; gray dashed line indicates end of the baseline period.](http://jn.physiology.org/fig5.b)
A reduction in mean firing rate (Fig. 7; \( P < 0.01 \)). This suggests that by the late planning stage (early MOVE epoch), task-related differences that occur during the early planning period become absent and movement-related differences are only observed following movement onset.

**Topographical Differences in Eye-Hand Decoupling**

**Oscillatory activity.** In support of our hypothesis, we also observed topographical differences between PMdr and PMdc in the modulation of oscillatory activity between conditions. The most striking finding was the significantly greater power in oscillatory activity during eye-hand decoupling within PMdr following peripheral cue onset (Fig. 4E, right). PMdc, on the other hand, demonstrated a reduction in power when the eyes were decoupled from the hand (Fig. 4F, right). To more explicitly evaluate these topographical differences during the IDP and MOVE epochs of a given condition, we plotted the relative power differences between PMdr and PMdc to more easily see between regions for each of four frequency bands (Fig. 8). Within the IDP epoch, a similar pattern of oscillatory activity can be seen between regions in the standard condition (Fig. 8A). Topographical differences were only observed in the 45- to 70-Hz range (Fig. 8A, left; \( P < 0.05 \)). During the nonstandard condition, however, clear differences in power can be observed across many frequencies ranges (10–70 Hz; Fig. 8A, right; \( P < 0.05 \)).

By the late planning and movement stage, PMdr and PMdc once again demonstrate similarity in the pattern of oscillatory activity during both conditions (Figs. 5, right, and 8B).

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**Fig. 5.** Time course of task-related differences in power during the IDP (A–D, left) and MOVE epochs (A–D, right), segregated by frequency band. Z-score values are broken up into frequency ranges of 0–10 (A), 10–30 (B), 30–45 (C), and 45–70 Hz (D). Black line represents peripheral cue onset (IDP period) and movement onset (MOVE period). Gray bars indicate when the power was significantly different between conditions (\( P < 0.05 \)).

**Fig. 6.** Mean discharge rates of single cells within PMd across the tasks. A and B: mean normalized firing rates for PMdr during standard (A) and nonstandard conditions (B). C and D: mean normalized firing rates for PMdc during standard (C) and nonstandard conditions (D). Black lines represent peripheral cue onset during the IDP epoch (A–D, left) and movement onset during the MOVE epoch (A–D, right).
summary, during the early planning of a nonstandard reach, PMd shows enhanced oscillatory activity compared with PMdc. By the late planning and early movement phase, although the oscillatory activity within PMd and PMdc are both modulated between conditions, there were less obvious topographical differences in the way that this activity was modulated.

**Spiking activity.** In support of our LFP finding, we also observed distinct topographical differences during the IDP epoch and subtle differences between PMd and PMdc during the MOVE period (Fig. 7B). Specifically, during the IDP epoch PMd showed a significantly greater mean discharge rate compared with PMdc for both conditions (Fig. 7A; $P < 0.01$). In line with the oscillatory activity results, the greatest mean discharge rate for PMd was observed during the nonstandard condition, when the eyes and hand were decoupled (Figs. 6B and 8A; $P < 0.01$). Figure 8B shows that by late planning (early MOVE epoch), no significant topographical differences are evident during the standard condition ($P > 0.05$), and only minor differences are observed during the nonstandard condition.

During this epoch we only observed clear topographical differences in the single-unit activity after movement onset (late MOVE epoch) and restricted to the standard condition (Fig. 7B; $P < 0.01$). Despite some minor differences from the LFP findings, the single-unit activity supports the hypothesis that functional differences exist between PMd and PMdc and how they contribute to the performance of visuomotor transformations.

**Gaze Effects**

In an effort to determine whether the changes in oscillatory and single-unit activity between conditions arose solely from the shift in gaze angle attributable to the different viewing planes, a gaze-only condition was analyzed (see METHODS). Since the visual display in the nonstandard condition was presented on a vertical monitor and that in the standard condition was presented on a horizontal monitor, the animal’s overall gaze angle (but not head position) changed between conditions. During the gaze-only condition, the animal fixated nine points on the horizontal and vertical screens. These fixation points corresponded to the location of the targets in the experimental conditions. The animal was instructed to fixate the points while maintaining its hands on levers to the side of the monitor. Any significant difference observed between conditions was considered to be related to gaze effects.

Within both PMd and PMdc, no effects of gaze were observed for the frequency bands we tested (0–70 Hz; Fig. 9; $P > 0.05$). These data suggest that the changes we observed in oscillatory activity between standard and nonstandard condi-

**Fig. 7.** Histogram demonstrating significant difference in the normalized mean firing rates between conditions and locations. A: normalized firing rates as a function of condition and location during the IDP period. B: normalized firing rates during MOVE epoch broken up into early (before movement onset) and late periods (after movement onset). *$P < 0.05$; **$P < 0.01$, significant difference between either conditions or locations, as indicated.

**Fig. 8.** Topographical difference in oscillatory activity during the IDP (A) and MOVE epochs (B). Positive $z$-score values reflect stronger oscillatory activity within PMd, whereas negative $z$-score values represent stronger activity within PMdc. Vertical black bars represent onset of peripheral cue (A) and movement onset (B). R > C, activity in rostral PMd is stronger than in caudal PMd; C > R, activity in caudal PMd is stronger than in rostral PMd.

**Fig. 9.** Gaze control analysis. Values are mean amplitude and SD during standard and nonstandard conditions for PMd (A) and PMdc (B). Bootstrapping methods revealed no significant difference in overall activity between conditions for each region ($P > 0.05$).
oscillatory activity that occurs within PMdr during the non-integration and might thus be reflected in the increased gamma coupled reaches would require enhanced local processing and might represent the crucial transformation of information that would make PMdr a crucial node in the sensorimotor transformation of nonstandard limb movements, although this interpretation raises further questions. In contrast, PMdc demonstrates a decrease in oscillatory activity during nonstandard relative to standard reach planning. This reduction in power may represent inhibitory inputs to PMdc, which would be important for allowing the eyes and hand to decouple. Recently, we have found that the mean discharge in single cells recorded from superior parietal areas during the same experiment presented here is significantly reduced during nonstandard task planning (Hawkins et al. 2013). The decoupling of goal/gaze spatial location and sensed limb location may underlie the reduction in activity in these bimodal cells. The decreased spiking activity observed in PMd during a nonstandard reaching movement reflects such an inhibiting signal to overrule the “default” visuomotor network for standard reaches (Everling et al. 1999; Gail et al. 2009; Schlag-Rey et al. 1997). This is in line with our spike data, which also demonstrate an increase in the discharge rate of single cells during the planning phase of nonstandard reaching movements. For PMd to provide an inhibitory output signal, it must first be able to receive information about the spatial disparity between gaze and limb position. The enhanced oscillatory activity observed within PMdr during nonstandard reach planning may serve this purpose, which may then drive the increase in output signals (single units) that would be needed to inhibit the “default” visuomotor network, as suggested by others (Gail et al. 2009; Gorbet et al. 2004). Such an arrangement would make PMdr a crucial node in the sensorimotor transformation of nonstandard limb movements.
be needed to decouple the actions of the limb and gaze to successfully perform the task used in these studies.

If the role of PMd is to instruct or inhibit other regions during nonstandard conditions, and across-region or more “global” synchronization is associated with lower frequency oscillations, then one might expect an increase in beta and lower frequencies for the nonstandard task. Subgamma frequencies including beta are observed when a region is integrating independent distant signals or for “top down” processing as opposed to engaging in local processing (Donner and Siegel 2011; Siegel et al. 2012). Consistent with this, our results demonstrate that within PMd there is an increase in low-frequency power during the early planning period of a nonstandard compared with a standard movement (Fig. 5B). On the basis of the aforementioned idea, the increase in low-frequency power seen in the early planning period of a nonstandard reach may reflect the integration of the cognitive rule into the motor action, allowing for a new relative position code of the eyes and hand.

Contrary to these observations, PMdc showed a reduction in low-frequency power during the planning and movement periods in the nonstandard condition (notice both epochs, Fig. 5, A and B). Under natural reaching situations, the brain must integrate hand and eye signals to arrive at the proper behavior, as in the standard version of the task. Perhaps within PMdc, performance during the nonstandard condition requires that these signals continue to be segregated, not integrated, just as the planes of movement of the hand and eyes are separated in space. Thus, although PMd normally functions to integrate the signals from various regions of the brain for successful eye-hand decoupling, the suppression of beta or lower frequencies would be expected during nonstandard reaching within PMd if this is an area of importance for eye-hand coupling. Cautious interpretation is warranted, however, since the oscillatory functions within PMd are largely unknown, and the present results suggest a complex repertoire of oscillations that reveal temporal and spectral differences even within PMd.

Functional and Anatomic Separation Within PMd

Most PMd single-unit studies have not separated PMd into its rostral-caudal subdivisions. A reexamination of the literature has revealed that the majority of cells that respond to conditional visuomotor associations appear to be located within PMd, whereas cells within PMdc demonstrate mostly movement-related activity (Grafton et al. 1998; Picard and Strick 2001). The authors of these reviews suggest the term “pre-PMd” for PMd, reflecting the cognitive conditional visuomotor associations that have been commonly observed there. The functional separation in the activity of PMd cell assemblies in the current study support this proposed distinction: PMd was more active during decoupled reaching movements, when the movement relied on a transformational rule to be incorporated into the reaching movement. By the late planning/early movement phase of both standard and nonstandard movements, PMd had stronger oscillatory and single-unit activity. During this period concerned with movement execution rather than planning, the salient features would be the biomechanical details of the movement, which did not vary with task in the present study (Boussaoud 2001; Picard and Strick 2001; Scott and Kalaska 1997; Scott et al. 1997; Toni et al. 2001). Left open is the question of how information within PMd affects the final movement programming in PMdc, supplementary motor area (SMA), M1, or spinal cord structures, given the evidence that there are few if any direct connections between PMd and these other areas (Barbas and Pandya 1987; Kurata 1991; Luppino and Rizzolatti 2000; Tachibana et al. 2004). It has been suggested that PMd, a rostral motor region, plays a large role in relaying prefrontal signals to pre-SMA and rostral cingulate motor area (CMAr) to eventually reach the more caudal motor regions such as SMA, PMd, and finally M1 (Lu et al. 1994; Morecraft et al. 2004; Takada et al. 2004). These connections are important because they may provide a pathway for information flow from DLPC and PMd, and thus allow signals important for decoupled reaching to reach the final motor plan. Another avenue for PMd activity to influence the motor plan may be via the corticostriatal connections between PMd and the basal ganglia (BG) (Tachibana et al. 2004). Considering that PMd is thought to play a role in inhibiting the natural tendency to couple the eyes and hand during decoupled reaching movements (Gail et al. 2009; Gorbet et al. 2004), its connection with BG structures may help mediate the inhibitory signal that would be necessary to accomplish this type of reaching movement. Recently, SMA has been shown to play a key role in proactive control, the ability to stop a movement based on endogenous signals (Chen et al. 2010; Jaffard et al. 2008). Proactive control is key to our ability to inhibit our natural tendency to couple the eyes and the hand. Thus this region may also play a key role in our ability to decouple the eyes and hand. Finally, based on the strong reciprocal connections that the premotor and parietal cortices share (Geyer et al. 2000; Lu et al. 1994; Luppino and Rizzolatti 2000; Matelli et al. 1998; Pandya and Yeterian 1984; Picard and Strick 2001), information from PMd may provide the parietal lobe with signals necessary to incorporate a rule into the ongoing movement.

In conclusion, the task-related oscillatory activity in PMd observed in the present study supports necessary but separate roles for rostral and caudal subregions in the control of nonstandard reaching, a behavior performed in everyday life. PMd activity appears to be more involved in integrating the rule-based aspects of a visually guided reach, whereas PMdc is more involved in the online updating of the decoupled eye and hand movements. We propose that PMd, particularly the rostral portion, plays a crucial role in breaking the tight linkage that exists for eye-hand coupling. We also provide physiological data that suggest distinct roles for PMd and PMdc in the control of visually guided reaching movements. On a practical level, these results indicate that caution should be taken when comparing data obtained from studies using direct object manipulation with those from studies using nonstandard, decoupled target/object interaction.

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