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Encoding of Reach and Grasp by Single Neurons in Premotor Cortex Is Independent of Recording Site

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Stark E, Asher I, Abeles M. Encoding of reach and grasp by single neurons in premotor cortex is independent of recording site. *J Neurophysiol* 97: 3351–3364, 2007. First published March 14, 2007; doi:10.1152/jn.01328.2006. Neural activity has been studied during reaching and grasping separately, yet little is known about their combined representation. To study the functional organization of reaching and grasping in the premotor cortex (PM), we trained two monkeys to reach in one of six directions and grasp one of three objects. During prehensile movements, activity of proximal (shoulder and elbow) muscles was mainly modulated by reach direction, whereas distal (finger) muscles were also modulated by grasp type. Using intracortical microstimulation, we identified spatially distinct PM sites from which movements of proximal or distal joints were evoked. In contrast to muscles, modulation of neural activity by reach direction was similar for single units recorded in proximal and distal sites. Similarly, grasp type encoding was the same for units recorded in the different sites. This pattern of encoding reach and grasp irrespective of recoding site was observed throughout the task: before, during, and after prehension movements. Despite the similarities between single units within different sites, we found differences between pairs of units. Pairs of directionally selective units recorded by the same electrode in the same proximal site preferred similar reach directions but not grasp types, whereas pairs of object-selective units recorded in the same distal site tended to prefer the same grasp type but not reach direction. We suggest that the unexpected "mixing neurons" encoding reach and grasp within distal and proximal sites, respectively, provide a neural substrate for coordination between reach and grasp during prehension.

INTRODUCTION

During prehension, reaching and grasping are combined into coherent movement (Jeannerod 1984). Reaching mainly involves movements of proximal joints, the shoulder and elbow, whereas grasping entails shaping the hand by manipulating distal joints, the fingers. Distinct brain regions have been implicated in the control of reach and grasp. In the premotor cortex (PM), inactivation (Fogassi et al. 2001; Kurata and Hoffman 1994), anatomical (He et al. 1993; Tanné-Gariepy et al. 2002), and neurophysiological studies (Luppino and Rizzolatti 2000; Wise et al. 1997) have related the caudal part of dorsal PM (PMd) to proximal arm movements and the control of reaching, whereas the rostral part of ventral PM (PMv) has been implicated in distal hand movements and the control of grasping.

Neurophysiological studies of PM have employed a combination of intracortical microstimulation (ICMS) (Godschalk et al. 1995; Stoney et al. 1968), informal mapping techniques (Gentilucci et al. 1988; Graziano et al. 1997), and single-unit recordings (Kurata and Tanji 1986; Rizzolatti et al. 1988) to study the functional organization of PMd and PMv. Generally, a good match was observed between single neuron activity, ICMS, and other mapping results: neurons recorded at sites mapped as proximal modulated their activity with reach direction (Crammond and Kalaska 1996; Gentilucci et al. 1988), and neurons recorded at sites in which ICMS evoked finger movements modulated their activity with grasp type (Murata et al. 1997; Rizzolatti et al. 1988).

Several issues suggest that the neural representations of reaching and grasping in PM are not completely separate. First, recent studies have shown that within PMd, there are sites related to distal movements (Dum and Strick 2005; He et al. 1993; Raos et al. 2004) and that neural activity in the PMv may be modulated by reach direction (Schwartz et al. 2004). Second, there are spatial differences between single neuron recordings and ICMS: whereas the former are local, the latter affect larger areas (Tehovnik et al. 2006). Third, most experiments to date have studied reach (Caminiti et al. 1991; Shen and Alexander 1997) and grasp (Hepp-Reymond et al. 1994; Murata et al. 1997) using separate experimental paradigms.

Here we studied the functional organization of PM in intact behaving monkeys as both reach and grasp were varied systematically. We found that during prehension, proximal muscles were modulated by reach direction more than distal muscles, and distal muscles were modulated by grasp type more than proximal muscles. We expected that neurons recorded in sites from which ICMS evoked proximal movements would be reach-related and neurons recorded in distal sites would be grasp-related. However, we found that the proportion and properties of single-units related to reaching and to grasping were the same regardless of the responses elicited by threshold ICMS through the same electrode at the recording point.

METHODS

Animals and behavioral task

Two monkeys (*Macaca fascicularis*, females, *D* and *J,* 2.5 and 3.2 kg, respectively) were trained to perform unconstrained prehension movements. All surgical and animal handling procedures were according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996), complied with Israeli law, approved by the Ethics Committee of the Hebrew University, and supervised by a veterinarian.

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During recording sessions, the monkey was seated in a primate chair with its head held and left arm restrained. A touch pad with three buttons arranged in a row (each 1.3 × 1.3 cm, with tricolor LEDs) was located 12 cm in front of the monkey's right mid-clavicular line at chest level, and the monkey rested its right hand on the central button. This resting position permitted equal amplitude movements (6.5/7.5 cm, *monkey D/J,* the difference due to differences in the monkeys' sizes) in six directions, equally spaced in the horizontal plane (a "center → out" arrangement). In each trial, an object was presented in one of the six locations, at the same height as the touch pad. During each session, two of the three objects shown in Fig. 1*A* were used, for a total of 12 different task conditions. During prerecording sessions, we observed that when different objects are presented in the same location, horizontally orientated objects result in considerably less variation of forearm orientation than vertically oriented objects. We therefore presented objects horizontally, requiring a similar orientation of the shoulder, elbow, and wrist joints in a given location. Different finger configurations were essential for a correct grasp of each object. As shown in RESULTS, extrinsic digit muscles were indeed modulated much more strongly by grasp type than shoulder, elbow, and wrist muscles. The precision grip object, for instance, consisted of a groove 8 mm wide, into which only one of the monkey's fingers (diameter, 6 mm) could fit; in this groove, two plates were installed, each connected to a micro-switch. For a correct grasp, both micro-switches had to be depressed simultaneously, possible only by inserting one finger in front of the plates, another behind, and pinching them together. Other objects were designed with other electro-mechanical constraints and gave rise to other grip types (see Fig. 1*A* for more details). Grip force was not controlled.

A trial was initiated after the light-emitting diode (LED) of the central button of the touch pad was illuminated in green and the monkey was required to press that button only (*Ready;* Fig. 1*B*). After a short period varied uniformly between 500 and 1,000 ms, a robotic arm transported an object into the workspace, concealed from the monkey by a half-mirror. If the monkey continued pressing the button, illumination conditions changed (*Cue On*) so the object was briefly visible through the half-mirror (200–400 ms, until *Cue Off*). After a delay (1,000–1,500 ms), all LEDs were illuminated in orange (*Go Signal*), and extinguished once the button was released (*Movement Onset*). During the delay, *Go Signal*, and movement, the monkey could not see the target object or its hand. Reaction time (from *Go Signal* to *Movement Onset*) was limited to 500/1,000 ms (*monkey D/J;* the differences due to the monkeys' performance levels) and movement time (from *Movement Onset* to *Correct Grasp*) to 1,000/2,000 ms. Actual reaction times were 202/362 ms (*monkey D/J* medians; 95% ranges, 172–245/105-604 ms) and actual movement times were 312/798 ms (186–533/470-1,564 ms). Following a correct grasp, the monkey was required to hold the object for a variable duration (580–620/700-1,000 ms) after which another visual cue prompted the monkey to return its hand to the central button of the touch pad (*Hold End*), whereby a reward was administered (*Reward*). Successful trials were reinforced by a juice reward, and only these trials were analyzed. Trials were separated from one another by 2–3 s. Direction and object assignment to trials was pseudo-random, so that objects were presented an equal number of times in different directions. During each session, monkeys completed 363 of these trials (median of 43 sessions; range: 139–585). All results were consistent between monkeys and are therefore reported together unless otherwise specified.

Following training, water-filled glass beads were glued onto the skull surface over the left hemisphere under aseptic conditions (medetomidine hydrochloride (Domitor) and ketamine anesthesia). A localizing MRI scan (Biospec Bruker 4.7 T animal system, fast spin echo sequence; effective echo time, 80 ms; repetition time, 2.5 s; 0.5 × 0.5 × 2 mm resolution) was then performed. Chamber (22 × 44 mm) implantation (halothane anesthesia, induced by Domitor and ketamine) was guided by the relative positions of the beads and cortical landmarks. Analgesia [pentazocine (Talwin) and carprofen (Rymadil)] and antibiotics (ceftriaxone) were administered peri-operatively. The dura mater was left intact. Sulci locations relative to chamber were then determined by another MRI scan.

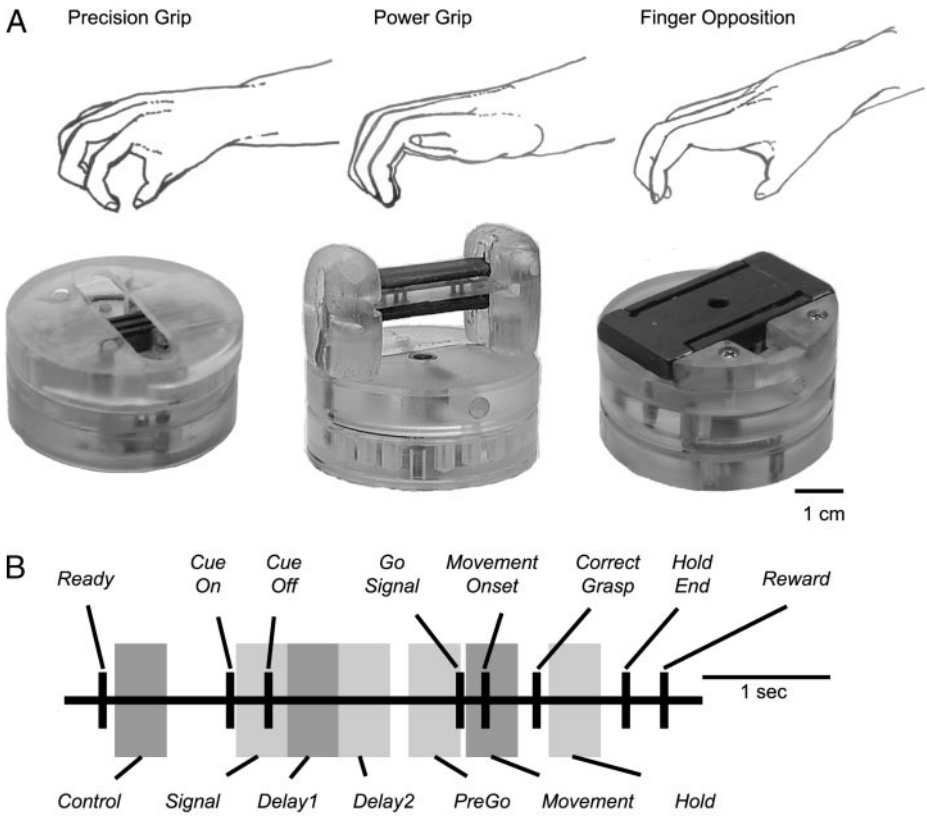

FIG. 1. Prehension task. *A*: grasp types and objects. Grips were drawn from video recordings of the monkeys performing the prehension task. Two objects were used in each session. In each trial, an object was presented in 1 of 6 locations arranged in a virtual circle around the central button of the touch pad. Each object was fitted with 2 micro-switches that made contact only when the object was grasped as shown. *Left*: precision grip. Two movable thin vertical plates were installed in a groove. They had to be pulled against a 3rd static plate for both micro-switches to close. *Middle*: power grip. Bottom and top black plates had to be pressed toward each other at the same time, accomplished by opposing the palm base and fingers. *Right*: finger opposition. Both the front plate (closer to the monkey) and the back plate (further away from the monkey) had to be pressed simultaneously, possible only by opposition of the thumb and 4 fingers. *B*: task description. The time sequence of a single trial is illustrated. Vertical lines: behavioral events; bars: analysis epochs. See METHODS for details.

Data acquisition and preprocessing

During each recording session, ≤16 glass-coated tungsten microelectrodes were employed (impedance 0.2–2 MΩ at 1 kHz). Electrodes were arranged in two circular guide tubes that were lowered down to ~1 mm above the dura mater (8 electrodes in each guide tube; inter-electrode spacing within tube ~300 μm; Double MT, Alpha-Omega Engineering, Nazareth, Israel). During each session, one guide tube was aimed toward PMd and another toward PMv. The border between primary motor cortex (M1) and PM was established based on stimulations made during separate mapping sessions (Fig. 4*A*): in M1 movements were typically evoked at low currents (≤40 μA) and visual responses during mapping were rare (for details of stimulation parameters and mapping protocol, see *Cortical mapping*). The border between PMd and PMv was defined as the line extending caudally from the arcuate spur (dotted lines in Fig. 4, *C* and *D*). Each electrode was lowered through the dura mater independently (EPS 1.31, Alpha-Omega Eng.) until spiking activity was encountered, inserted an additional distance into the cortex (median, 0.71 mm), and left in the same site during the entire recording and mapping session. The signal from each electrode was amplified (10,000), band-pass filtered (1–10,000 Hz), and sampled at 25 kHz (Alpha-Map 5.4, Alpha-Omega Eng.).

An off-line procedure was applied to identify and sort spike waveforms in the 25-kHz digitized traces. First, ~50-Hz line influences were removed by cycle-triggered averaging: the signal following AC polarity change in a main was averaged over many cycles and subtracted from the original signal in an adaptive manner. Second, spikes were detected by computing a modified second derivative (7 samples backward and 11 forward), accentuating "spiky" signal features (bimodal, skewed, and sharp). Segments that crossed a threshold (4.5 SDs from the mean derivative) were identified. Within each segment, the occurrence time of the putative spike was defined as the time of the maximal derivative. If a sharper spike was not encountered within 1.2 ms, then 64 samples, starting 10 before the peak, were extracted. We also detected spikes based on amplitudes (≥5 times the SD of the 300- to 6,000-Hz band-passed trace): spikes detected using the two methods were the same in 95% of the cases, and results were independent of the detection method. Third, extracted spike waveforms were linearly de-trended and aligned so each started at the point of maximal fit with two library principal components accounting, on average, for 93% of the waveform variance (Abeles and Goldstein 1977). Finally, waveforms were projected onto the principal component basis to arrive at two coefficients. These coefficients were subjected to manual spike-sorting in the environment of Alpha-Sort (4.0, Alpha-Omega Eng.).

Eye movements were recorded using an infra-red beam system tracking movements of one eye (Oculometer, Dr. Bouis, Karlsruhe, Germany). The horizontal and vertical signals from this system were sampled at 400 Hz and low-pass filtered (40 Hz). Behavioral events (LEDs, switches, lights, and so on) were sampled at 6 kHz. The workspace and monkey's movements were monitored using three infra-red cameras and recorded on VHS tapes.

During some sessions intra-muscular electromyograms (EMGs) were recorded in parallel to neurons. Wire pairs were inserted into shoulder (acromion deltoid, latissimus dorsi, pectoralis major), elbow (biceps brachii, brachialis, triceps brachii), wrist (extensor carpii radialis longus, extensor carpii ulnaris, palmaris longus), and extrinsic digit (extensor digitorum communis, flexor digitorum profundus, flexor digitorum sublimis) muscles. Wire positions were verified by stimulation (90-ms trains of 0.2-ms biphasic pulses at 330 Hz) at currents of 50–500 μA. The signals from these wires were amplified (30,000), band-pass filtered (30–3,000 Hz), and sampled at 6.25 kHz. Root mean square (RMS) values of EMG were computed by raising signals to the second power, applying a low-pass filter (100 Hz), and taking the square root. Each muscle was recorded during 2–10 separate sessions for a total of 61 recordings; each recording was 184–527 (median, 414) trials long. Different recordings of the same muscle were consistent (correlation-coefficient between mean activity in each of the 12 task conditions, 0.86 ± 0.05, mean ± SE; 164 pairs of same-muscle recordings).

Cortical mapping

During each session, immediately following neural recordings and without moving the electrodes, two standard methods were used to characterize each electrode's recording site: threshold ICMS and sensory-motor mappings (SMM). In the SMM, we assessed recording site properties by listening to the modulation of the multi-unit rustle of each electrode during proprioceptive input (passive movements of individual joints, palpation of muscles), tactile input (stroking of skin, stroking of facial skin while eyes were covered), visual input (3 dimensional objects, LEDs, hand movements of the experimentalist; at reaching distance and beyond), and active movements (see Gentilucci et al. 1988; Graziano et al. 1997; Kakei et al. 2001; Schwartz et al. 2004 for similar SMM protocols). The latter were examined using a Kluver board variant: a Perspex plate with nine holes in three rows, 6.5 cm apart. Each hole contained a small food pellet and was covered by an object requiring a different grasp (ball, cork, plate, string, and so on). The SMM in each site was summarized by two properties: response modality (motor, somatosensory, and/or visual) and organ (elbow, eye, face, finger, multi-joint, mouth, pinna, shoulder, and wrist were observed).

In the ICMS we noted, for each electrode's recording site separately, the movement elicited by the lowest possible current during at least half of the stimulation trials (0.2-ms biphasic pulses at 330 Hz for 90 ms at currents of 5–90 μA) (Godschalk et al. 1995; see Gentilucci et al. 1988; Kakei et al. 2001 for similar ICMS protocols employed in PM). Movement was summarized by two properties: movement type (flexion, extension, adduction, abduction, opposition, and so on) and organ (same as SMM organs in the preceding text). We classified evoked forelimb movements as follows: *proximal*: movements around the shoulder and elbow flexion/extension; *wrist*: forearm supination/pronation and wrist movements; and *distal*: finger movements. Only results from proximal and distal sites are reported in detail in this paper. Although at suprathreshold currents we sometimes observed complex multi-joint movements (Godschalk et al. 1995; Graziano et al. 2002), lower currents always yielded single-joint movements with the exception of distal movements that combined several fingers in 2/3 of the cases. We did not observe transitions from proximal to distal (or vice versa) movements as current was changed.

Neural database and data analyses

One baseline (control) and six task epochs were defined for analyses, all 400 ms long (Fig. 1*B,* bars). During the *Control* epoch, starting 100 ms after *Ready*, the monkey did not know in which direction it would have to reach and what type of grip it would have to use. During the *Signal* epoch, starting 50 ms after *Cue On*, the identity and location of the target objects was briefly visible yet no movement was required. During *Delay* epochs, starting 450 ms after *Cue On*, there was no visual cue and no movement was required. These exact same conditions were maintained throughout the *PreGo* epoch (starting 400 ms prior to the *Go Signal*). The *Movement* epoch started 150 ms before the hand left the touch pad, and the *Hold* epoch started 100 ms after a *Correct Grasp*. Note that the *Delay*2 and *PreGo* epochs overlap on average by 100 ms, and the *Movement* epoch includes both reaction and movement time (as in Weinrich and Wise 1982).

Units fulfilling the following criteria were analyzed. *1*) *Anatomy*. Only units recorded at PM sites were considered. *2*) *Mapping*. Only units recorded at sites classified as proximal or distal by ICMS and/or SMM were included. *3*) *Isolation.* Quality of single-unit isolation was determined by the homogeneity of spike waveforms, separation of the

projections of spike waveforms onto principal components during spike-sorting and clear refractory periods in ISI histograms. Only well-isolated units were considered. *4*) *Number of trials*. Each unit had to be recorded for at least five trials per task condition and exhibit stationary activity. This was determined by visual inspection of mean firing rates and raster plots of individual trials. *5*) *Firing rate*. Only units with mean firing rates $\geq$1 spike/s during at least one task epoch were used. *6*) *Task dependency*. Spike counts for all task conditions together, during the *Control* and during each task epoch, were compared using a two-sample, two-tailed *t*-test. Only units with *P* values $<$0.01/6 during at least one of the six epochs were included.

We made a total of 617 PM penetrations, 531 during recording sessions, of which 378 were in sites classified as proximal or distal. A total of 724 units, recorded from 326 of these sites, passed the preceding criteria (Table 1, 1st 3 columns, lists the sites, units, and their classification). Although the number of units varied when changing parameters used for inclusion criteria 4–6, results were not sensitive to specific parameter values. These units were recorded during 70–582 (median, 257) trials.

For each unit a preferred direction (PD) and a preferred object were estimated. PDs were estimated by summation of six vectors: the direction of each vector was the instructed movement direction, and its amplitude was the mean spike count over all trials in that direction. The direction of the vector sum is the PD. Estimates of PDs made by fitting a cosine function were virtually identical (mean absolute PD difference, 2.7 $\pm$0.28°, mean $\pm$ SE; 724 units). A preferred object was defined as the object that elicited the maximal spike count.

We used a two-way ANOVA, with direction and object as factors, to determine relation of neural activity to task parameters (effects were considered significant at $P < 0.01$ levels). This analysis assumes that spike counts distribute normally: because spike counts are nonnegative and distribute as a Poisson counting process, for some units a normal distribution was not a good approximation (Bera-Jarque test of normality: 76/724 units with $P < 0.01$). We therefore repeated analyses using a nonparametric ANOVA (2-way Kruskal-Wallis test); results were almost identical for the two ANOVA tests. Because vector summation was used to estimate PDs and ANOVA to determine significance of directional tuning, we compared the vector summation PD estimate with a discrete estimate, the direction in which a unit fired maximally: the mean absolute difference between the two estimates was 30 $\pm$ 0.9° (SE) (724 units). Thus for the neural data used in this study, an ANOVA is a reasonable method for estimating the significance of tuning. The same results were obtained when estimating significance using a resampling test (Crammond and Kalaska 1996).

To estimate effect sizes we computed eta-squared, defined as $\eta^2 = \sigma^2_{\text{effect}}/\sigma^2_{\text{total}}$ (Fisher 1925). Effect variance is defined as the variance of the mean discharge in each relevant task condition (e.g., σ^2_{task} is the variance of 12 numbers, and σ^2_{dir} is the variance of 6 numbers). η^2 is a unit-less measure of the fraction of the total variance associated with an effect. If the signal-to-noise ratio (SNR) is defined as $\sigma^2_{\text{effect}}/(\sigma^2_{\text{total}} - \sigma^2_{\text{effect}})$ then η^2 equals SNR/(1 + SNR) and is thus a monotonic function of the SNR. η^2 is bounded between 0 and 1 and accounts for linear and nonlinear effects. Because η^2 is additive for different effects, the total variance associated with the prehension task parameters is equal to the sum of direction, object, and interaction effect sizes: $\eta^2_{\text{task}} = \eta^2_{\text{dir}} + \eta^2_{\text{obj}} + \eta^2_{\text{int}}$. To compare variance associated with direction with that not associated with direction alone, we employed a "reach-grasp index": RGI = $(\eta^2_{\text{dir}} - \eta^2_{\text{obj}} - \eta^2_{\text{int}})/\eta^2_{\text{task}}$. This index equals 1 when there is only a direction effect and -1 when there is no direction effect.

We used multiple linear regression, model $F^{u,t} = b_0 + b_1 \cdot X^t + b_2 \cdot Y^t + \varepsilon^t$, to test for relations between neural activity and eye positions. In this model, *F* is the spike count of unit *u* during the relevant epoch of trial *t*, *X* and *Y* are the horizontal and vertical eye positions during the same time, and ε is an error term. All values were standardized prior to regressing (the mean subtracted and divided by the SD) for each of the 12 task conditions separately to remove possible task-related effects.

RESULTS

Proximal and distal muscles are active differently during prehension

We measured the activity of shoulder, elbow, wrist, and extrinsic digit muscles during the prehension task (Fig. 1) as monkeys reached in different directions and grasped various objects. Muscles were generally quiescent before the *Go Signal* and became active just before *Movement Onset* as the monkey's hand released the touch pad (Fig. 2). To quantify the temporal relations between reach and grasp movement components and muscle activity, we identified, for each muscle, the time of peak activity. Activity of all muscles peaked during movement, and activation of proximal (shoulder and elbow) and distal (extrinsic digit) muscles overlapped (Fig. 3*A*). However, activity of proximal muscles peaked 125 ms after *Movement Onset*, whereas activity of distal muscles peaked 221 ms

TABLE 1. *Reach-grasp indices (RGIs) during the Movement epoch*

Parsing Criterion	Number of Sites	Number of Units	Median RGIs	*P* Value (*U* Test)
ICMS & SMM				
(proximal/distal)*	246/80	551/173	0.35/0.36	0.46
Positive ICMS (proximal/distal)	133/44	302/97	0.34/0.38	0.88
Negative ICMS (proximal/distal)	113/36	249/76	0.36/0.21	0.18
Dorsal/ventral regions†				
Monkey D	137/105	320/238	0.3/0.3	0.63
Monkey J	51/33	100/66	0.56/0.44	0.24
Both	188/138	420/304	0.36/0.36	0.31
Caudal/rostral sites‡				
Dorsal	94/94	202/218	0.41/0.34	0.35
Ventral	69/69	154/150	0.38/0.29	0.35
Both	163/163	356/368	0.38/0.33	0.17
PMd/PMv§				
Monkey D	185/57	438/120	0.3/0.28	0.86
Monkey J	51/33	100/66	0.56/0.44	0.24
Both	236/90	538/186	0.34/0.38	0.99

*The criterion used throughout this study. †Regions were defined as dorsal/ventral to the dashed lines in Fig. 4. ‡In each region, sites were divided into two equal sets based on distances from the central sulcus. §Areas were defined as dorsal/ventral to the acruate spur (dotted line in Fig. 4).

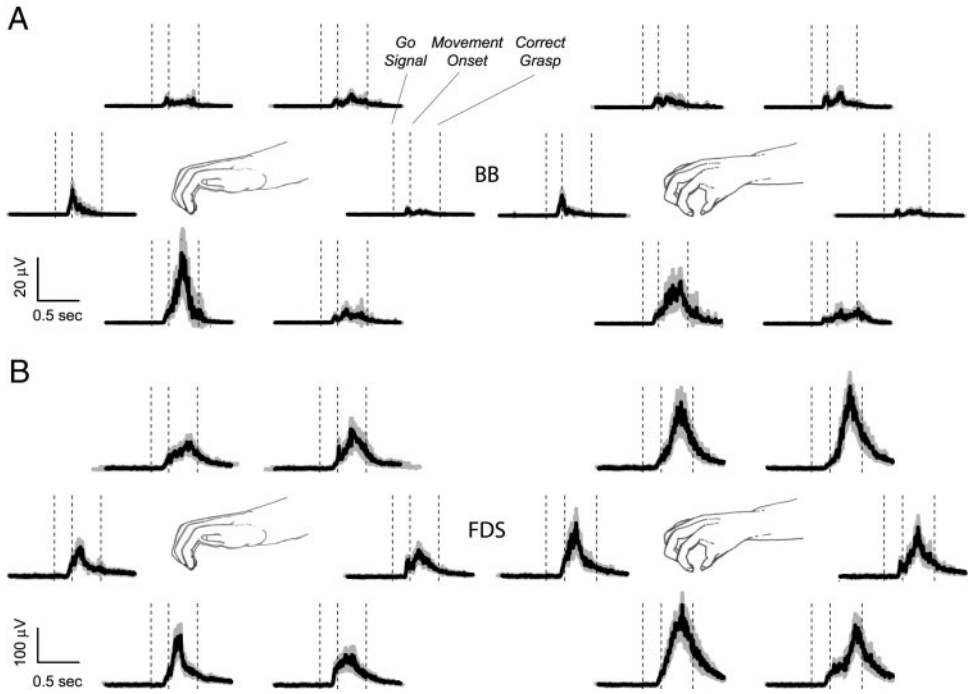

FIG. 2. Muscle activity. *A*: activity of an elbow muscle (BB, biceps brachii). Direct stimulation of the muscle resulted in elbow flexion and forearm supination. *Left*: activity during power grip trials; *right*: activity during precision grip trials. Each of the 6 panels in a set is shown in the direction corresponding to the movement direction in the actual trials. RMS activity of every trial was aligned on *Movement Onset* and averaged over trials for each direction and grip type separately (black; gray shadings show 95% confidence intervals, computed separately for each sample). In each panel, vertical dashed lines indicate mean event times. For both grip types, the muscle is most active during movements toward the monkey and slightly to the left [reach-grasp index (RGI), 0.9]. *B*: activity of an extrinsic digit muscle (FDS, flexor digitorum sublimis). Conventions are the same as in *A*. Direct stimulation of this muscle resulted in flexion of the index finger alone. For each direction, this muscle was active during a precision grip to a higher degree than during a power grip (RGI, 0.05).

after *Movement Onset* (medians; Mann-Whitney *U* test: $P < 0.001$). Similar results were obtained when the time of median (instead of peak) activity was considered. Thus although activation times of proximal and distal muscles were not completely distinct, activity of proximal muscles tended to peak before the activity of more distal muscles.

To quantify the modulation of muscle activity by reach and grasp during the *Movement* epoch, we employed a reach-grasp index (RGI) that equaled 1 when all task-related variance was attributed to reach direction and −1 when all task-related variance was related grasp type (see METHODS). For instance, the biceps brachii (an elbow flexor and forearm supinator; Fig. 2*A*) had an RGI of 0.9, reflecting a dominant influence of reach over grasp, whereas the FDS (an extrinsic digit flexor; Fig. 2*B*) had an RGI of 0.05, reflecting a more balanced effect of reach and grasp. Proximal muscles had consistently higher RGIs than distal muscles (medians: 0.85 and 0.15, respectively; Mann-Whitney *U* test: $P \ll 0.001$). In fact, the distributions of RGIs of proximal and distal muscles were completely distinct (Fig. 3*B*). Wrist muscles had high RGIs, similar to the proximal muscles (median: 0.94; *U* test: $P = 0.11$). This is in accordance with the experimental design where all grasp types at a given location required the same wrist orientation. Thus in the prehension task, proximal and wrist muscles showed larger effects of reach direction than did distal muscles, while distal muscles showed larger effects of grasp type.

Proximal and distal sites aggregate in different regions of premotor cortex

We mapped the PM using two methods: ICMS and SMM. During ICMS, we identified the weakest (threshold) current that evoked perceptible movement by stimulating a given site, and during SMM, we listened to the multi-unit rustle during passive and active manipulations (see METHODS). We made a total of 617 electrode penetrations in PM. Movements were observed following ICMS in 288 of these sites (47%; Fig. 4*B*. The mean threshold for obtaining a response was 58.7 ± 1.4 (SE) μA. SMM yielded clear classification in 549 of the sites (89%). In 68 sites (11%), no movement was evoked and no specific organ could be determined. There was a good correspondence between the two methods: when both yielded positive results, the ICMS organ was usually the same as the SMM organ (283/288 sites, 98%).

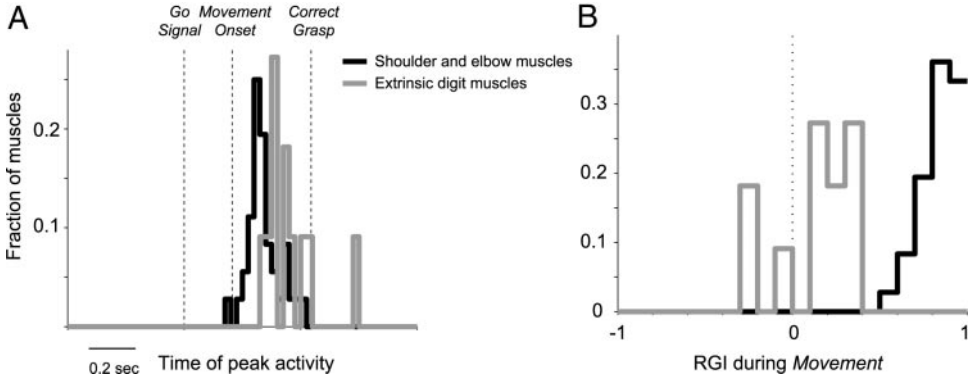

FIG. 3. Muscle properties. *A*: time of peak activity of individual muscles. Horizontal axis measures time (bin size, 25 ms) and vertical axis measures the fraction of muscles with peak activity in the corresponding bin (36 recordings of shoulder and elbow muscles and 11 recordings of extrinsic digit muscles). Vertical dashed lines indicate the mean event times. Proximal muscles were activated ~100 ms before distal muscles. *B*: RGIs of muscles during the *Movement* epoch. Horizontal axis measures RGIs (bin size, 0.1) and vertical axis measures the fraction of RGIs in the corresponding bin. Muscles are the same as in *A*. Proximal muscles were related mainly to reach direction while distal muscles were also related to grasp type.

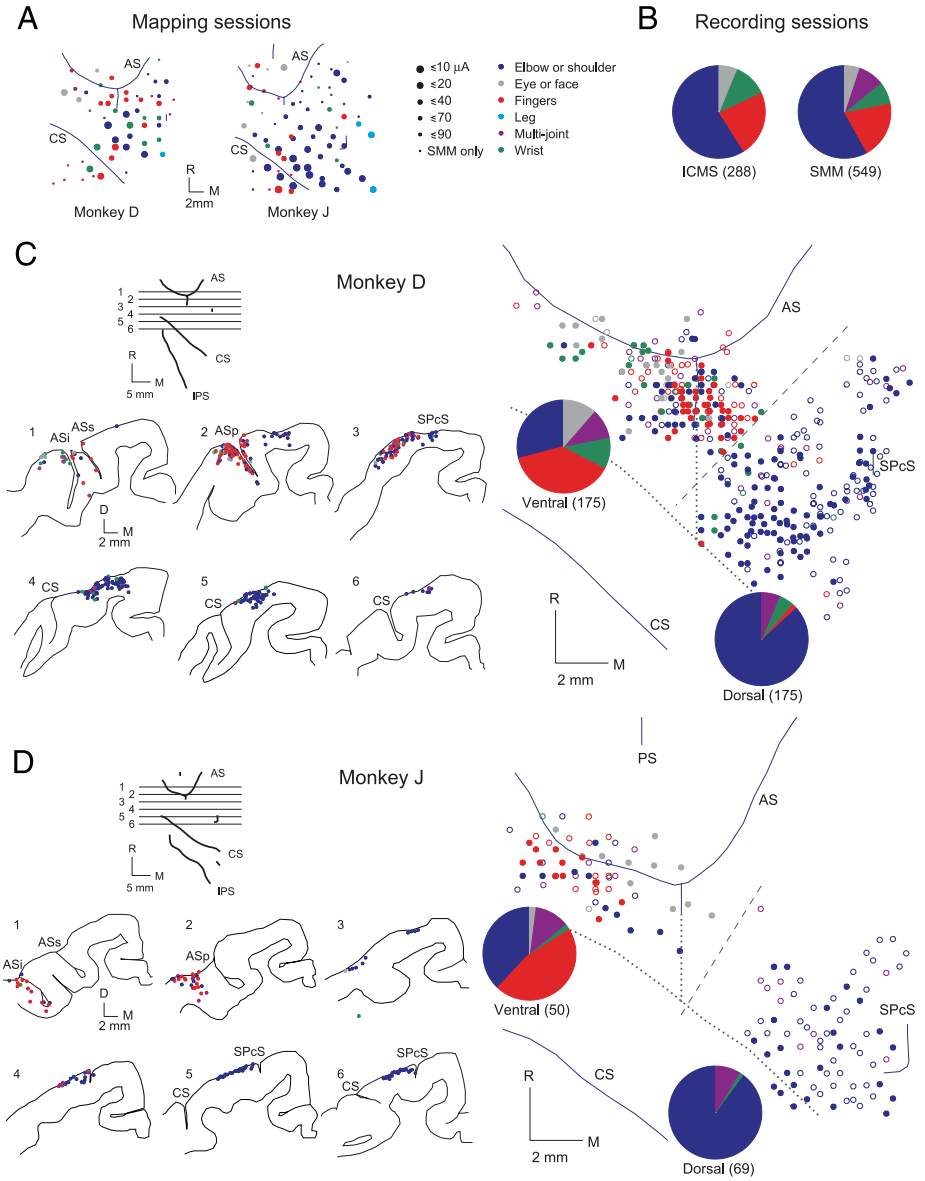

FIG. 4. Cortical maps of 2 monkeys. *A*: surface maps reconstructed from MRI scans for *monkeys D* and *J*. Each dot represents 1 site, tested by intracortical microstimulation (ICMS) and sensory-motor mappings (SMM) during prerecording mapping sessions. Dot color and size correspond to body part and ICMS current, respectively. *B*: distributions of body parts mapped by ICMS and SMM in both animals during recording sessions. The total number of sites included is shown in parentheses. *C* and *D*: coronal sections and surface maps of sulci reconstructed from MRI scans for *monkeys D* and *J*. *Insets*: location of the coronal sections on the surface maps. In the coronal sections, each dot represents 1 site, and its color corresponds to the body part. In the surface maps, sites mapped by ICMS (full circles) and SMM (empty circles) are distinguished. Dotted lines show borders between M1 and dorsal and ventral premotor cortex (PMd and PMv; see METHODS). Dashed lines show borders between the sampled regions, and pie charts show distributions of sites in each region (see RESULTS). In both monkeys, the base of the inferior limb of the arcuate sulcus (AS) is about 2 mm rostral to the surface location, so sites apparently rostral to the AS were deep and caudal to the AS and thus within PM. ASp, arcuate spur; ASs, AS superior limb; CS, central sulcus; D, dorsal; IPS, intra-parietal sulcus; M, medial; PS, principal sulcus; R, rostral; SPcS, superior precentral sulcus.

We did not attempt to map the entire PM cortex. Instead, we focused on regions in which proximal (shoulder or elbow) or distal (finger) forelimb representations were found. Two attributes of the resulting maps are noteworthy (Fig. 4, *C* and *D*). First, in each monkey, there was one region consisting mainly of shoulder and elbow sites (henceforth designated as proximal sites) and a separate region in which finger sites (henceforth designated as distal sites) were most common. Second, in both monkeys, proximal sites were interspersed within the primarily distal region, whereas the opposite was very rare, and multi-joint and wrist sites were scattered among both proximal and distal regions.

To formally quantify these observations, we divided recording sites, in each monkey separately, into two regions using a *K*-means algorithm ($K = 2$). Note that this partition is based only on the relative locations of the sites. The borders between the resulting regions are shown by dashed lines in Fig. 4, *C* and *D*, and the distributions of sites within each region by the pie charts. In both monkeys, most proximal sites were located in the dorsal regions (75 and 77%, *monkeys D* and *J*, respectively), and most distal sites were in the ventral regions (96 and 100%). In both monkeys, the dorsal regions roughly corresponded to the PMd. In one monkey, the region with the predominantly distal sites corresponded to the rostral PMv (Fig. 4*D*), and in the other monkey, to a region intermediate between the PMd and the PMv (Fig. 4*C*). In both monkeys, there was a gross anatomical separation between the bulk of proximal and distal sites.

Single-units in proximal and distal sites are active similarly during prehension

We recorded the activity of 724 task-dependent PM units, 551 (76%) from sites classified as proximal and 173 (24%) from distal sites. These two sets of units form the neural database for all analyses. As may be expected, some single-units displayed activity that was consistent with recording site properties. These included reach-related activity of units recorded in proximal sites (Fig. 5*A*) and grasp-related activity of units recorded in distal sites (Fig. 5*B*). However, other units

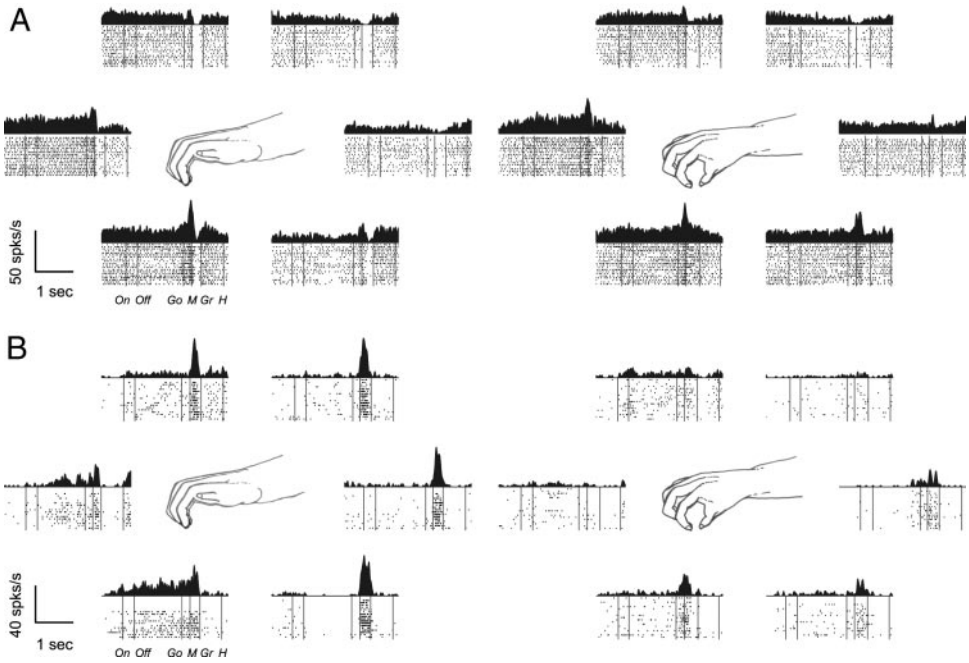

FIG. 5. Single-unit activity consistent with the mapping results. *A*: raster displays and peri-event time histograms (PETHs) of a unit recorded in a proximal site in PMd where ICMS resulted in shoulder extension. Panels are laid out as in Fig. 2*A*. *Bottom part* of each panel shows spike times (short black lines) and behavioral events (long gray lines, from left to right: *On, Cue On; Off, Cue Off; Go, Go Signal; M, Movement Onset; Gr, Correct Grasp; H, Hold End*). *Top*: PETH obtained by averaging the single trial spike times and convolving with a Gaussian kernel (SD, 15 ms). The unit was most active during the *Movement* epoch of trials in which movement direction was left, regardless of grip type (RGI, 0.8). *B*: activity of a unit recorded in a distal site in PMv, where ICMS resulted in flexion of all fingers. For each and every direction, the unit's discharge during the *Movement* epoch was stronger for a power grip than for a precision grip (RGI, −0.56).

displayed activity that was unexpected in terms of the mapping responses of the corresponding recording sites. For instance, the unit the activity of which is shown in Fig. 6*A* was recorded in a proximal site but exhibited grasp-related activity in addition to reach-related activity. Still other units had discharge patterns that were completely inconsistent with the mapping results; for example, strictly reach-related activity of a unit recorded in a distal site (Fig. 6*B*).

Single-units were active throughout the task. To assign specific values to the time of activity, we measured the time of peak activity of single units: for 318/724 (44%) units, activity peaked prior to movement. In contrast to muscles, the time of peak activity of neurons recorded in proximal and distal sites did not differ (Mann-Whitney *U* test: $P = 0.76$; Fig. 7*A*). The same results were obtained when times of median activation were considered instead of the peaks. To quantify and compare the involvement of units recorded in proximal and distal sites in the prehension task at the same time muscles were active (that is, during the *Movement* epoch), we employed the RGI. In contrast to muscles, units in proximal and distal sites had similar RGI values (median RGIs: 0.35 and 0.36; *U* test: $P = 0.46$; Fig. 7*B*). Thus units were very different from muscles in terms of activation time, activation sequence, and relative contribution of reach direction and grasp type to their activity (compare Figs. 3 and 7).

We further tested how many units modulated their activity by reach direction and/or grasp type. During the *Movement* epoch, the fractions of units recorded in proximal and distal sites with a significant direction effect (2-way ANOVA, $P < 0.01$) were roughly the same (386 and 126 units in proximal

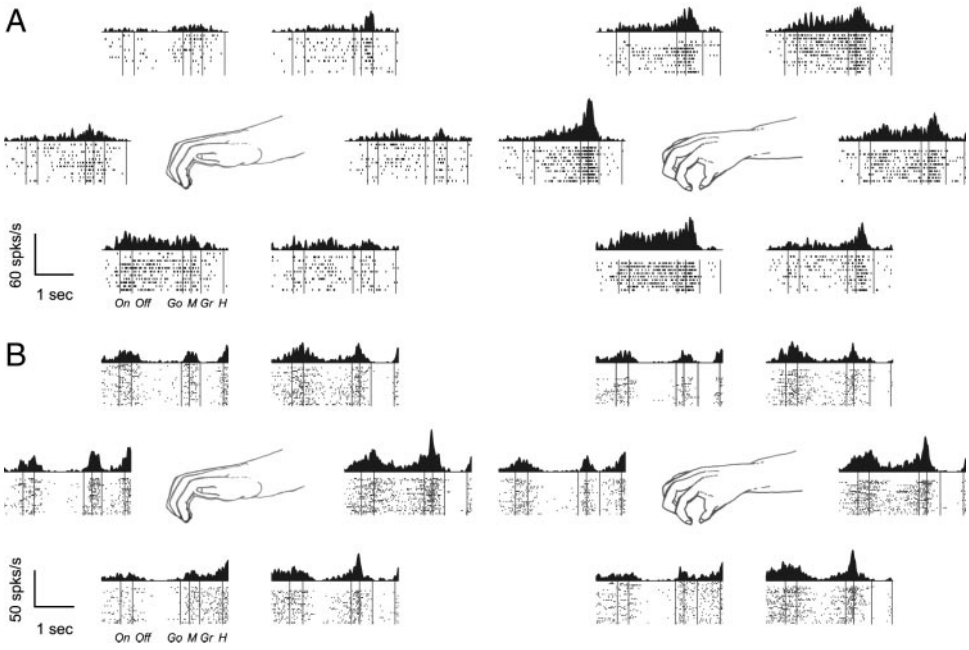

FIG. 6. Single-unit activity inconsistent with the mapping results. *A*: activity of a unit recorded in a proximal site in PMd where ICMS resulted in elbow extension. Yet for each direction, the unit's discharge during a precision grip was stronger than during a power grip (RGI, −0.65). Conventions are the same as in Fig. 5. *B*: activity of a unit recorded in a distal site in PMv where ICMS resulted in flexion of fingers 2–5. Regardless of grip type, the unit's discharge was strongest during movements to the right (RGI, 0.65).

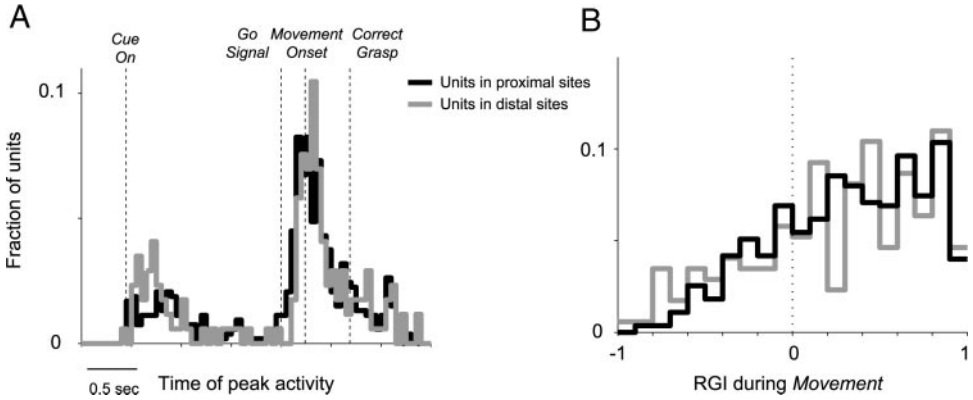

FIG. 7. Single-unit properties. *A*: time of peak activity of single-units (551 and 173 units recorded in proximal and distal sites, respectively; bin size, 50 ms). Activity of units in proximal and in distal sites peaked throughout the task. *B*: RGIs of single-units during the *Movement* epoch. Conventions are the same as in Fig. 3*B*. Units in proximal and distal sites alike were related to reach and grasp.

and distal sites, respectively; 70 and 73%; χ^2 test of independence: $P = 0.77$; Fig. 8*A, left*). The fractions of units in proximal and distal sites with an object effect were equal (29% in both sets of units; χ^2 test: $P = 0.99$; Fig. 8*A, right*). Finally, the fractions of units in proximal and distal sites that exhibited interaction effects were similar one to another (25 and 32%; χ^2 test: $P = 0.14$). Thus the probability of observing single-unit activity related to task parameters did not correlate with recording site classification.

Similarity of fractions of units with significant direction, object, and interaction effects in proximal and distal sites does not necessarily imply that task parameters are encoded equally by individual units in the two populations. To quantify encoding strength, we employed a measure of effect size ranging from 0 to 1 (see METHODS). During the *Movement* epoch, effect sizes for direction, η^2_{dir}, among units with a significant direction effect were roughly the same for units in proximal and distal sites (means, 0.19 and 0.21, respectively; Mann-Whitney *U* test: $P = 0.5$; Fig. 8*B, left*), and so were the effect sizes for object, η^2_{obj} (0.07 and 0.09 for units in proximal and distal sites, respectively; *U* test: $P = 0.08$; Fig. 8*B, right*). The effect sizes for interaction, η^2_{int}, were also similar for units in proximal and distal sites (0.09 and 0.11; *U* test: $P = 0.27$). Moreover, the distributions of preferred directions (PDs) of units in proximal and distal sites were both uniform (Rayleigh test: $P = 0.11$ and $P = 0.34$; Fig. 8*C*). Both distributions of preferred objects were similar to the distributions expected based on the numbers of units with significant object effects that were tested with each object (χ^2 test: $P = 0.68$ and $P = 0.59$, units in proximal and distal sites; Fig. 8*D*). In short, encoding of reach and grasp parameters by units in proximal sites during the *Movement* epoch appeared to be similar to encoding of those parameters by units in distal sites in all tested aspects.

Reach and grasp encoding by units in proximal and distal sites: spatial and temporal considerations

As reported in the preceding section, similar properties were observed for single-units recorded in proximal and distal forelimb representations as determined by ICMS and/or SMM (Table 1, 1st row). The same results were obtained when considering only units recorded at sites in which ICMS was positive (Table 1, 2nd row) or when considering only units recorded at sites in which ICMS was negative but SMM yielded clear classification (Table 1, 3rd row). Moreover, similar results were observed when parsing sites according to anatomical criteria such as dorsal versus ventral regions, caudal versus rostral parts of the PM, or PMd versus PMv, for each monkey separately and for the two monkeys together (Table 1). Specifically, the properties of units recorded in distal sites in the PMd and in the PMv were the same, and the properties of units recorded in proximal sites within the primarily distal regions were the same as for the rest of the units.

Using multiple regression analysis, we tested the influence of gaze angle, reaction time, and/or movement time on neural activity (APPENDIX). We found that single-unit activity during the *Movement* epoch was only weakly modulated by these parameters and to a similar extent as during the *Control* epoch. Thus similarities between units in proximal and distal sites

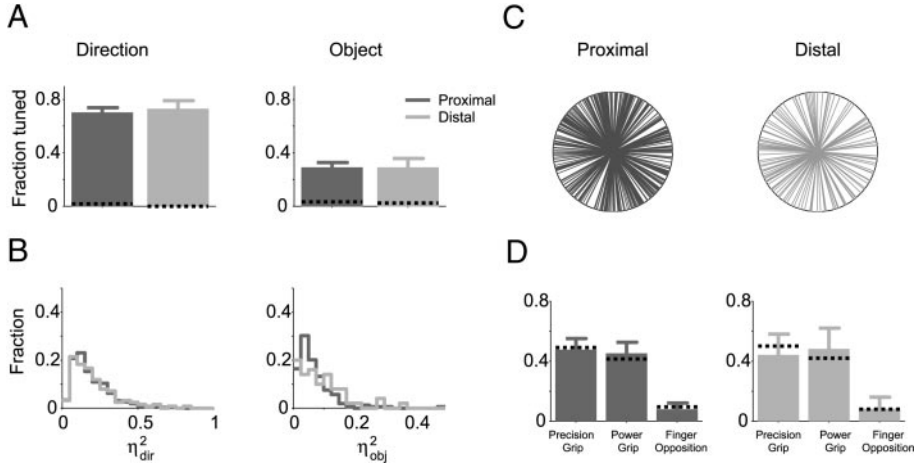

FIG. 8. Details of single-unit properties during the *Movement* epoch. *A*: fractions of single-units tuned to direction (*left*) and to object (*right*). Error bars show 95% binomial confidence levels. Fractions were not significantly different between units in proximal and distal sites (551 and 173, respectively). For reference, the corresponding fractions during *Control* are also shown (dotted lines). *B*: effect sizes of direction and object. Population is all units with a significant effect of direction (*left;* 386 and 126 units in proximal and distal sites, respectively; bin size, 0.05) or object (*right;* 159 and 50 units in proximal and distal sites; bin size, 0.025). *C*: distributions of PDs for the directionally tuned units in proximal and distal sites. *D*: distributions of preferred objects for the object-tuned units in proximal and distal sites. Bars correspond to the fraction of tuned units preferring a given object, and dotted lines show the fraction of tuned units tested with each object. Error bars show 95% binomial confidence levels.

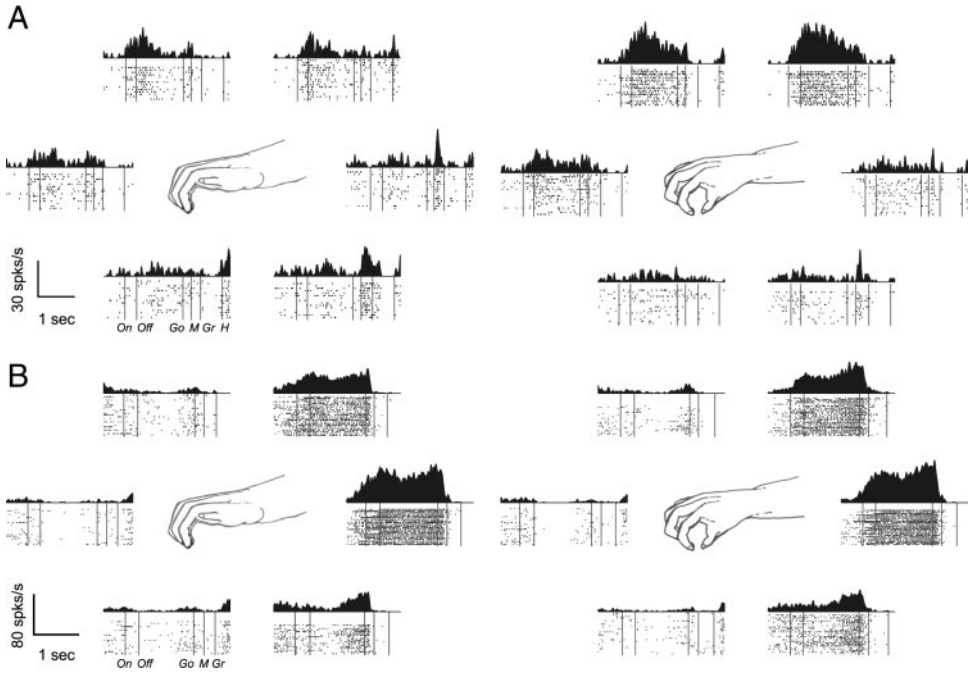

FIG. 9. Single-unit activity inconsistent with the mapping results during the *Delay* epochs. *A*: unit was recorded in a proximal site in PMd where ICMS resulted in shoulder external rotation, and yet its discharge before a precision grip was stronger than before a power grip (RGI, −0.16). *B*: unit was recorded in a distal site in PMv where ICMS resulted in finger flexion, but firing was modulated mainly by movement direction (RGI, 0.98).

observed during the *Movement* epoch are unlikely to result from modulations by the tested parameters.

Single-unit activity was not limited to the *Movement* epoch (Fig. 7*A*). Most units modulated their activity by reach direction (668/724 units, 92%) or grasp type (441 units, 61%) during at least one of the six task epochs (excluding *Control*, Fig. 1*B*). Some units exhibited activity that was confined to specific periods, such as the *Delay* epochs (Fig. 9), whereas others were modulated during several epochs. In the latter case, tuning properties were usually retained. For instance, during the *Movement* epoch, the RGIs of the units illustrated in Figs. 5 and 6 were 0.8, −0.56, −0.65, and 0.65, and during the delay, the corresponding values were 0.79, 0.09, −0.23, and 0.99. Over the entire sample of units, the mean between-epoch RGI difference was 0.032 ± 0.0086 (SE) (724 units). Thus RGIs of a given unit were quite consistent throughout the trial. Moreover, tuning specifics were consistent. For instance, 184 units (25%) had significant directional modulation that persisted from *Signal* to *Movement* epochs, and the mean between-epoch correlation-coefficient was 0.75± 0.02 (184 units; computed between mean spike counts in 6 directions for each pair of epochs), with an inter-epoch PD difference of 38 ± 2.3° (mean ± SE).

During all task epochs, single-unit properties (fractions of units with significant direction, object, and interaction effects and effect sizes) were essentially the same for the samples of units recorded from proximal and distal sites. In particular, RGIs of units in proximal and distal sites were similar one to another during all epochs (2-way nonparametric ANOVA on RGI values, site effect: $P = 0.66$; site/epoch interaction effect: $P = 0.16$; Fig. 10). Notably, single-unit activity became more grasp-dependent during the *Hold* epoch (ANOVA, epoch effect: $p \ll 0.001$, corrected for multiple comparisons) regardless of the recording site (post hoc *U* test, *Hold* epoch RGIs, units in proximal vs. distal sites: $P = 0.26$). Thus single-unit activity seemed to be modulated by reach direction and grasp type regardless of the recording site during all task epochs.

Consistent units tend to be anatomically aggregated

Differences not directly related to the encoding of reach and grasp might distinguish units with properties consistent with the recording site (for instance, reach-related units recorded in proximal sites) from units with inconsistent properties (for instance, reach-related units recorded in distal sites). To test this possibility, we defined consistent/inconsistent units according to RGI values of all 724 units (consistent unit: unit in proximal site with RGI value in the upper 3rd of all RGIs during the *Movement* epoch or unit in distal site with RGI in the lower 3rd; inconsistent unit: unit in distal site with RGI value in the upper 3rd or unit in a proximal site with RGI in the lower 3rd; 249 consistent, 234 inconsistent). Because none of the units that were recorded together with muscles expressed postspike facilitation effects (114 spike-triggered averages, estimated using ≥1,000 triggers/average, 30 ms before and after the spike) (Fetz and Cheney 1980), we could not test the

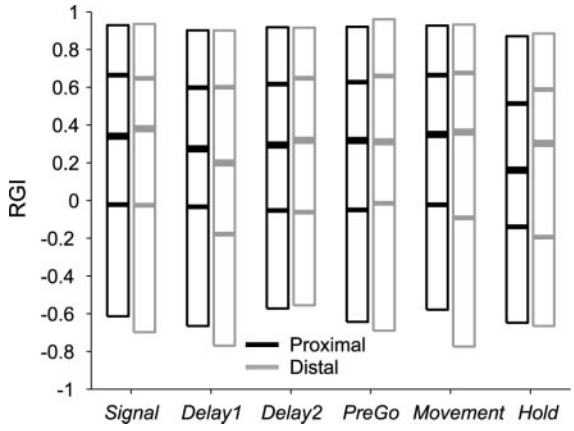

FIG. 10. Distributions of single-unit RGIs during various task epochs. In each box plot, thickest lines show medians, adjacent thin lines show quartiles, and thinnest lines (*top* and *bottom*) delimit 95% ranges. RGIs were not significantly different between units recorded in proximal and distal sites (see RESULTS).

tendency of consistent units to have direct output (corticomotoneuronal) connections. We examined other features that included firing rates during the *Control* epoch (spike/s), trial-to-trial variability (of firing rates), spike waveform peak-to-peak amplitude (μV), ICMS thresholds (μA), and recording depth (mm). However, no significant differences between consistent versus inconsistent units were observed for *any* of the tested features.

Even if all properties of individual neurons were identical for proximal and distal sites, it would still be possible for groups of neurons to be organized differently with respect to PDs and preferred objects in proximal and in distal sites. To test this possibility we estimated PD differences (PDDs) among pairs of directionally tuned units in proximal and distal sites separately. During the *Movement* epoch, the mean PDDs among pairs of directionally tuned units recorded in the same proximal site, that is, by the same electrode, was 62.7 $\pm$ 3.4° (SE) (235 pairs), smaller than the mean PDDs for all possible pairs of directionally tuned units in proximal sites, which was 89.2 $\pm$ 0.24° (SE) (47,278 pairs; *U* test: $P \ll 0.001$). This property was observed for proximal sites during all epochs (Fig. 11*A, left*) but was weaker or absent among distal sites (Fig. 11*A, right*) and was maintained even when differences in the number of proximal and distal sites were accounted for.

An opposite pattern was seen for preferred objects, which tended to be identical among pairs of units recorded in the same distal but not proximal site. During the *Movement* epoch, preferred objects were the same in 70% (26/37) of the pairs of object-tuned units recorded in the same distal site, significantly more than the fraction of identical preferred objects among all possible pairs of object-tuned units in distal sites, 50% (447/903; binomial test: $P < 0.01$). This tendency was maintained also during the *Signal* epoch but not during the *Hold* epoch (Fig. 11*B, right*); during other epochs the number of same-site object-tuned units was too small to allow comparisons. In contrast, among pairs of object-tuned units recorded in the same proximal site, preferred objects did not tend to be the same, a pattern not significantly different from that observed for all possible pairs of object-tuned units recorded in proximal sites during all tested epochs (Fig. 11*B, left*).

DISCUSSION

We studied the functional organization of reaching and grasping in PM. During prehension, proximal muscles were modulated by reach direction more than distal muscles, and distal muscles were modulated by grasp type more than proximal muscles. However, modulation of neural activity by reach or grasp parameters did not correlate with recording site identity, and properties of individual neurons were the same in PMd and PMv sites where ICMS evoked proximal and distal movements. Thus a neuron recorded at a proximal site in a proximal region is not necessarily a "proximal neuron, " and a neuron recorded at a distal site in a distal region is not necessarily a "distal neuron."

Mixing of reach and grasp representations

Although psychophysical studies showed that during prehension, reaching and grasping are interdependent and processed in parallel (Jeannerod 1984; Soechting and Flanders 1993), the prevailing view from both neurophysiological and anatomical studies is that separate brain regions and pathways process reaching and grasping and that proximal and distal sites tend to aggregate in anatomically distinct regions, PMd and PMv (Luppino and Rizzolatti 2000; Tanné-Gariepy et al. 2002; Wise et al. 1997). The latter tendency was supported by our observations (Fig. 4). However, there have been several

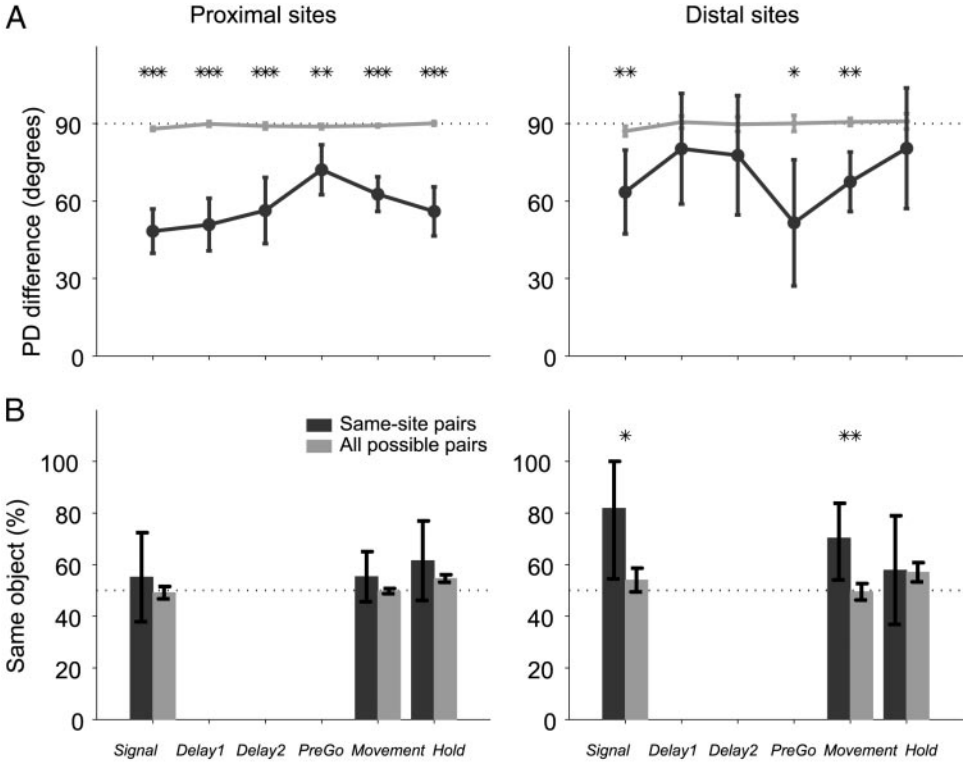

FIG. 11. *A*: PD differences (PDDs). *Left*: PDDs among pairs of directionally tuned units recorded in the same proximal site (black) or among all possible pairs of directionally tuned units in proximal sites (gray). Single/double/triple stars indicate that the 2 sets are significantly different at the 0.05/0.01/<0.001 level (Mann-Whitney *U* test). Error bars show 95% confidence intervals. The theoretical mean PDD assuming independent sampling from a uniform distribution of PDs is 90° (dotted line). *Right*: same as the *left* for pairs of units recorded in distal sites. Comparing the *right* and *left panels,* PDs of pairs of units recorded in the same proximal but not distal site tended to be similar. *B, left*: fraction of pairs of object-tuned units recorded at the same proximal site (black) or all possible proximal sites (gray) that preferred the same object. The theoretical fraction of identical object preferences is 50% (dotted line). Fractions were compared using a binomial test. Other conventions are the same as in *A*. *Right*: same as the *left* for pairs of distal units. Objects preferred by pairs of units recorded in the same distal but not proximal site tended to be the same.

reports of anatomical mixing of muscle fields, both in M1 (finger muscles; Rathelot and Strick 2006; Sato and Tanji 1989) and in the PM (proximal and distal muscles; Dum and Strick 2005; Godschalk et al. 1995; Raos et al. 2004). This was also confirmed in the present study.

The main result of this study, mixing of single neurons encoding reach and grasp *within a given site*, is novel. How is this possible, given the large number of studies that tested PM activity in relation to reach and grasp? One possibility is related to learning-induced changes in cortical circuitry during task performance. Alternatively, mixing may not have been demonstrated because of the novel way reach and grasp were combined in the current task. Previous experiments testing directional modulation of PM neurons have typically altered reach direction in an orderly manner without manipulating grasp type (Caminiti et al. 1991; Shen and Alexander 1997), whereas experiments examining grasping used reach-to-grasp movements, varying the grasped object while keeping reach direction fixed (Hepp-Reymond et al. 1994; Murata et al. 1997). The current prehension task systematically varied both reach direction and grasp type, opening the possibility to observe neural activity related to either component.

We focused on PM areas to study the neural control of prehension, but other brain regions are clearly involved. Cerebellar activity has been related to reaching and grasping (van Kan et al. 1994) but not at the same time (Mason et al. 2006). Here we showed that although activity of proximal muscles tended to precede activity of distal muscles (Fig. 3*A*), the activity of single PM neurons was related to reach and grasp throughout preparation and execution of prehension (Fig. 7*A*). We did, however, observe a general tendency of PM neurons to become more grasp-related during the *Hold* period (Fig. 10). Whether these differences are due to anatomical or methodological differences is an issue for future studies.

Behavioral separation between reach and grasp

The current task aimed to differentiate between reach and grasp by systematically varying reach direction and grasp type. As the analysis of muscle activity showed, this was largely successful (Fig. 3*B*). There are other possible experimental paradigms that may be used for the same purpose. One possibility is to impose time restrictions on the reach and grasp phases, inducing artificial temporal separation. However, even during unconstrained reaching and grasping, the activity of proximal and distal muscles does not peak simultaneously (Fig. 3*A*). A second venue may be to vary reach alone, with no grasping whatsoever, and then vary grasp alone without reaching. This approach was partially adopted in the current study by requiring reaching from one touch pad to another identically shaped pad (APPENDIX). The main advantage of the current task was to enable characterization of muscle and neural activity during unconstrained reaching and grasping in a systematic manner.

None of the measured muscles displayed activity related only to reach or grasp. Although this may seem surprising, during natural movements reach and grasp are in fact biomechanically dependent. For instance, activity of wrist muscles accompanies digit movements (Schieber 1995), and shoulder movements are accompanied by stabilization of distal joints. Accordingly, activation patterns of proximal and distal muscles are expected to overlap. There were, however, some clear differences between proximal and distal muscles: proximal muscles were related mainly to reach direction while more distal muscles were also related to grasp type (Fig. 3*B*).

Activity of intrinsic hand muscles (Lemon et al. 1986) was not monitored in this study due to technical constraints. Because the activity of extrinsic digit muscles was modulated by grasp type much more than the activity of proximal muscles, we expect that if monitored, intrinsic muscle activity would be modulated mainly by grasp type.

The task did not call for any changes in wrist orientation when grasping different objects. Although we did not measure hand orientation directly, we videotaped movements from three directions. Examination of these records did not reveal any gross changes in wrist orientation while moving to either object at the same location. Wrist orientation is influenced by activity of wrist muscles, which depends on the movement task. In previous studies, wrist orientation and muscles were modulated by grasp type when grasping different vertically oriented objects at the same location (Brochier et al. 2004). In other behavioral paradigms, the activity of wrist muscles was related to movement direction (Kakei et al. 2001). In the present task, wrist muscles had RGI values close to one and were therefore mainly related to reach direction.

Anatomical and functional considerations

For most analyses, we parsed the sample of neurons into two sets, recorded in proximal and distal sites. There were two reasons for this "functional" rather than an "anatomical" choice. First, there were some differences between the monkeys. Although in both monkeys, the majority of proximal sites were aggregated in similar regions, the caudal PMd (Wise et al. 1997), the predominantly distal regions differed between monkeys, corresponding to the rostral PMv in one monkey and a region between PMd and PMv in another (Luppino and Rizzolatti 2000). This variability is not unique: functional cortical fields are known to vary with respect to sulcal landmarks between animals (Gabernet et al. 1999; Merzenich et al. 1975). Second, it was previously shown that proximal and distal sites are mixed in PM (Dum and Strick 2005; Godschalk et al. 1995). This was also observed in the current study. Thus it was more meaningful to parse sites according to the results of ICMS and/or SMM than by anatomy. Yet parsing sites according to anatomical criteria did not reveal any differences either (Table 1). Nevertheless, it is possible that in other PM sites, not explored in the current study, neurons with different properties do exist.

Possible explanations for the lack of correlation between single-unit properties and recording site identity

Single-unit activity was measured during the prehension task while recording site properties were tested using threshold ICMS and SMM. Whereas recording single-unit activity is equivalent to making an observation, presumably with minimal disruption of spontaneous activity, ICMS involves interference with the ongoing neural activity in a nonbiological yet causal manner. Whereas single-units are related to local circuit properties, mapping results represent more distributed properties: SMM involves listening to multi-unit activity, and the number

of neurons activated by threshold ICMS depends on the excitability of individual neurons (Stoney et al. 1968). Moreover, ICMS may result in complex activation of large areas (Butovas and Schwarz 2003) and remote targets (Tokuno and Nambu 2000). Despite these differences, a good match between single-unit properties, ICMS, and SMM has been reported repeatedly (Aflalo and Graziano 2006; Asanuma et al. 1968; Gentilucci et al. 1988; Murphy et al. 1978; Strick and Preston 1982). How can the discrepancy between single-unit and recording site properties observed in the current study (Figs. 7, 8, and 10) be accounted for?

One possibility is that some neurons influence output more than others, and the firing properties of these neurons differ for distinct sites. For instance, a directionally tuned neuron in a proximal site might have stronger influence on a proximal muscle than a neuron with the same PD and effect sizes recorded in a distal site (Fig. 12*A*). This is possible if the neuron in the proximal site has lower excitability threshold or stronger synapses on downstream neurons that in turn synapse on proximal muscles. Then differences between consistent and inconsistent units in terms of ICMS threshold, postspike-facilitation occurrence, and so on are expected. Although none of the latter differences were observed in the current data, this possibility cannot be excluded.

A second possibility is that in a site with a given property, neurons are particularly efficient *as a group* in influencing downstream neurons related to the same property. This may be achieved by organizing neurons into networks differently in distinct sites. Previous studies of the motor cortex suggested that directionally tuned neurons recorded in proximal sites tend to aggregate according to their PDs (Amirikian and Georgopoulos 2003; Ben-Shaul et al. 2003). If directionally tuned neurons in a given proximal site have similar PDs, whereas neurons in a distal site have the same firing rates and effect sizes as the proximal site neurons but more diverse PDs, then ICMS in the proximal site may be more prone to yield directional movement by virtue of averaging. In contrast, the similarity of preferred objects among object-tuned neurons in a given distal site may be greater than the similarity among object-tuned neurons in a proximal site (Fig. 12*B*). Results reported here (Fig. 11) are consistent with this possibility.

Concluding remarks

Psychophysical studies showed that reach and grasp are coordinated with one another during prehension, but neurophysiological studies suggested that they are processed separately at the motor cortical level. Complete segregation between two entities makes coordination between them difficult to achieve. Anatomical (Dum and Strick 2005; Huntley and Jones 1991) and physiological (Kwan et al. 1987; Matsumura et al. 1996) studies suggested horizontal connections between motor cortical sites millimeters apart. The current results complement and extend these findings by showing a mixing of neurons encoding reach and grasp between proximal and distal forelimb representations.

What is the functional role of neurons with properties *inconsistent* with the recording site properties, such as reach-related neurons recorded at distal sites? Such neurons may partake in horizontal connections between distant sites, for instance, with other reach-related neurons at proximal sites. In that case, the former neurons may relay reach-related information from the proximal site to grasp-related neurons at the distal site, facilitating movement coordination. Clearly further research is required to test these ideas.

APPENDIX

Similarities between units in proximal and distal sites are not due to eye position or movement time effects

Because monkeys were not required to maintain fixation during the task, gaze angle (Boussaoud et al. 1998; Mushiake et al. 1997) varied with reach direction during all 43 sessions (1-way MANOVA, $P < 0.01$). To control for this potentially confounding factor, we tested whether neural activity was related to eye position using regression analysis (METHODS). During the *Control* epoch, activity of 60/724 units (8%) was modulated by eye position (F test, $P < 0.01$). During the *Movement* epoch, the fraction of gaze-related units was similar (81 units, 11%; χ^2 test: $P = 0.06$). Mean R^2 values of gaze-related units were 0.08 during both epochs. Finally, gaze effects did not correlate with recording site (62/551 and 19/173 gaze-related units in proximal and distal sites, respectively; 11% in both sets; χ^2 test: $P = 0.85$). Thus gaze angle varied with task parameters but neural activity during the *Control* and *Movement* epochs was related to gaze angle to similar extents. Similar results were obtained for the other epochs.

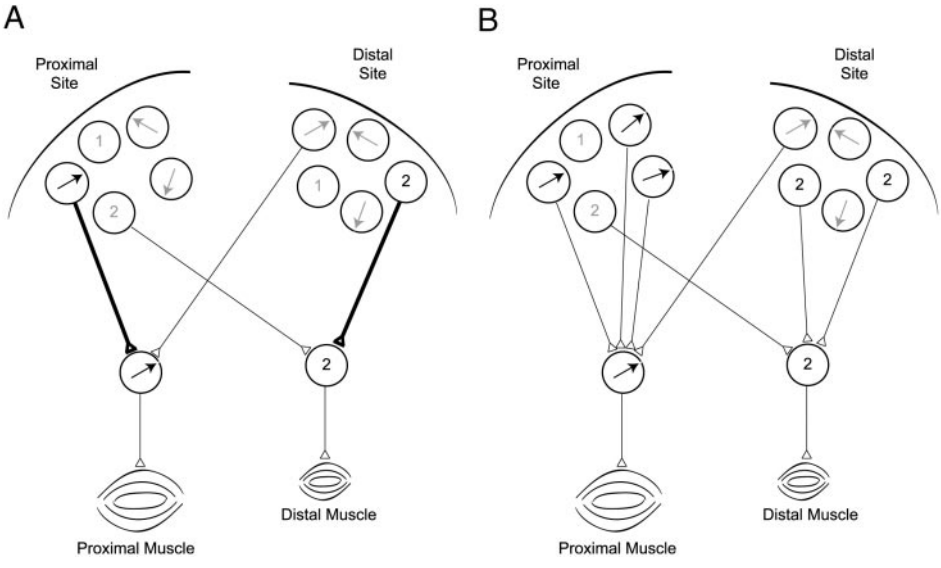

FIG. 12. Possible mechanisms that may resolve inconsistencies between recording site and single-unit properties. Within each site, reach-related properties are illustrated by arrows pointing in the direction of the PD and grasp-related properties by numbers representing different grasp types. The thickness of a line connecting 2 neurons represents the strength of the neuron's effect on downstream circuits and through them on the putative muscle. For illustrative purposes only, neurons are shown as though they are 2 stations away from muscles. *A*: neurons with properties consistent with the recording site properties have dominant influence. These neurons have a lower excitability threshold and/or stronger synapses on downstream neurons. *B*: neurons with properties consistent with the recording site properties are aggregated according to that property. Reach-related neurons in a proximal site have similar PDs, and grasp-related neurons in a distal site have the same preferred grasp type.

Another potential confound stems from reaction and movement times (inversely related to speed) which varied with reach direction, grasp type, or both during all sessions (2-way ANOVA, $P < 0.01$, corrected for multiple comparisons). We tested whether neural activity was modulated by reaction and/or movement time using regression analysis as for gaze angle. During the *Movement* epoch, the activity of 77/724 units (11%) was modulated by reaction/movement times; the mean R^2 was 0.05. Modulation was similar for units in proximal and distal sites (10 and 13%, respectively; χ^2 test: $P = 0.36$). During the *PreGo* epoch similar results were obtained (9 and 6% of units in proximal and distal sites, respectively; χ^2 test: $P = 0.3$), but during other epochs, the number of units modulated by reaction/movement times was at chance level. Thus neural activity during the *PreGo* and *Movement* epochs was weakly modulated by reaction and movement times, regardless of the recording site.

Dominance of reach-related single-unit activity

Reach-related modulations were larger and more frequent than grasp-related modulations (Figs. 8, *A* and *B,* and 10). This could result from a statistical bias: for each unit, six directions but only two objects were sampled. To control for this factor, we diluted reach directions by keeping trials from two randomly selected directions, so diluted data included an equal number of sampled directions and sampled objects. In these data, the dominance of direction over object tuning was smaller yet maintained (fractions of tuned units: 33 vs. 19%; χ^2 test: $P \ll 0.001$; median RGI, -0.11). Thus the apparent dominance of reaching reported in RESULTS resulted only in part from the experimental design.

Neural activity modulated solely according to whether grasping is or is not performed will not result in an object effect. Therefore viewing object effects as reflecting grasp-related activity is a conservative estimate. To test this possibility, single-unit activity during the prehension task, requiring reaching and grasping, was compared with the activity of the same units during a task that required only reaching, in which targets were touch pads identical to the resting position touch pad (a total of 367 units were recorded in *monkey D* during both tasks, uniformly from all sites). During the *Movement* epoch, 66/238 units (28%) that did not exhibit an object effect modulated their activity according to whether grasping was or was not instructed, yielding a total of 195/367 units (53%) related to grasp performance. Thus the grasp-related activity reported in this study may be regarded as a lower bound estimate. Although we did not conduct a "grasp without reach" experiment, the same logic presumably applies to that case.

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REFERENCES

Abeles M, Goldstein MH. Multispike train analysis. *Proc IEEE* 65: 762–773, 1977.

Aflalo TN, Graziano MS. Partial tuning of motor cortex neurons to final posture in a free-moving paradigm. *Proc Natl Acad Sci USA* 103: 2909–2914, 2006.

Amirikian B, Georgopoulos AP. Modular organization of directionally tuned cells in the motor cortex: is there a short-range order? *Proc Natl Acad Sci USA* 100: 12474–12479, 2003.

Asanuma H, Stoney SD Jr, Abzug C. Relationship between afferent input and motor outflow in cat motorsensory cortex. *J Neurophysiol* 31: 670–681, 1968.

Ben-Shaul Y, Stark E, Asher I, Drori M, Nadasdy Z, Abeles M. Dynamical organization of directional tuning in the primate premotor and primary motor cortex. *J Neurophysiol* 89: 1136–1142, 2003.

Boussaoud D, Jouffrais C, Bremmer F. Eye position effects on the neuronal activity of dorsal premotor cortex in the macaque monkey. *J Neurophysiol* 80: 1132–1150, 1998.

Brochier T, Spinks RL, Umilta MA, Lemon RN. Patterns of muscle activity underlying object-specific grasp by the macaque monkey. *J Neurophysiol* 92: 1770–1782, 2004.

Butovas S, Schwarz C. Spatiotemporal effects of microstimulation in rat neocortex: a parametric study using multielectrode recordings. *J Neurophysiol* 90: 3024–3039, 2003.

Caminiti R, Johnson PB, Galli C, Ferraina S, Burnod Y. Making arm movements within different parts of space: the premotor and motor cortical representation of a coordinate system for reaching to visual targets. *J Neurosci* 11: 1182–1197, 1991.

Crammond DF, Kalaska JF. Differential relation of discharge in primary motor and premotor cortex to movement posture during reaching movements. *Exp Brain Res* 108: 45–61, 1996.

Dum RP, Strick PL. Frontal lobe inputs to the digit representations of the motor areas on the lateral surface of the hemisphere. *J Neurosci* 25: 1375–1386, 2005.

Fetz EE, Cheney PD. Postspike facilitation of forelimb muscle activity by primate corticomotoneuronal cells. *J Neurophysiol* 44: 751–772, 1980.

Fisher RA. *Statistical Methods for Research Workers.* London: Oliver and Boyd, 1925.

Fogassi L, Gallese V, Buccino G, Craighero L, Fadiga L, Rizzolatti G. Cortical mechanism for the visual guidance of hand grasping movements in the monkey: a reversible inactivation study. *Brain* 124: 571–586, 2001.

Gabernet L, Meskenaite V, Hepp-Reymond MC. Parcellation of the lateral premotor cortex of the macaque monkey based on staining with the neurofilament antibody SMI-32. *Exp Brain Res* 128: 188–193, 1999.

Gentilucci M, Fogassi L, Luppino G, Matelli M, Camarda R, Rizzolatti G. Functional organization of inferior area 6 in the macaque monkey. I. Somatotopy and control of proximal movements. *Exp Brain Res* 71: 475–490, 1988.

Godschalk M, Mitz AR, van Duin B, van der Burg H. Somatotopy of monkey premotor cortex examined with microstimulation. *Neurosci Res* 23: 269–279, 1995.

Graziano MS, Hu XT, Gross CG. Visuospatial properties of ventral premotor cortex. *J Neurophysiol* 77: 2268–2292, 1997.

Graziano MS, Taylor CS, Moore T. Complex movements evoked by microstimulation of precentral cortex. *Neuron* 34: 841–851, 2002.

He SQ, Dum RP, Strick PL. Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral side of the hemisphere. *J Neurosci* 13: 952–980, 1993.

Hepp-Reymond MC, Husler EJ, Maier MA, Ql HX. Force-related neuronal activity in two regions of the primate ventral premotor cortex. *Can J Physiol Pharmacol* 72: 571–579, 1994.

Huntley GW, Jones EG. Relationship of intrinsic connections to forelimb movement representations in monkey motor cortex: a correlative anatomic and physiological study. *J Neurophysiol* 66: 390–413, 1991.

Jeannerod M. The timing of natural prehension movements. *J Mot Behav* 16: 235–254, 1984.

Kakei S, Hoffman DS, Strick PL. Direction of action is represented in the ventral premotor cortex. *Nat Neurosci* 4: 1020–1025, 2001.

Kurata K, Hoffman DS. Differential effects of muscimol microinjection into dorsal and ventral aspects of the premotor cortex of monkeys. *J Neurophysiol* 71: 1151–1164, 1994.

Kurata K, Tanji J. Premotor cortex neurons in macaques: activity before distal and proximal forelimb movements. *J Neurosci* 6: 403–411, 1986.

Kwan HC, Murphy JT, Wong YC. Interaction between neurons in precentral cortical zones controlling different joints. *Brain Res* 400: 259–269, 1987.

Lemon RN, Mantel GW, Muir RB. Corticospinal facilitation of hand muscles during voluntary movement in the conscious monkey. *J Physiol* 381: 497–527, 1986.

Luppino G, Rizzolatti G. The organization of the frontal motor cortex. *News Physiol Sci* 15: 219–224, 2000.

Mason CR, Hendrix CM, Ebner TJ. Purkinje cells signal hand shape and grasp force during reach-to-grasp in the monkey. *J Neurophysiol* 95: 144–158, 2006.

Matsumura M, Chen D, Sawaguchi T, Kubota K, Fetz EE. Synaptic interactions between primate precentral cortex neurons revealed by spike-triggered averaging of intracellular membrane potentials in vivo. *J Neurosci* 16: 7757–7767, 1996.

Merzenich MM, Knight PL, Roth GL. Representation of cochlea in primary auditory cortex in the cat. *J Neurophysiol* 38: 231–249, 1975.

Murata A, Fadiga L, Fogassi L, Gallese V, Raos V, Rizzolatti G. Object representation in the ventral premotor cortex (area F5) of the monkey. *J Neurophysiol* 78: 2226–2230, 1997.

Murphy JT, Kwan HC, MacKay WA, Wong YC. Spatial organization of precentral cortex in awake primates. III. Input-output coupling. *J Neurophysiol* 41: 1132–1139, 1978.

Mushiake H, Tanatsugu Y, Tanji J. Neuronal activity in the ventral part of premotor cortex during target-reach movement is modulated by direction of gaze. *J Neurophysiol* 78: 567–571, 1997.

Raos V, Umilta MA, Gallese V, Fogassi L. Functional properties of grasping-related neurons in the dorsal premotor area F2 of the macaque monkey. *J Neurophysiol* 92: 1990–2002, 2004.

Rathelot JA, Strick PL. Muscle representation in the macaque motor cortex: an anatomical perspective. *Proc Natl Acad Sci USA* 103: 8257–8262, 2006.

Rizzolatti G, Camarda R, Fogassi L, Gentilucci M, Luppino G, Matelli M. Functional organization of inferior area 6 in the macaque monkey. II. Area F5 and the control of distal movements. *Exp Brain Res* 71: 491–507, 1988.

Sato KC, Tanji J. Digit-muscle responses evoked from multiple intracortical foci in monkey precentral motor cortex. *J Neurophysiol* 62: 959–970, 1989.

Schieber MH. Muscular production of individuated finger movements: the roles of extrinsic finger muscles. *J Neurosci* 15: 284–297, 1995.

Schwartz AB, Moran DW, Reina GA. Differential representation of perception and action in the frontal cortex. *Science* 303: 380–383, 2004.

Shen L, Alexander GE. Preferential representation of instructed target location versus limb trajectory in dorsal premotor area. *J Neurophysiol* 77: 1195–1212, 1997.

Soechting JF, Flanders M. Parallel, interdependent channels for location and orientation in sensorimotor transformations for reaching and grasping. *J Neurophysiol* 70: 1137–1150, 1993.

Stoney SD Jr, Thompson WD, Asanuma H. Excitation of pyramidal tract cells by intracortical microstimulation: effective extent of stimulating current. *J Neurophysiol* 31: 659–669, 1968.

Strick PL, Preston JB. Two representations of the hand in area 4 of a primate. II. Somatosensory input organization. *J Neurophysiol* 48: 150–159, 1982.

Tanné-Gariepy J, Rouiller EM, Boussaoud D. Parietal inputs to dorsal versus ventral premotor areas in the macaque monkey: evidence for largely segregated visuomotor pathways. *Exp Brain Res* 145: 91–103, 2002.

Tehovnik EJ, Tolias AS, Sultan F, Slocum WM, Logothetis NK. Direct and indirect activation of cortical neurons by electrical microstimulation. *J Neurophysiol* 96: 512–521, 2006.

Tokuno H, Nambu A. Organization of nonprimary motor cortical inputs on pyramidal and nonpyramidal tract neurons of primary motor cortex: an electrophysiological study in the macaque monkey. *Cereb Cortex* 10: 58–68, 2000.

van Kan PL, Horn KM, Gibson AR. The importance of hand use to discharge of interpositus neurons of the monkey. *J Physiol* 480: 171–190, 1994.

Weinrich M, Wise SP. The premotor cortex of the monkey. *J Neurosci* 2: 1329–1345, 1982.

Wise SP, Boussaoud D, Johnson PB, Caminiti R. Premotor and parietal cortex: corticocortical connectivity and combinatorial computations. *Annu Rev Neurosci* 20: 25–42, 1997.