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Delay of Movement Caused by Disruption of Cortical Preparatory Activity

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Churchland MM, Shenoy KV. Delay of movement caused by disruption of cortical preparatory activity. *J Neurophysiol* 97: 348–359, 2007. First published September 27, 2006; doi:10.1152/jn.00808.2006. We tested the hypothesis that delay-period activity in premotor cortex is essential to movement preparation. During a delayed-reach task, we used subthreshold intracortical microstimulation to disrupt putative "preparatory" activity. Microstimulation led to a highly specific increase in reach reaction time. Effects were largest when activity was disrupted around the time of the go cue. Earlier disruptions, which presumably allowed movement preparation time to recover, had only a weak impact. Furthermore, saccadic reaction time showed little or no increase. Finally, microstimulation of nearby primary motor cortex, even when slightly suprathreshold, had little effect on reach reaction time. These findings provide the first evidence, of a causal and temporally specific nature, that activity in premotor cortex is fundamental to movement preparation. Furthermore, although reaction times were increased, the movements themselves were essentially unperturbed. This supports the suggestion that movement preparation is an active and actively monitored process and that movement can be delayed until inaccuracies are repaired. These results are readily interpreted in the context of the recently developed optimal-subspace hypothesis.

INTRODUCTION

Voluntary movements are believed to be "prepared" or "planned" before they are executed (Day et al. 1989; Ghez et al. 1997; Keele 1968; Kutas and Donchin 1974; Riehle and Requin 1993; Rosenbaum 1980; Wise 1985). Important evidence comes from tasks where a temporal delay separates an instruction stimulus from a go cue. Behaviorally, longer delays yield shorter reaction times (RTs, the time from the go cue to movement onset), suggesting that movement preparation is given a head start by the delay (Crammond and Kalaska 2000; Riehle and Requin 1989; Rosenbaum 1980). Physiologically, neurons in a number of brain areas show changes in activity during the delay (Godschalk et al. 1985; Kurata 1989; Padoa-Schioppa et al. 2002; Riehle and Requin 1989; Snyder et al. 1997; Tanji and Evarts 1976; Weinrich and Wise 1982; Weinrich et al. 1984). This "delay-period" activity typically shows tuning for the instructed movement, and its magnitude can be predictive of RT (Bastian et al. 2003; Churchland et al. 2006b; Riehle and Requin 1993). It is therefore widely suspected that such activity is related to, perhaps even the substrate of, movement preparation occurring during the delay (Bastian et al. 2003; Churchland et al. 2006b; Cisek and Kalaska 2002; Crammond and Kalaska 2000; Godschalk et al. 1985; Riehle and Requin 1993; Weinrich et al. 1984; Wise 1985; Wise and Kurata 1989). Yet despite such correlative evidence (appropriate activity at the appropriate time), there exists no evidence of a *causal* nature linking delay-period activity with movement preparation. In particular, it is not known if movement preparation is impaired after disruption of activity via intracortical microstimulation, inactivation, or lesion. Lesion and inactivation studies have demonstrated a role for dorsal premotor cortex (PMd) in producing "conditional" motor responses when using unusual or arbitrary stimulus-response associations (Kurata and Hoffman 1994; Passingham 1988). Yet it is unclear whether these results indicate a role for PMd in the preparation of straightforward movements (Kurata and Hoffman 1994) or in making nonstandard associations. Importantly, although movement preparation is thought to be a time-consuming process, lesions and pharmacological inactivation cannot be used to disrupt movement preparation in a temporally specific way. In contrast, intracortical microstimulation can be used to transiently disrupt neural activity at well-defined times. If movement preparation can indeed be disrupted, then one of two effects seems likely. The subsequent movement may be perturbed when the disrupted movement "plan" is put into action. On the other hand, movement preparation may be an actively monitored process, allowing the brain to detect inaccuracies, and delay execution until they can be corrected. In this case, disruption might cause movements to be *delayed* but otherwise near normal.

The latter possibility is suggested by recent experiments (Churchland et al. 2006b) in which we used firing rate *consistency* as a "proxy" index for firing rate *accuracy* (i.e., how close firing rates are to some "optimal" movement plan). We found that, on trials where firing rates were less consistent (and thus presumably less accurate) RTs were longer. This suggests that the brain can detect errors and delay movement until they can be corrected. A direct prediction, which we test here, is that an externally introduced disruption should lead to a delay in RT.

We used subthreshold intracortical microstimulation to disrupt preparatory activity in PMd during a delayed-reach task. The impact was remarkably specific: microstimulation increased RT, but the movements themselves were essentially unchanged. A variety of comparisons and controls further demonstrate the specificity of this effect.

METHODS

Subjects

Animal protocols were approved by the Stanford University Institutional Animal Care and Use Committee. Microstimulation was applied to the left hemisphere of two adult male monkeys (*Macaca mulatta*) using ~1–2 MΩ tungsten microelectrodes (Frederick Haer, Bowdoinham, ME) introduced through an implanted cylinder (19 mm

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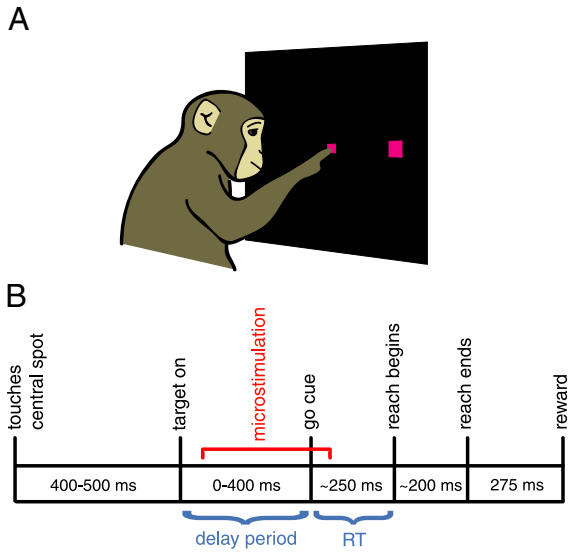

FIG. 1. Illustration of the task. *A*: monkeys sat in a primate chair 30 cm from a fronto-parallel display. *B*: each trial began with the appearance of a central spot. After this was touched and held, the target appeared, and initially jittered slightly (2 mm SD). Cessation of target motion, along with the disappearance of the central spot, provided the go cue. Reaches were rewarded if they were brisk and accurate, with reaction time (RT, estimated on-line) between 150 and 500 ms.

ID, Crist Instruments). The head and nonreaching (left) arm were comfortably restrained. Hand and eye position were tracked optically (Polaris, Northern Digital, Canada; Iscan, Burlington, MA). Details of these methods have been reported previously (Churchland et al. 2006b).

Task

Figure 1 illustrates the structure of the task. Targets could appear either to the left or the right of the central spot. For *monkey A*, target locations were 10 cm distant, at 5 and 185°. For *monkey B,* we used more distances (10 and 12 cm) and directions (rightward/leftward targets at 0, 10, 20°/160, 170, 180°) and also employed two instructed speeds (instructed by target color). This was done in the hopes of making the task more challenging and increasing both the behavioral effect of the delay period on RT and the possible effect of disrupting movement preparation. There was no evidence that this manipulation was successful (effects were no larger in this monkey), probably because the monkey was very familiar with the task, having performed hundreds of thousands of trials during training and prior recording experiments.

The delay period (from target onset to the go cue) varied randomly. A few early experiments using *monkey B* included delays up to 800 ms, but for most experiments the delay-period ranged from 0 to 400 ms (*monkey A*) or 30 to 400 ms (*monkey B*). We were initially unsure whether very short delays (of ≤1 video frame) might have a disorienting effect that could influence RT in a manner unrelated to movement preparation per se. We therefore used very short delays on one monkey but not the other. In retrospect this was probably an unnecessary concern, as similar results were obtained for both monkeys, and RT data for delays <30 ms fell on a continuum with that for longer delays (e.g., they did not produce dramatically longer—or shorter—RTs).

The relatively short (0–400 ms) range of delay durations was chosen to ensure that microstimulation occurred near the go cue for a nontrivial subset of trials, (microstimulation had to be delivered at random times throughout the delay, see following text) while still spanning the time consumed by movement preparation (probably 100–200 ms for this task, see Churchland et al. 2006b).

Intracortical microstimulation

Microstimulation (333 Hz for 57 ms) consisted of biphasic pulses from a programmable pulse generator (Master 8, AMPI, Jerusalem, Israel) and isolator (Frederick Haer). Figure 2 shows the *surface* locations of the penetrations leading to the stimulation sites. A typical penetration yielded one to three microstimulation sites, spaced ~500 μm apart in depth. Penetrations in M1 often produced more sites as gray matter persisted over a greater depth as penetrations continued into the central sulcus. During surgery, the cylinder was positioned so that its center would lie at the junction of PMd and M1, approximated as a line parallel to the central sulcus, and intersecting the precentral dimple just posterior to its middle. Sites anterior to cylinder-zero are thus considered to lie within PMd (30 and 73 sites for *monkeys A* and *B*), and sites posterior are considered to lie within M1 (12 and 32 sites,

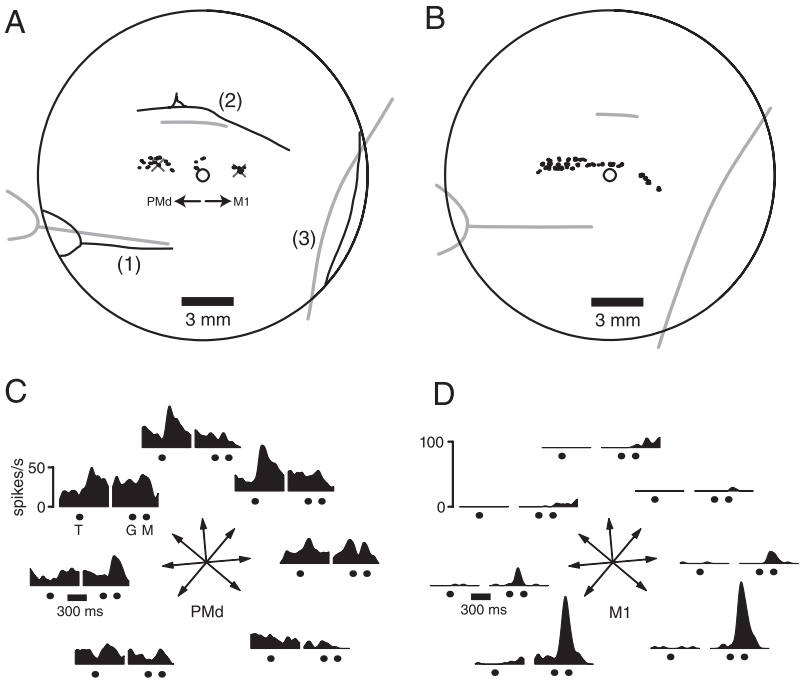

FIG. 2. Surface locations of stimulation sites in the left hemisphere of both monkeys (note that sites in M1 could be located in the central sulcus well below the surface locations of the penetrations). *A*: locations of stimulation sites for *monkey A*. Anterior is leftward. Lateral is down. The large circle outlines the limits of the implanted cylinder. The small circle shows its center. Gray lines give the locations, inferred from MRI, of the spur of the arcuate sulcus (1), the precentral dimple (2), and the central sulcus (3). Black lines give the actual locations, measured at autopsy. *B*: similar illustration but for *monkey B*. This monkey is still actively involved in experiments, so landmarks are estimated solely by MRI. *C*: single-neuron activity recorded from a site in dorsal premotor cortex (PMd; location given by the more anterior of the 2 small gray crosses in *A*). Each subpanel shows the mean firing rate for 1 of the 7 target directions. The dots at bottom give the time of target onset (T) the go cue (G) and the mean time of movement onset (M). Mean firing was computed once locked to target onset (left hand side of each subplot), and again locked to the go cue (right hand side). *D*: similar plot but for a site in M1 (location given by the more posterior of the 2 small gray crosses in *A*).

respectively). This definition, though common, is admittedly rough. However, it is worth noting that, for penetrations posterior to cylinder zero, the stimulated sites were often in deep gray matter (up to 7.5 mm, with gray matter sometimes continuing deeper still) and were thus unambiguously in M1. Thus M1 was more thoroughly explored than it appears given the surface locations of the penetrations.

Sites were tested if microstimulation evoked movements of the contralateral (right) arm with contractions originating in the muscles of the shoulder (most often) or upper arm (occasionally). At a few sites, microstimulation evoked movements of both the arm and the leg/body. Such sites were tested only if arm movements had the lower threshold. A few PMd sites were tested, based on their location and/or neural activity, even though high levels (>200 μA) of microstimulation evoked no movement. Neural recordings were often performed before microstimulation, both to provide data for other experiments, and to verify the presence of task-related activity. During these recordings, we observed both preparatory delay-period activity, and movement-related activity. As expected, the former was more prominent in PMd (Fig. 2*C*) and the latter more prominent in M1 (Fig. 2*D*). The sites from which the recordings in *C* and *D* were made are shown by the small gray crosses in Fig. 2*A*. Further examples can be seen in Churchland et al. (2006a; 2006b). The recording sites in those studies overlapped heavily with (and often shared the same penetration with) the PMd stimulation sites in the current experiment.

For each site, we estimated the threshold current for evoking movement by visually observing the effects of microstimulation as the animal continuously held his hand to a target for juice reward and/or palpating the muscle groups of the arm while delivering microstimulation. Thresholds varied from as low as 23 μA in M1 to >200 μA in PMd. At sites in PMd, we used currents close to (~80–90%), but below our estimate of threshold. For sites in M1, we used currents either at or just below threshold (~90–100%). This was to ensure that any tiny movements evoked during the task (something that occasionally occurred in PMd due to the difficulty of perfectly estimating threshold) would be at least as common for M1.

The range of microstimulation thresholds we observed (see Fig. 7*A*) is somewhat higher than is typically reported (e.g., Crammond and Kalaska 1996; Kakei et al. 1999; Raos et al. 2003). It is unclear how large a discrepancy there is to be explained: microstimulation thresholds cannot be reliable expressed with respect to current as they depend on a variety of factors including pulse frequency, train duration, and the degree of separation between paired pulses (and the interaction of that separation with the filtering properties of the electrode). Furthermore, the current density will depend on the shape of the electrode tip (Tehovnik 1996) and on how much current is spent charging/discharging the capacitance of the electrode shank. FHC epoxylite-insulate electrodes lack a well-defined tip and have considerable shank capacitance (impedance drops noticeably as electrodes are lowered into saline). Thus we don't believe there is a meaningful discrepancy between our thresholds and those reported previously. If there is a real discrepancy (i.e., one not related to stimulation parameters and electrode type), then it is likely related to *1*) higher thresholds in the shoulder and upper-arm "representation," as opposed to the wrist and digit representation, *2*) the fact that we did not seek to find the minimum threshold, but tested sites throughout the layers of cortex, across which thresholds can vary considerably, and/or *3*) the use of paired (as opposed to cathodal-only) pulses. For example, Raos et al. (2003) reports microstimulation thresholds of 3–40 μA in comparison with which our thresholds appear large. However, that study employed cathodal-only pulses. Furthermore, sites with thresholds >40 μA were actually typical (512/904 sites) but were simply not tested/analyzed further. Finally, in that study sites with thresholds <10 μA were in fact quite uncommon even in M1 and were essentially absent in PMd. Thus we don't see any discrepancy between our results and prior work. Certainly there can be no concern that we were stimulating the wrong cortical areas as these were localized with MRI and we have made extensive recordings from these areas in both monkeys (Churchland et al. 2006a,b).

Given that high (e.g., 200 μA) currents were passed at some sites with high thresholds, there is the possibility of some tissue damage at those sites. Asanuma and Arnold (1975) suggests that tissue damage can occur with currents as low as 50 μA, although other examinations suggest that the threshold for damage is considerably higher, especially when using biphasic pulses (Tehovnik 1996). On occasions where we attempted to record neural activity after microstimulation, we were typically able to observe neural activity at or near the stimulation sites. Thus if there was any damage due to microstimulation, it was probably very local. We also note that tissue damage cannot be a cause of the observed effects. First, stimulated and unstimulated trials were interleaved. Second, the effects of microstimulation were temporally specific.

Microstimulation was delivered on 41 and 18% of trials for *monkeys A* and *B*. Microstimulation onset times were drawn from a uniform distribution, from 325 ms before the go cue until 60 ms after. If the time selected was earlier than target onset, microstimulation was not delivered. This method prevented microstimulation from predicting the go cue (something that pilot experiments indicated was important). Unfortunately, this method also insured that the time of microstimulation only occasionally fell in the most relevant range, around the time of the go cue. Many of our analyses therefore combine data across sites to gain statistical power.

Analysis

Hand-position signals were low-pass filtered (25 Hz cutoff). We then computed both absolute hand speed and hand velocity in the direction of the target (i.e., the dot-product of 2-D hand velocity with a unit vector pointing from the central spot to the target). For each trial, we estimated RT (latency of movement onset after the go cue) by finding the peak speed, and searching backward in time until hand speed dropped <10 cm/s (~9% of mean peak speed). Reach endpoints were measured 100 ms after the estimated end of the movement. Plots of reach endpoint, mean hand speed, and mean velocity are based on all trials including failures (0.4 and 2% of trials for the 2 monkeys). To ensure that RT could be accurately measured for individual trials, plots of mean RT are based only on successful trials, where the target was hit accurately with an RT of 150–450 ms. Saccadic RT was measured as the time when eye position passed a threshold distance in the direction of the target (30 mm from the central spot for *monkey A,* 60 mm for *monkey B*).

Our measurements of RT slightly overestimate the "true" RT as it takes time following reach/saccade onset for the threshold criterion to be reached. By inspection, measurements of both reach and saccade RT appear to overestimate the true value by ~15 ms. Predictably, changing the threshold alters the mean RTs, but relative effects (e.g., the increase in RT after microstimulation) are preserved. For example, similar results were obtained using lower (2.5 cm/s) and higher (20 cm/s) thresholds for reach RT. Unless otherwise noted, statistical comparisons were made via *t*-test.

EMG recordings

In separate dedicated sessions, we recorded EMG activity using hook-wire electrodes (44 gauge w/ 27 gauge canula, Nicolet Biomedical, Madison, WI) placed in the muscle for the duration of single recording sessions. Recordings were made from four muscle groups (deltoid, biceps brachii, pectoralis, latissiumus dorsi) for *monkey A* and six (deltoid, biceps brachii, triceps brachii, trapezius, latissimus dorsi, pectoralis) for *monkey B.* EMG recordings confirmed that there was little or no change in muscle activity following target onset and during the delay (see supplementary materials of Churchland et al.

2006b).[1] Thus the activity present at that time (and which microstimulation is intended to disrupt) is most naturally interpreted as being related to movement preparation.

RESULTS

Disrupting PMd activity increases RT

Two monkeys were trained on a delayed-reach task (Fig. 1). Intracortical microstimulation was delivered, on a random subset of trials, to PMd, an area rich in delay-period activity (e.g., Fig. 2*C*). Microstimulation was delivered below the threshold for actually evoking movement (see METHODS) and lasted 57 ms with a random start time from 325 ms before the go cue until 60 ms after (this broad range was necessary to prevent microstimulation from predicting the go cue). For the purposes of analysis, the continuous range of micro-stimulation times was binned into epochs. In particular, we defined the "peri-go" epoch to be microstimulation that began 0–60 ms after the go cue. From separate recording experiments, we estimate that the latency of PMd to respond to the go cue is ~70 ms. Peri-go microstimulation could thus begin up to ~70 ms before the go cue is registered in PMd, and could end up to ~47 ms afterward (hence the term peri-go). It would seem that the integrity of movement preparation would, from a behavioral standpoint, be most critical around the time of the go cue, when movement preparation is presumably about to give rise to movement generation. If so, then peri-go microstimulation ought to be near-ideal, if we wish to observe the behavioral impact of a disruption. We also define an "early" epoch: microstimulation starting >100 ms before the go cue (and thus ending >~113 ms before the go cue is registered by PMd). If we assume that recovery from disruption is at least as rapid as the natural progress of movement preparation, then microstimulation in this epoch ought to have little effect on behavior. The effect of microstimulation just prior to the go cue will be considered later.

Figure 3*A* plots mean hand speed as a function of time for an example stimulation site in PMd. For unstimulated trials (black trace), mean hand speed began to rise ~210 ms after the go cue. After peri-go microstimulation (red trace), the initial rise in mean hand speed occurred ~45 ms later. Early-epoch microstimulation (green) had little effect. Figure 3*B* shows the same analysis, applied to data collapsed across all 30 sites in PMd of *monkey A*. The initial rise in mean hand speed occurred ~25 ms later for trials with peri-go stimulation, relative to trials with no stimulation. Similar results were obtained for *monkey B* (Fig. 3*C*, 73 sites in PMd) with hand speed rising ~26 ms later than normal after peri-go microstimulation.

For sites where we collected larger numbers of trials (e.g., >350), we observed that the effect of microstimulation on RT at a given site often waned with time, being much weaker past *trial number 200* and often absent for *trial numbers >300–400.* It is unclear whether the effect waned due to active compensation by the monkey, to depletion of neurotransmitter, or to damage local to the stimulated site. The effect was usually regained once the electrode was moved. Because of this, when performing analyses that collapse or compare across sites (e.g., Figs. 3, *B* and *C,* and 7*C*), we always restricted analysis to the first 200 trials/site. This concentrates analysis on trials with an effect, and allows fair comparisons across sites (which could have different numbers of total trials, but usually had ≥200).

[1] The online version of this article contains supplemental data.

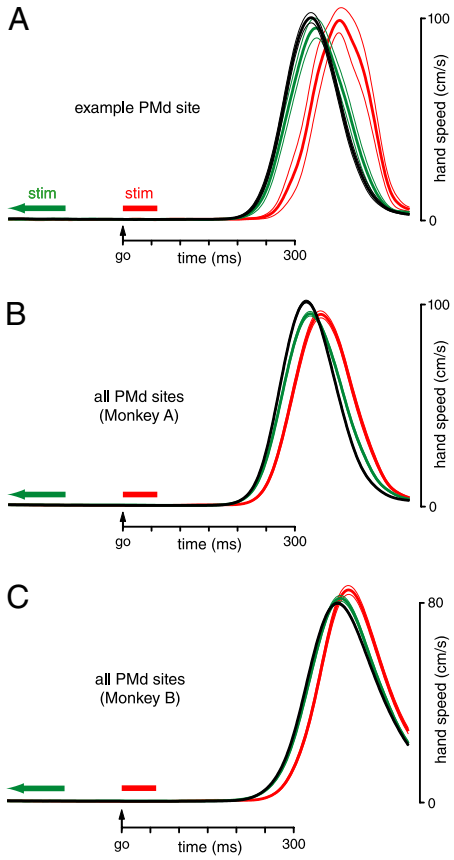

FIG. 3. Effect of PMd microstimulation on reach timing. Traces plot mean absolute hand speed with flanking traces showing the SE. Data are aligned to the go cue (arrow), so that RT can be roughly inferred from when hand speed begins to climb. Different colors plot data for trials with no microstimulation (black), early microstimulation (green), and peri-go microstimulation (red). Colored bars indicate the range of microstimulation onset times. Analysis is restricted to trials with delay periods >100 ms, so that movement preparation had time to begin before the go cue. *A*: data for 1 PMd site from *monkey A* (122, 37, and 13 trials for the unstimulated, early, and peri-go conditions). *B*: similar analysis, but pooling trials across all (30) sites in PMd of *monkey A* (1,968, 870, and 337 trials for the 3 conditions). *C*: pooling across all (73) sites in PMd of *monkey B* (9,038, 1,099, and 386 trials for the 3 conditions).

One can roughly infer RT from plots of mean hand speed: the first rise is a good indicator of the shortest RTs. However, RTs form a distribution with different RTs on different trials even within a condition. To examine those distributions, we measured the RT on each trial using a speed threshold (see METHODS). Figure 4, *top,* plots cumulative RT distributions and includes data from all stimulation sites in PMd. For both monkeys, the distribution of RTs is shifted to the right following peri-go microstimulation (red), relative to unstimulated trials (black). Mean RT increased 26 and 19 ms for the two monkeys ($P < 10^{-6}$ for each). Early-epoch microstimulation (green) had less impact (increases of 7 and 2 ms, $P < 10^{-6}$ and $P > 0.05$). Increases in RT following peri-go microstimulation were similar for leftward (ipsilateral) and rightward (contralateral) reaches (distributions not show). Mean RTs for left and right reaches increased 22 and 27 ms for *monkey A* and 18 and 19 ms for *monkey B* (also see supplementary Fig. 1). This is consistent with the observation that PMd activity relates roughly equally to ipsi- and contralaterally directed movements of the contralateral limb (e.g., Moran and Schwartz 1999).

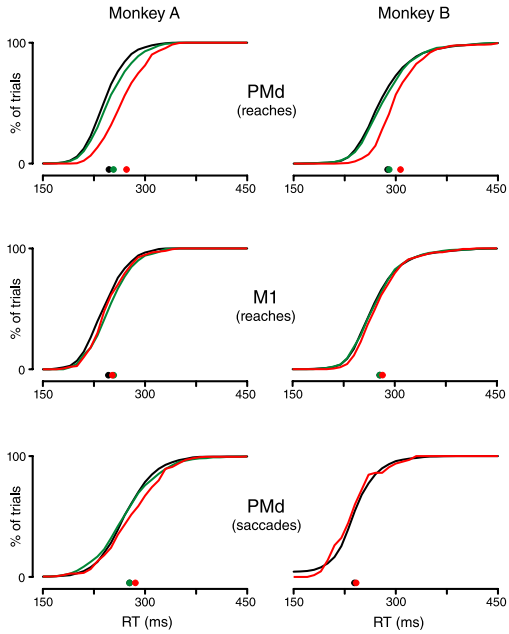

FIG. 4. RT distributions for both monkeys. Traces show cumulative RT distributions for trials with no microstimulation (black), early-epoch microstimulation (green), and peri-go microstimulation (red). Circles plot distribution means. *Top:* reach RTs for trials from all sites in PMd. As in Fig. 3, analysis was restricted to trials with delay-period durations >100 ms. Total trial counts for the 3 conditions (in the same order as above) were 1,965, 863, and 336 (30 sites for *monkey A*) and 8,842, 1,079, and 378 (73 sites for *monkey B*). These counts are slightly reduced from those in Fig. 3 because the current analysis excludes those (rare) trials in which the target was not accurately hit or where the RT did not fall within the 150- to 450-ms range and/or could not be measured accurately on that trial. M*iddle*: identical analysis for sites in M1. Total trial counts were 943, 372, and 150 (12 sites for *monkey A*) and 3,839, 417, and 185 (32 sites for *monkey B*). *Bottom*: saccade RTs for all sites in PMd. For *monkey A,* the data set was the same as that in the *top row* except we excluded trials in which his usual pattern of fixation was not observed. Total trials counts were thus slightly reduced: 1,876, 832, and 323. For *monkey B,* analysis was applied only to trials with delay periods <100 ms (to ensure the saccade had not yet taken place, see text) and could therefore not be applied to the early-epoch condition. Again, trials were excluded if the usual pattern of saccades was not observed (e.g., if no saccade occurred, or if the central spot was never fixated). Total trials counts were 1,287 and 65. For this restricted dataset, mean reach RT increased 18 ± 4 ms.

Microstimulation had little effect on reach trajectory or endpoint

Despite its pronounced effect on RT, PMd microstimulation produced only very small changes in the reaches themselves. Figure 5 plots reach endpoint for both unstimulated trials (black) and trials with peri-go microstimulation (red). Interpretation is simplest for *monkey A,* for which we used only two target locations. For each of these, there was near-complete overlap in the distribution of endpoints for stimulated and unstimulated trials (Fig. 5*A*). The only statistically detectable effect was a tendency for leftward reaches to be slightly longer on average (3 mm = 3%, $P < 0.001$) after microstimulation. For rightward targets, there was very little effect (0.6 mm shorter reaches after stimulation, $P = 0.15$). Results were essentially identical for *monkey B.* For this monkey, we used 12 target locations (arranged in 1 rightward and 1 leftward cluster). We also used two instructed speeds: "fast" reaches to square red targets and "slow" reaches to round green targets. (The use of multiple reach targets and 2 instructed speeds—features of the task with which the monkey was very familiar—were not critical to the present study but were included to insure that the task remained reasonably challenging). As for *monkey A,* reach endpoints were very similar regardless of the application of microstimulation, although some small changes were seen. For the instructed-fast condition (Fig. 5*B*), rightward reaches ended, on average, at a slightly lower point after microstimulation (0.9 mm, $P < 0.05$). This was also true for leftward reaches in the instructed-slow condition (Fig. 5*C*), which ended on average 0.7 mm lower ($P < 0.05$) after microstimulation. Endpoints were otherwise statistically indistinguishable between stimulated and unstimulated trials.

The preceding analysis collapses data across stimulation sites and might tend to underestimate the effect of microstimulation on endpoint. For example, microstimulation might produce rightward deviations at one site and leftward deviations at another, resulting in little change in mean endpoint across sites. A similar effect could occur at the level of individual trials. One presumes this did indeed occur, but it cannot have been a large effect: endpoint variability was typically quite comparable for stimulated and unstimulated trials (Table 1). We also looked for effects separately for each stimulation site/target direction (and in the case of *monkey B,* each instructed speed). Significant effects of microstimulation on reach endpoint were observed slightly more often than expected by chance: 11 and 11% for *monkeys A* and *B* (5% being chance), respectively, but were small. Mean changes were 1.8 and 1.4 mm for the two monkeys, only slightly more than that expected due to sampling error (1.3 and 1.2 mm, estimated by re-sampling). Thus analysis at the level of single sites is consistent with analysis

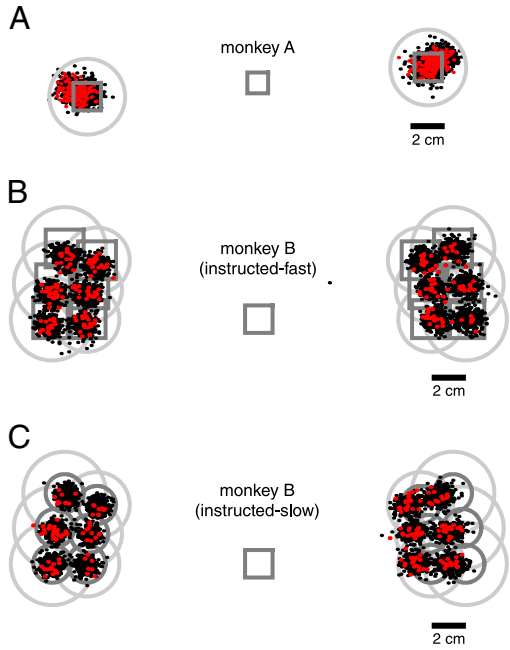

FIG. 5. Effect of PMd microstimulation on reach endpoint. Datasets (same as in Fig. 3, *B* and *C*) pool across all stimulations sites in PMd. Each dot plots reach endpoint on 1 trial. Black and red dots correspond, respectively, to trials with no microstimulation and with peri-go microstimulation. *A*: data for *monkey A.* The central square marks the position and size of the central touch point. The flanking squares mark the 2 locations at which the reach target could appear. Circles show acceptance windows. *B*: data for *monkey B.* This panel plots endpoints for "instucted-fast" reaches. There were 12 possible target locations, all of which are shown, along with their surrounding acceptance windows. *C*: also for *monkey B,* endpoints for "instucted-slow" reaches.

TABLE 1. *SDs of reach endpoint and initial direction*

	Leftward Reaches			Rightward Reaches		
	Horizontal Endpoint, mm	Vertical Endpoint, mm	Initial Direction, °	Horizontal Endpoint, mm	Vertical Endpoint, mm	Initial Direction, °
Monkey A	5.3 **5.2**	4.2 **4.2**	5.4 **5.7**	5.6 **5.7**	4.6 **4.2**	6.6 **6.4**
Monkey B						
Fast	3.7 **3.7**	3.7 **3.8**	8.5 **7.6**	4.0 **4.0**	3.3 **3.4**	6.6 **5.6**
Slow	3.3 **3.6**	3.2 **3.2**	9.7 **7.8**	4.1 **4.1**	3.2 **3.0**	9.9 **9.0**

SDs for unstimulated trials (plain text) and trials where microstimulation was delivered during the peri-go epoch (bold text). Data are shown separately for leftward and rightward reaches, and in the case of *monkey B* for instructed-fast and instructed-slow reaches. For *monkey B,* six rightward and six leftward targets were presented. Prior to computing SDs, we therefore expressed reach endpoints relative to the target location and then collapsed data across the six nearby targets. Initial reach direction was measured from 100 ms prior to movement onset until the time of peak velocity.

after collapsing across sites. Microstimulation occasionally had detectible effects on movement endpoint, but these were small, especially in relation to the size of the acceptance windows for obtaining reward (radii of 19–24 mm depending on monkey and target distance).

The near-normalcy of reach endpoints after microstimulation was not the result of on-line correction: the entire reach trajectory was altered little by microstimulation. Figure 6 plots mean reach velocity, computed after aligning each trial's data to its own peak velocity (as opposed to Fig. 3, where data were aligned to the go cue). Reach velocity profiles were very similar for unstimulated (black) trials and trials with peri-go microstimulation (red). Flanking traces show SDs (not standard errors, as in Fig. 3). This was done to allow comparison of variability in the velocity profile for stimulated and unstimulated trials. Microstimulation did not produce an increase in variability. Thus it was not the case that large disruptions at different sites canceled out on average. The data for *monkey B* are from the longer of the two distances (12 cm). Velocity profiles were as similar (between stimulated and unstimulated trials) for the shorter (10 cm) distance.

In addition to computing the mean velocity trajectory, we have also made a variety of measurements at the level of individual trials. These included peak initial acceleration (~50 ms after reach onset) and initial reach direction. Results support what is observed in the mean velocity trajectories in Fig. 6. Differences between stimulated and unstimulated trials were rare and small. The largest such difference was for *monkey B,* where, for instructed-slow reaches to leftward targets, peak initial acceleration was 13% higher after microstimulation ($P < 0.001$). This can also be seen (if only just) in Fig. 6, *bottom left.* However, in most cases reaches from stimulated and unstimulated trials were negligibly different. Changes in the mean initial reach direction were small (on the order of 1°), and the variance of the initial direction did not increase after microstimulation (Table 1). In summary, the impact of microstimulation on reach trajectory was remarkably minor, and this was true whether measurements were made early or late in the reach. This stands in contrast with the moderate but systematic increase in RT.

Controls: specificity of the effect of microstimulation

The preceding results indicate that microstimulation disrupts some internal process. This process appears to recover rapidly: early-epoch microstimulation, starting >100 ms before the go cue, had little effect. As PMd is rich in delay-period activity, one suspects that the internal process in question is movement preparation. However, other possibilities must be considered. Might microstimulation of almost *any* brain area have a distracting effect that could increase RT? Might the disrupted process be attention rather than movement preparation per se? Two findings serve as important controls and illustrate the specificity of the effect of microstimulation.

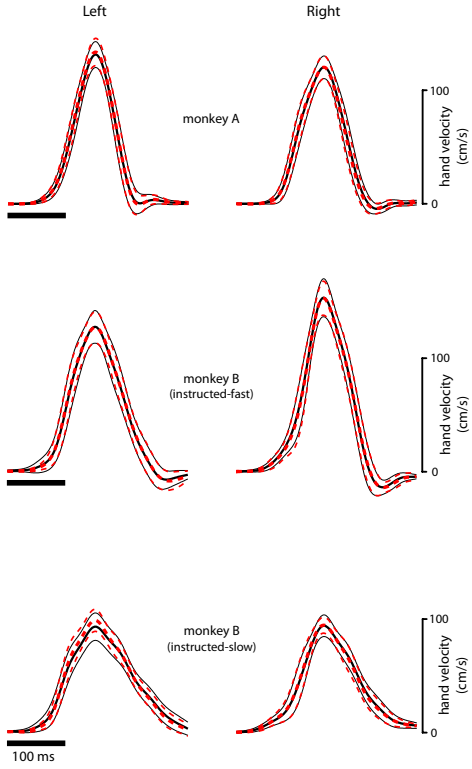

FIG. 6. Influence of PMd microstimulation on the profile of reach velocity. Traces plot mean reach velocity (in the target direction, rather than absolute speed as in other figures), *with each trial's data aligned to its peak velocity* before averaging. Black traces show data for unstimulated trials, and red-dashed traces show data for trials with peri-go microstimulation. Flanking traces show SDs. *Left* and *right*: data for leftward and rightward reaches. The 3 rows plot data for *monkey A,* and for the 2 instructed-speeds that were used for *monkey B.* Data for *monkey B* are for the more distant (12 cm) targets. Data for *monkey B* were collapsed across the 3 nearby directions (e.g., 0, 10, and 20° for the rightward panel) to allow sufficient numbers of trials (*32–56*) for the microstimulation condition to be able to compute reliable means. Collapsing across directions is tolerable because the unstimulated velocity profiles were exceedingly similar for nearby directions (hand velocity was computed in the target direction prior to averaging).

First, microstimulation of nearby primary motor cortex (M1) had much less impact on RT. Mean RT after microstimulation of M1 increased only slightly (6 and 4 ms, $P = 0.03$ and 0.15 for the 2 monkeys, Fig. 4). This was true even when we restricted our analysis to sites with similar absolute currents for the two areas (Fig. 7). Although microstimulation currents were on average lower in M1 (microstimulation was delivered below the threshold for evoking movement, which is typically lower for M1), there was considerable overlap in the currents used (Fig. 7*A*). This overlap is unsurprising: within a given area thresholds typically vary across layers and penetrations. Furthermore, microstimulation sites were in the upper arm and shoulder "representation" (i.e., suprathreshold microstimulation evoked movements of the upper arm and shoulder), where thresholds in M1 tend to be higher than for the wrist/digit representation. Finally, we intentionally used slightly higher currents—relative to threshold—in M1, for reasons explained in the following text. The considerable overlap allows comparison between PMd and M1 after restricting analysis to sites with similar mean currents (Fig. 7*B*, see legend for details). For both monkeys, the change in mean RT was still significantly larger ($P < 0.001$) for PMd (23 and 18 ms) than for M1 (8 and 3 ms).

The difference between PMd and M1 can also be captured by regressing the effect size (change in RT after microstimulation) versus anterior-posterior location (Fig. 7*C*). As expected, effects are smaller at more posterior sites, with the transition appearing continuous rather than abrupt (although this is difficult to say with any certainty). Similar results are obtained when each trial (rather than each site) contributes a data-point (regression slopes of −4.2 and −3.0 ms/mm for the 2 monkeys, $P < 0.001$). As a further control, regarding whether PMd sites are more effective due to larger currents, we regressed the change in RT versus the anterior-posterior location of that site and the current passed. If the impact of location were indirectly due to current level, then that impact should be eliminated by the inclusion of current in the regression. For both monkeys, there was still a significant dependence of effect size on location ($P < 0.001$) the slope of which was changed little by the inclusion of current as an independent variable (increased slightly for one monkey, decreased slightly for the other). Thus the greater efficacy of PMd stimulation is not simply due to more current being passed there. Of course, current level will be imperfectly correlated with the degree of excitation caused by microstimulation. For example, it is possible that microstimulation of PMd has a greater effect (in terms of the number of neurons excited) for a given current, even though it is less likely to cause movement. As an aside, we also found a nonsignificant tendency for effects to be larger at more superficial sites (correlation of ΔRT with depth for PMd sites, rho = −0.15, $P = 0.24$). If real, such an effect might suggest that the more superficial layers play a greater role in movement preparation. Alternately, it might simply follow from the fact that superficial sites tended to have higher thresholds, allowing more current to be passed.

The preceding results indicate that the effect of microstimulation on reach RT is area-specific and is not a trivial consequence of passing current anywhere in cortex. However, these results should not be over-interpreted and do not necessarily rule out a role for M1 in movement preparation (see DISCUSSION). Furthermore, the comparison of PMd and M1 cannot address a deeper issue: whether the process disrupted by microstimulation might be something other than movement preparation: perhaps visual processing or attention. To address this issue, we asked how microstimulation of PMd impacted saccade RT. We found that microstimulation had only a weak effect on saccade RT (Fig. 4, *bottom*). Fixation was not enforced, but each monkey exhibited a fairly consistent pattern of saccades. *Monkey A* typically fixated the central spot and made a saccade to the target only after the go cue. Mean saccade RT was slightly longer than that for reaches: 277 versus 247 ms (although by virtue of their faster velocity, saccades generally reached the target first). After peri-go microstimulation, saccadic RT increased significantly (mean of

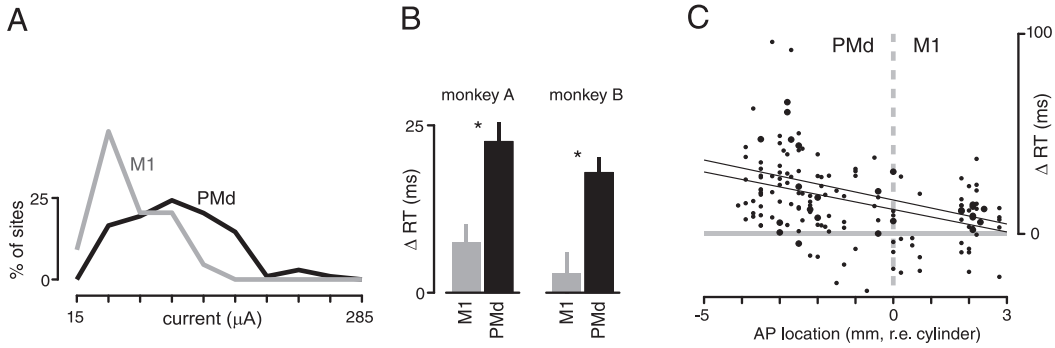

FIG. 7. The effect of M1 and PMd microstimulation: relationship to absolute current used. *A*: distributions of microstimulation currents across sites in M1 (gray) and PMd (black). *B*: effect of peri-go microstimulation on RT after restricting analysis so that average microstimulation currents were similar for M1 and PMd. For *monkey A,* we excluded M1 sites with currents <50 μA and PMd sites with currents >110 μA. For *monkey B,* we excluded M1 sites with currents <40 μA and PMd sites with currents >125 μA. These values were picked to roughly equalize the mean current in M1 and PMd: 81 ± 21 and 78 ± 24 μA for *monkey A;* 77 ± 27 and 75 ± 24 μA for *monkey B* (ranges are SDs). The mean change in RT after peri-go microstimulation is shown for M1 (gray) and PMd (black). Bars show SEs. *C*: effect of peri-go microstimulation as a function of the anterior-posterior location of the stimulation site. The vertical axis plots the difference in mean RT for trials with and without microstimulation. Data are for trials with delay-period durations >100 ms. Each symbol corresponds to data from 1 site. We used only the 1st 200 trials for each site. This was done to avoid finding artifactual site-to-site differences due to the different number of trials collected at each site, a concern given the tendency of the effect to wane with time. Data are combined for the 2 monkeys. Large symbols show sites that had ≥10 trials with peri-go microstimulation and indicate where some confidence can be had in the value of a given point. Smaller symbols show all sites, regardless of the number of trials with peri-go microstimulation. Regression slopes were similar for the restricted and complete datasets (black lines; −4.7 and −3.7 ms/mm; $P = 0.01$ and $P < 10^{-5}$) and for the two monkeys when analyzed separately (data not shown, slopes of −4.8 and −3.3 when all sites were included, $P < 0.001$ for each).

9 ± 3 ms SE, $P < 0.001$) but much less than did reach RT (26 ± 2 ms). *Monkey B* typically made a saccade from the central spot to the target soon after target onset often without waiting for the go cue. We therefore restricted this analysis to trials with delay periods <100 ms so that the saccade had not yet occurred by the time of the go cue. Mean saccadic RT was 240 ms relative to the go cue versus 266 ms for reaches. Microstimulation produced essentially no change in mean saccadic RT (+2 ± 4 ms, $P = 0.7$).

Thus the effects of PMd microstimulation on reach RT cannot be primarily due to a disruption of attention or visual processing or else the effect on saccade RT should be at least as large. However, there are a few notable caveats. First, the relatively small increase in saccade RT for *monkey A* could be due to a weak disruption of attention/saccade preparation. This would be consistent with the finding that eye movements can be evoked from the more rostral portions of PMd (Fujii et al. 2000), suggesting that it may play a role in saccadic, as well as reach preparation (on the other hand, our microstimulation sites were all within caudal PMd). Alternately, the effect on saccade RT may be entirely secondary to the effect on reach RT, as this monkey typically initiated a saccade only after initiating a reach. A second caveat is that interpretation for *monkey B* is somewhat complicated by the fact that he typically made a saccade to the target during the delay, necessitating that we restrict analysis to short delays (<100 ms) so that microstimulation preceded the saccades. Importantly, this analysis restriction alone cannot be blamed for the lack of effects: reach RT for the same trials increased by 18 ± 4 ms after peri-go epoch microstimulation. Nevertheless, it could be that saccades, being triggered by the target appearance, are more resistant to microstimulation that is locked to the subsequent go cue. In either case, it is noteworthy that although saccades and reaches are occurring around the same time (compare *monkey B* in Fig. 4, *top* and *bottom*), only the reaches are delayed by microstimulation.

Perhaps most critically, we note that an effect on saccadic RT was not a necessary condition for observing an effect on reach RT. This is most clear for *monkey B* but can also be demonstrated for *monkey A*. To do so, we consider only those sites where there was either no effect on saccade RT, or a slight *shortening* of saccade RT after peri-go microstimulation. Even among these (7) sites, reach RT was delayed by stimulation: with a mean increase of 21 ± 6 ms and a range of 2–48 ms.

A remaining potential concern is that the disruption of movement preparation may be indirect. Microstimulation may cause tiny muscle twitches, the feedback from which could impact movement preparation. We attempted to deliver PMd microstimulation below movement threshold, yet microstimulation of some sites did cause tiny movements. These evoked movements were of similar magnitude to the minute movements (jitter, drift, and small corrections) naturally present as the monkeys held their hand on the screen. The evoked movements were thus not noticed when initially estimating threshold, but could be revealed when averaging across trials. This is shown in the left column of Fig. 8, which employs an expanded scale (~10 times relative to Fig. 3) to reveal these small movements. After microstimulation (red bar), there is a small increase in mean hand speed above the baseline for unstimulated trials, and this was seen for both monkeys. However, it is unlikely that these tiny movements are responsible for the

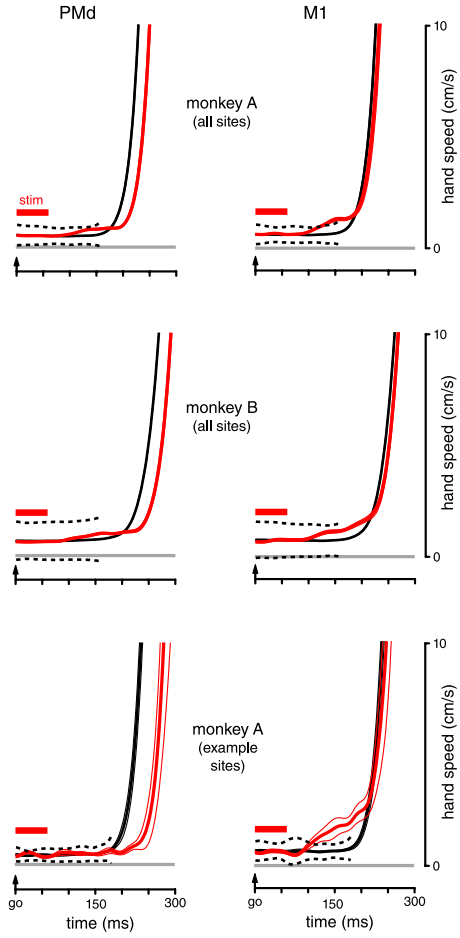

FIG. 8. Small movements evoked by microstimulation. Identical analysis to that in Fig. 3 but extended to sites in M1, and shown with a magnified (10 times) vertical scale to reveal small effects. Traces plot mean absolute hand speed (±SE, often less than the line width) for trials without microstimulation (black) and with peri-go microstimulation (red). Data are aligned to the go cue. Dashed black lines show the SD of baseline hand speed for unstimulated trials. *Top:* data for all sites in PMd and M1 of *monkey A*. Note that even for unstimulated trials, average baseline speed is not 0 due to jitter and other tiny hand movements. *Middle:* same analysis for *monkey B*. *Bottom:* 1 example site each for PMd and M1. The example site for PMd is the same as that shown in Fig. 3*A* and illustrates that many sites showed an impact of microstimulation on RT even though no discernable movement was evoked. The example site for M1 illustrates the converse: even when small but clear movements were evoked from M1, there was typically little change in RT. All data are aligned to the go cue. In principal, this might make microstimulation-evoked movements appear smaller than if data were locked to the onset of microtimulation. In practice, there was only a slight (~5%) difference in the magnitude of microstimulation-evoked movement between the 2 alignment methods.

increase in RT. First, their magnitude was small (an increase in mean speed that was ~0.3% of peak reach speed) and typically fell within the SD of baseline hand speed for unstimulated trials (dashed traces). Second, many PMd sites showed no hint of evoked movement after microstimulation yet still showed a robust increase in RT (Fig. 8, *bottom left*). Finally, microstimulation of M1 was *even more* likely to produce tiny movements (Fig. 8, *right*). This is probably true both because of its stronger spinal projection and also because we intentionally delivered M1 microstimulation very near (90–100%) threshold, with the specific goal of determining whether small muscles twitches were the basis of the RT effect. Increases in mean hand speed, measured 150 ms after microstimulation, were 0.61 and 0.81

cm/s (*monkeys A* and *B,* respectively) averaged across sites in M1 versus 0.26 and 0.24 cm/s for sites in PMd. Yet M1 microstimulation had much less impact on the subsequent RT (as seen in Figs. 4 and 7 and also in the mean speed traces in Fig. 8).

We did not record EMG activity during microstimulation experiments. It might seem that such recordings could provide an appealing control: allowing us to determine whether the putatively subthreshold microstimulation might have produced small undetected muscle contractions that could be an indirect source of RT changes. However, the presence of very small muscle twitches could never be ruled out via electromyographic (EMG) recordings from a handful of muscles. This is true for a number of reasons, not the least of which is the rather poor signal-to-noise ratio of EMG recordings. Indeed, we generally found that the precise (resolution of 0.3 mm) measurement of the hand was the more sensitive measure. Plots of hand velocity frequently reveal small movements that are difficult to detect from EMG recordings alone (supplementary Fig. 3). Of course, the reverse is presumably also true: EMG recordings might detect muscle contractions that did not cause the hand to move. However, this ability is limited by poor signal to noise. For this reason, we decided to instead perform the positive control of producing small contractions via M1 microstimulation.

Microstimulation reverses the RT advantage provided by the delay period

The preceding results argue that PMd microstimulation directly disrupts reach-related movement preparation. If so, then a further stringent test is possible. From a behavioral standpoint, it is believed that longer delay periods produce shorter RTs because movement preparation can occur before the go cue, saving time later (Crammond and Kalaska 2000; Riehle et al. 1994; Rosenbaum 1980). This hypothesis predicts that the impact of microstimulation, delivered just *before* the go cue, ought to depend on the duration of the preceding delay. If that delay is long, then microstimulation at the end of the delay ought to reduce the advantage it would normally confer. If that delay is short, then microstimulation ought to have little effect, as minimal movement preparation has yet been accomplished.

To test this prediction, we analyzed the impact of pre-go microstimulation, with onset times from 100 ms before until 10 ms after the go cue. The logic behind this interval is as follows. Pre-go microstimulation (which can begin as late as 10 ms after the go cue and lasts 57 ms) therefore ends, at the latest, ~3 ms before premotor cortex senses the go cue (at a latency of ~70 ms). Thus pre-go microstimulation should disrupt the product of delay-period movement preparation with minimal disruption of any movement preparation that begins after the go cue.

Figure 9*A* plots mean hand speed versus time and shows the effect of both delay-period duration and pre-go microstimulation (*monkey A*). As expected, longer delays led to shorter RTs: hand speed rises earlier for delay-periods >100 ms (black) than for delay periods <100 ms (gray). The advantage of a longer delay is nearly eliminated by pre-go microstimulation (red). In contrast, pre-go microstimulation has very little effect for trials with short delays (gray and pink traces overlap). A slightly weaker but otherwise similar effect can be seen for *monkey B* (Fig. 9*B*).

Unsurprisingly, the same pattern can be seen when computing mean RT across trials (Fig. 9, *C* and *D*). In the absence of microstimulation (black trace), RTs are longest for the shortest delays (0–100 ms) and are lower for longer delays. This effect is greatly reduced by pre-go microstimulation (red). This effect was statistically significant for both monkeys ($P < 0.005$ and 0.02, ANOVA interaction). Thus the impact of pre-go microstimulation is as expected given previous conclusions regarding movement preparation.

Other changes in RT

A drop in RT over the first 100–200 ms of the delay is a consistent feature of monkey performance (see supplementary Fig. 2) (also see Churchland et al. 2006b). However, as more time passes (later in the delay), it is common to observe additional changes in RT. In our experience, these later changes are often inconsistent across monkeys and can be highly dependent on the details of the task (e.g., the range and distribution of delays). In the current task, the RT of *monkey A* rises slightly for the longer delays (>200 ms), whereas the RT of *monkey B* continues to fall. This may have been due to the larger number of trials (~3 times) performed by *monkey B* for this task and a resulting awareness of the statistics of the delay (allowing anticipation of the go cue; see supplementary Fig. 2 for further discussion of this issue). This is of course speculation: there are a variety of processes other than movement preparation that likely influence RT. However, it is reasonable to suppose—as most studies have—that the initial decline in RT is due in large part to movement preparation. This inference is supported by our finding that the initial decline can be largely reversed by disruption of activity in a brain area linked to movement preparation.

DISCUSSION

We found that disruption of putatively preparatory activity in PMd, via microstimulation, increases RT. The effect is quite specific. Microstimulation had little effect unless it was delivered to PMd around the time of the go cue. Furthermore, there was little or no (depending on the monkey) increase in saccade RT. Finally, disruption of activity just before the go cue had an impact only if there was sufficient preceding delay for movement preparation to have made progress.

Causal evidence for a role in movement preparation

Taken together, the preceding results provide causal evidence for the hypothesis that movement preparation depends (at least in part) on activity in PMd. By causal evidence, we mean evidence gleaned by *causing* a change in the system, for example via microstimulation, inactivation or lesion. Various lines of causal evidence support a role of PMd (and of course M1) in movement *generation*. However, causal evidence linking PMd (or any brain area) to limb movement *preparation* had been lacking. A possible exception is the finding that RT can be lengthened by a transcranial shock (electrical or magnetic, typically above threshold) delivered to human motor (Day et al. 1989) or premotor (Schluter et al. 1999) cortex. However, this effect is widely believed to involve the temporary suppression of descending motor drive (Cracco et al. 1999; Day et al. 1989; Hallett 2000; Romaiguere et al. 1997; Taylor et al. 1995;

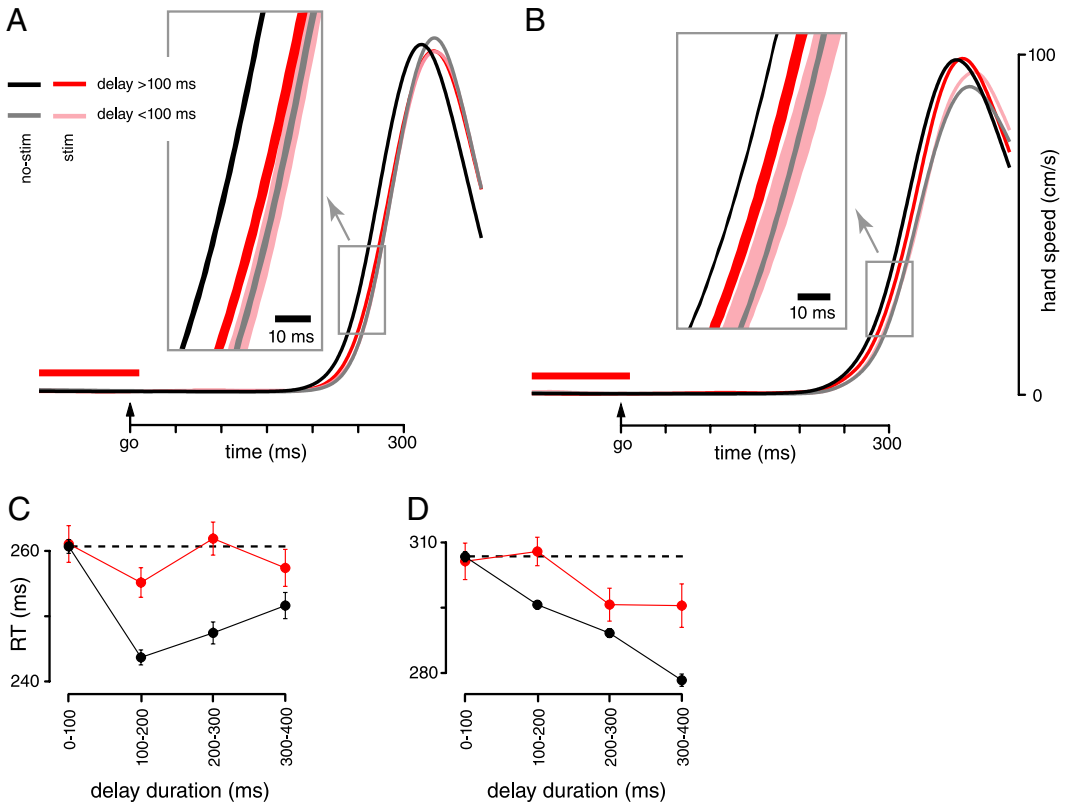

FIG. 9. Impact of pre-go microstimulation of PMd on RT. Analyses was restricted to those sites where *peri*-go microstimulation (*after* the go cue) had increased RT by ≥5 ms (19 and 50 sites for *monkeys A* and *B*) *A*. traces of mean hand speed for *monkey A* with data aligned to the go cue. The solid red bar shows the range of microstimulation onset times. As indicated by the legend, trials were segregated by the presence or absence of microstimulation, and by delay-period duration. An expanded scale shows data for the key range of time points with trace width indicating ±SE. *B*: similar plot for *monkey B*. *C*: analysis of mean RT (measured per trial) for the same dataset as in *A* (*monkey* A). Mean RT (±SE) is plotted as a function of delay-period duration. Red and black symbols plot data for trials with and without microstimulation. For the microstimulation conditions, trial counts (an average of 127 trials with pre-go microstimulation per epoch) are lower than might be expected, due to selection for both delay duration and microstimulation time. *D*: similar analysis but for *monkey B* (average of 138 trials with pre-go microstimulation per epoch).

Wilson et al. 1993; Ziemann et al. 1997)—the result of massive stimulation of inhibitory interneurons. Views have differed on whether movement preparation is also disrupted (Day et al. 1989; Schluter et al. 1999), but the suppression of motor drive is generally thought to be the primary mechanism. In contrast, the subthreshold microstimulation used in the current study appears to disrupt movement *preparation* (presumably by causing an inappropriate change in the state of activity, which could be due to either excitation or inhibition) rather than suppressing motor drive directly. We infer this because, after microstimulation, monkeys had no difficulty maintaining the outstretched position of the hand on the screen, a posture requiring continual muscle contraction. The dependence on delay-period duration further argues that the effect of microstimulation on RT is due to a disruption of movement preparation. Finally, the impact of microstimulation was greater in PMd than in M1, a pattern opposite that for transcranial stimulation (Taylor et al. 1995).

Prior lesion (Passingham 1988) and inactivation (Kurata and Hoffman 1994) studies have provided causal evidence that PMd is involved in making conditional motor responses: responses requiring an unusual or arbitrary stimulus-response association. Yet in those studies directly cued movements were unimpaired, making it unclear whether PMd plays an important role for straightforward movements. In Kurata and Hoffman (1994), it was suggested that it likely does but that the relevant network is sufficiently large that straightforward movements can still be planned and executed even when PMd is impaired. Our results are compatible with this interpretation. We suggest that microstimulation may be efficacious (when inactivation was not) because it is delivered very suddenly, making compensation by other circuits more difficult. Furthermore, the disruption in PMd may well spread (in a way that inactivation probably wouldn't) to other areas involved in movement preparation, perhaps via the pathways that are normally important in movement preparation.

A final caveat is that our results shed little light on the *type* of preparation-related processing that is putatively disrupted and whether it is primarily visual or motor. Although saccadic RTs are at most weakly impacted, the disrupted information could still be visual, if one was to presume that the reach system possesses its own dedicated visual signals. Alternately, the disrupted information could be related to low-level planning of muscle contractions. What can be concluded from the current results is that the disrupted information—visual, motor or sensorimotor—is probably largely effector specific, as reach RT suffered much more than saccade RT.

PMd versus M1

The differential effects of microstimulation in M1 and PMd can be summarized as follows: for a given current, it is easier

to evoke movement in M1 but easier to increase RT in PMd. This does not, of course, rule out a role for M1 in movement preparation, nor a role for PMd in direct movement generation. It does suggest that the relative strength of these roles is reversed between the two areas. However, nothing in our data indicates a sudden functional transition between PMd and M1. Indeed the gradient of effect size appears continuous. On a related note, the presence of preparatory activity in PMd presumably depends on its recurrent connections with other brain areas, including prefrontal and parietal cortex. To the degree that movement preparation is accomplished by the whole circuit, one suspects that similar effects on RT could be produced by disruption of activity at other critical nodes.

The idea that M1 and the premotor areas exist in a hierarchy has recently been challenged on a number of fronts (Dum and Strick 2002; Graziano et al. 2002; Haiss and Schwarz 2005). Although our results indicate that PMd and M1 are not functionally identical, they don't necessarily argue for a hierarchy. They do argue that for a reaching task, PMd plays the more prominent role in movement preparation. However, for a different task, (perhaps one involving fine control of the digits), that might not be true. In summary, the current results suggest important differences in the roles of PMd and M1, but what exactly those differences are (planning vs. execution, reaching vs. fine motor control) are still unclear. From the standpoint of the present study, the lack of RT effects after M1 microstimulation is most important as a control: it largely rules out any concern that the effect of PMd microstimulation on RT might be indirectly due to tiny undetected muscle twitches.

Test of the optimal-subspace hypothesis

We have previously suggested that movement preparation involves bringing preparatory activity to a state that is appropriate, when some trigger is applied, to generate the desired movement [optimal subspace hypothesis (Churchland et al. 2006b)]. Based on comparisons of neural recordings with RT, we suggested that movement preparation is essentially an optimization and that RT is delayed if errors are still present. This hypothesis makes the strong prediction that *externally* introduced errors should increase RT. This is indeed the effect of microstimulation in the current study. The hypothesis further predicts that the disruption of preparatory activity should have a modest effect on the reaches themselves (as movement is delayed until errors are corrected). We propose that this is why, in the current study, microstimulation had a clear effect on RT, but quite minimal impact on reach trajectory and endpoint.

In the current study, we found that the ability of microstimulation to increase RT depends on the duration of the preceding delay period. Microstimulation at the end of a long delay period greatly reduces the advantage conferred by that delay. However, when there had been only a short delay period prior to microstimulation, then there was little change: RTs were as long as expected for a short delay period but no longer. This result is consistent with the idea that disrupting movement preparation is inconsequential if movement preparation has not yet made much progress. This idea can also be expressed in terms of the optimal-subspace hypothesis. Disrupting activity when it is still variable/inaccurate is expected to have little impact: a perturbation merely moves the system from one nonoptimal state to another. However, once activity has reached the optimal subspace, any change in its state is likely to be harmful and will act to reverse the time savings earned during movement preparation.

Possible neural underpinnings of voluntary movement

We speculate that the type of mechanism described in the preceding text—the ability to delay or abort a movement if the movement plan contains errors—may be at the heart of what makes voluntary movements voluntary. The hallmark of an *in*voluntary movement is presumably that it occurs regardless of the intent of the "rest" of the nervous system (although it can perhaps be modulated or suppressed in advance). A voluntary movement, one then presumes, has exactly the opposite property: the rest of the brain is given access to information about the upcoming movement, and can refine or cancel it if necessary. The effects of microstimulation argue that it is indeed the case that errors in movement planning can be detected and corrected before movement initiation—although at the cost of a longer RT. Still, the current results do not indicate where or how this occurs. It may be, as suggested in the preceding text, that other brain areas are involved. Alternately, the putative monitoring of movement preparation may be entirely internal to PMd. In either case, this process must operate on very short time scales (tens of milliseconds), and—at least in the current task—is therefore probably more automatic than cognitive. Note that we are suggesting that the planning of voluntary movements allows time for their correction or cancellation. This may be true even if the corrections/cancellations are not themselves voluntary or conscious.

Needless to say, identifying the mechanisms by which movement preparation is monitored—and the movement eventually triggered—will require further experiments. It may also necessitate the development of new computational ideas. Along these lines, recent work has focused on the possible role of internal models in optimizing control signals during movement (Kawato 1999; Kawato et al. 1987; Wolpert et al. 1995). Such theory is naturally extended to the realm of movement preparation, which we suggest involves both the optimization of motor plans, and a mechanism to detect when optimization is (or isn't) complete.

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