Sequential Activation of Motor Cortical Neurons Contributes to Intralimb Coordination During Reaching in the Cat by Modulating Muscle Synergies

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Yakovenko S, Krouchev N, Drew T. Sequential activation of motor cortical neurons contributes to intralimb coordination during reaching in the cat by modulating muscle synergies. J Neurophysiol 105: 388–409, 2011. First published November 10, 2010; doi:10.1152/jn.00469.2010. We examined the contribution of the motor cortex to the control of intralimb coordination during reaching in the standing cat. We recorded the activity of 151 pyramidal tract neurons (PTNs) in the forelimb representation of three cats during a task in which the cat reached forward from a standing position to press a lever. We simultaneously recorded the activity of muscles in the contralateral forelimb acting around each of the major joints. Cell activity was recorded with and without the presence of an obstacle requiring a modification of limb trajectory. The majority of the PTNs (134/151, 89%) modulated their discharge activity at some period of the reach while 84/151 (56%) exhibited a significant peak or trough of activity as the limb was transported from its initial position to the lever. These phasic changes of activity were distributed sequentially throughout the transport phase. A cluster analysis of muscle activity in two of the cats showed the presence of five muscle synergies during this transport period. One of the synergies was related to the lift of the paw from the support surface, two to flexion of the limb and dorsiflexion of the paw, one to preparation for contact with the lever, and one to the transport of the entire limb forward; a sixth synergy was related to the production of electromyographic (EMG) activity or force. This aspect of motor control is exemplified by the early studies of Evarts (1968; Evarts et al. 1983; see also Smith et al. 1975) and particularly by those groups using spike triggered averaging (STA) to show causal relationships between cell and muscle activity (Cheney and Fetz 1980; Fetz and Cheney 1980; Griffin et al. 2008; Maier et al. 1993; Schieber and Rivilis 2005). However, there have been relatively few studies that have specifically asked how motor cortical activity ensures intralimb coordination during a reaching movement. One of the earliest studies to address this issue was that of Murphy et al. (1985) who recorded EMG and motor cortex activity when monkeys reached forward to press a button. They identified two populations of cells based on the effects of microstimulation at the recording sites. One population was considered to contribute to the control of more proximal muscles and the other group to more distal muscles. They found that the proximal population discharged on average 60 ms before the more distal population and proposed that intralimb coordination was facilitated by the nested-zone organization of the motor cortex (Murphy et al. 1978). Since then few studies have addressed the specific issue of how sequential activation of movement during reaching is produced, although several groups have addressed related issues (e.g., Scott 1997). Particularly pertinent is the study of Holdefer and Miller (2002), who used a cross-correlation analysis to show that cells in the motor cortex may be related to different muscle groups activated at different times during a reaching sequence (see also Morrow et al. 2009).

In work from this laboratory on the contribution of the motor cortex to the control of locomotion (Drew 1993), we showed that pyramidal tract neurons (PTNs) in the motor cortex of the cat are activated sequentially during locomotion and especially in situations in which a voluntary modification of gait was required. On the basis of this study, we have suggested that PTNs in the motor cortex serve to specify the spatiotemporal pattern of EMG activity that is needed to coordinate the movement of the limb during the gait modification. More recently, these ideas were formalized by...
the identification of clusters of synergistic muscles that were activated in a precise temporal pattern during locomotion (Drew et al. 2008b; Krouchev et al. 2006). We suggested that the sequential activation of the cells recorded in our earlier studies would serve to modify the activity of these groups of synergistic muscles and to specify the changes in the pattern of EMG activity that are needed to modify the limb trajectory to step over obstacles of different shapes or sizes.

In the present study, we examined whether a similar organization underlies the coordination of whole arm reaching movements made predominantly in the sagittal plane. We simultaneously recorded the activity of PTNs as well as the EMG activity of multiple muscles acting around each of the major joints of the forelimb as the cat reached forward and then pressed a lever. Cell activity was also recorded when an obstacle was introduced into the path of the cat in different locations and the cat modified limb trajectory to avoid it. This had the effect of producing greater variability and some dissociation of the activity of different groups of muscles as in our previous locomotion study (Drew 1993). The results are compatible with a view that the motor cortex regulates intralimb coordination during reaching by modifying the activity of a small number of multiarticular synergies.

Preliminary results have been published in abstract form (Yakovenko and Drew 2005).

METHODS

Training and surgery

These experiments were performed on the same three cats, MC28, MC29, RS24 used in a previous report from this laboratory (Yakovenko and Drew 2009). The weight of these three cats was, respectively, 6.6, 4.2, and 5.6 kg. Details of the task, the surgery, and the basic protocol can be found in Yakovenko and Drew (2009). In brief, the cats were trained to reach to a target and depress a lever to obtain a food reward (Fig. 1A, inset). They were trained to use either limb in response to auditory cues of different frequencies. The signal to begin the movement (GO signal) was the simultaneous end of the auditory cue and the opening of a shutter overlaying the lever. Following training the cats were prepared for surgery in aseptic conditions.

The cats were intubated and surgery was performed under general anesthesia (2–3% isofluothane with oxygen). A catheter was inserted stereotaxically to the pericruciate cortex. An array of 6 Tri-ML insulated stainless steel microwires (50 μm diam) was inserted stereotaxically into the right pyramidal tract (P7, L1.0) to allow antidromic activation of PTNs (Drew 1993). Pairs of Teflon-insulated, braided, stainless steel wires were implanted into selected muscles of the fore- and hindlimbs to record EMG activity during the task. All connectors were cemented to the cranium with dental acrylic. Following surgery, the steel wires were implanted into selected muscles of the fore- and hindlimbs to allow antidromic activation of PTNs (Drew 1993). Cells were classified as PTNs and were recorded during the task. Cell discharge activity was initially recorded during 3–5 min of treadmill locomotion (data not reported) before the cat was moved to the reaching apparatus which was placed adjacent to the treadmill. For each cell, we then recorded blocks of activity during reaching with each limb. Neuronal activity was recorded initially in the unobstructed condition, during 5 reaches with the left, contralateral, limb, 10 reaches with the right, ipsilateral, limb, and then another 5 with the contralateral limb. An obstacle was then introduced between the limb and the lever to induce a modification of limb trajectory during the reach. This obstacle was visible to the cat, and the limb never contacted the obstacle during the reach.

Data were recorded initially with the obstacle 7–8 cm in front of the limb and 6 cm above the plane of the paw. We refer to this as the distal position of the obstacle. A block of 10 reaches was recorded with the contralateral and then the ipsilateral limb. The obstacle was then displaced so that it was positioned ~2 cm from the paw but at the same height. We refer to this as the proximal position of the obstacle. Another block of 10 reaches with each limb was then recorded. Occasionally cell activity was also recorded with the obstacle in other positions within the working space. In these additional trials, the obstacle position was always slightly further away from the paw than in the distal condition (by 2–4 cm) and in some cases, slightly higher (also by 2–4 cm). We consider these positions a variation on the standard distal location, and these trials, when present, are classified together with the distal trials.

At the end of the recording session with any given cell, we tried to determine the receptive field of the cell. Neurons that responded to light touch or brushing were classified as having a cutaneous receptive field. In the absence of any detectable cutaneous receptive field, we manipulated the limb to determine if the cell received deep or proprioceptive inputs. We then replaced the cat in the treadmill and tried to isolate further neurons. At the end of each experimental session, we applied microstimulation (11 pulses, each of 0.2 ms at 330 Hz, ≤5 μA) through the recording electrode at the depth of the last recorded PTN. In selected penetrations, we made marking lesions (35 μA cathodal current for 10 s) to aid in histological reconstruction.

Neuronal data were digitized at 100 kHz and saved to disk simultaneously with the EMG activity and behavioral markers; the latter were series of signals were digitized at 1 kHz. Video recordings of all experiments were also recorded to DVD using a Panasonic video camera (60 frame/s; shutter speed = 1 ms).

Data analysis

We first screened all of the recorded trials and removed those in which the onset of the EMG activity in the brachialis (Br) or cleidobrachialis (CIB) muscle (forelimb flexors) preceded the GO signal. As in our previous studies (Schepens and Drew 2003; Yakovenko and Drew 2009), the onset of activity in these two muscles defined the onset of the movement. The neuronal trace was then displayed, and a custom-program was used to interactively discriminate single units by using up to three time/amplitude windows that allowed us to isolate the waveform of a given cell, both from the background activity and from any other cells present in the recording. A custom program was also used to interactively mark the onset and offset of the activity in selected EMGs as well as other behavioral cues. These muscles and events always included: the onset of the increase in EMG activity of the Br or CIB muscle in the reaching limb, which was used as an indicator for the onset of movement (see preceding text); the onset of the lever depression; and the onset of the period of EMG activity in the latissimus dorsi (LtD) muscle that preceded the retraction of the limb following the lever press. We refer to this burst of activity in the LtD, occurring just prior to limb
retraction, as LtD2 to distinguish it from the earlier period of activity occurring during the transport period of the limb (see Fig. 1).

The data were subject to two major stages of analysis. In the first case, we used averaged data from all cells to examine the general characteristics of the database. In the second, we analyzed data on a trial by trial basis to examine the quantitative relationships between cell and muscle activity.

**GENERAL CHARACTERISTICS.** In the initial analysis, averages of the cell activity for each condition (unobstructed + with each obstacle), as well as for all conditions combined, were triggered on the onset of the change in EMG activity in the Br or CIB. Significant task-related changes in neuronal activity with respect to the 500 ms period of activity prior to the GO signal (control activity) were determined for three separate periods of the reach. The first was defined as the period from the change in activity of the CIB until the moment of the lever press; this corresponds to the transport period of the reaching movement as the limb is moved toward the lever. The second was the time from the onset of the lever press until the onset of activity in the LtD that preceded retraction (lever-press period). The third was the period beginning with the onset of activity in the LtD and continuing for a period of 0.5 s (retraction period). Significant changes in activity with respect to control were calculated based on a threshold crossing analysis (Yakovenko and Drew 2009). In brief, each of the three periods was normalized to unity, and the averaged activity of the cell during each period was filtered at 30 Hz. If the cell discharge exceeded the interval of confidence ($P < 0.05$) of the SD of the SD of the
control activity for >25% of any given period, then the cell was considered to be significantly modified during that period.

Significant changes of activity could be long-lasting and considered as tonic or brief and considered as a phasic change in activity. In the latter case, we refer to these as peaks or troughs of activity for increased and decreased discharge frequency, respectively. To qualify as a peak or trough, cell discharge had to decrease to 50% of maximum or minimum either within the same period or within the first 50% of the subsequent period. For the retraction period, the value had to be attained by the time the paw was replaced on the platform. The phase of the maximum (peak) or minimum (trough) cell discharge frequency within a period was generally expressed as a positive value between 0.0 (onset of a given period) and 1.0 (end of a given period). The exceptions are those few peaks that precede the onset of activity in the CIB. These are expressed as a negative value with respect to the transport period. In some examples in the manuscript, the phase values are concatenated so that values in the lever-press period are expressed as phase values between 1.0 and 2.0 and those in the retraction period as values between 2.0 and 3.0. Peak discharge frequency was calculated from the normalized and filtered averages.

To determine whether the addition of an obstacle modified cell discharge, we compared the average activity during reaching with an obstacle to the interval of confidence (P < 0.05) of the SE trace during reaching without an obstacle. Deviations from the interval of confidence for >25% of any of the three normalized periods were considered to be significant changes in activity.

TRIAL BY TRIAL ANALYSIS. The second major stage of the analysis was designed to detail the relationship between neuronal activity and muscle activity on a trial by trial basis. For this analysis, cells had to fulfill two criteria: they had to exhibit a significant peak or trough during one of the three defined periods (transport, lever press, or retraction) and they had to show abrupt changes in activity from which we could determine the phase of onset and offset of activity in single trials (see following text). All peaks and troughs that fulfilled these two criteria were included in our analysis, which consisted of five steps.

First, we identified the muscle synergies active during the reach. In brief, we measured the onset and offset of the periods of activity of the recorded muscles and defined these values as a phase value in the same way as for the cell activity (see preceding text). We then plotted the phase of offset versus the phase of onset; we refer to this as a phase space plot. Synergies were then identified by using the associative cluster analysis that we previously used to define synergies during locomotion (Krouchev et al. 2006). In brief, synergies were defined as groups of muscles whose activity was coincidental and in which the onset and offset of activity occurred at the same time. From each synergy, we identified one muscle as being representative of that synergy.

Second, we interactively measured the time of the changes in cell activity in single trials using a custom program. The onset (and offset) of cell discharge was defined as the time when activity diverged from ±2 SD of the control activity for a period of ±75 ms (Yakovenko and Drew 2009).

Third, we determined if there was any overlap in phase space between the spatial location of the cell activity and any of the representative muscles. For this, we identified the mean activity in phase space of both the cell and the muscles (mean of the phase of onset and the phase of offset for the cell and muscle in each trial). Similar to Krouchev et al. (2006), we then calculated an ellipse of 2 SD around the mean of the cell and of each muscle activity. Any overlap of the cell ellipse with any muscle ellipse was taken as evidence of a possible relationship between the cell and one or more of the synergies.

Fourth, for cells showing an overlap with at least one muscle, we calculated the Euclidean (linear) distance between the mean of the cell in phase space and the mean of each representative muscle. We then performed a paired t-test between the distances for the two muscles with the smallest distances. If these were significantly different (P < 0.05), then we assigned the cell to the muscle with the smallest distance. This complemented the overlap analysis by showing that the relationship was significantly better with muscles representing one synergy than with any others.

Last, we used linear regression analysis to determine the relationships between the temporal aspects of cell and muscle discharge and between the magnitude of the cell discharge frequency and EMG amplitude. The magnitude of cell discharge frequency and EMG activity was determined by integrating the activity between the time of onset and the time of offset of the cell and the periods of muscle activity respectively. Cell discharge frequency is expressed in Hz*s and EMG amplitude as mV*s.

Digitized video frames were analyzed using custom software to mark parasagittal positions of reflective markers placed over bony landmarks on the left forelimb. Elbow joint position (which is complicated by skin slippage) was reconstructed using shoulder and wrist marker positions in a kinematic planar model based on morphometric segment measurements. Signals were low-pass filtered (6 Hz) by using a fourth order Butterworth digital filter.

Histology

At the end of the recordings, lesions (35 μA, 10 s, DC cathodal) were made in selected locations to aid in histological reconstruction. The cats were perfused with formaldehyde and the cortex blocked and then sectioned (40 μm) in the sagittal plane. They were then stained with cresyl violet. The location of the penetrations made in each track was plotted on flattened maps of the pericruciate cortex (see Yakovenko and Drew 2009).

RESULTS

Behavior

The general features of the reach were similar to those that we have previously described in this (Schepens et al. 2008; Yakovenko and Drew 2009) and a similar task (Schepens and Drew 2003). The basic features of the kinematics and the activity of selected EMGs during unobstructed reaches are illustrated by the black lines in Fig. 1. In general, the cat produced a smooth limb trajectory from the platform on which the limb was positioned to the lever on which it pressed (Fig. 1A, central trace). The reach was characterized initially by a retraction of the shoulder and a flexion of the elbow together with ventroflexion of the wrist (black lines, Fig. 1B). Subsequently there was a protraction of the shoulder and an extension of the elbow together with a dorsiflexion of the paw prior to and during the lever press. The limb was then retracted to replace it on the platform (Fig. 1B).

The initial, movement-related, changes in EMG activity (Fig. 1, C and D) were seen in the shoulder retractors, such as the LtD and the teres major (TrM, not illustrated). These were followed by an activation of the CIB; the Br was also active at this time but for a briefer period than the CIB. Very shortly after, there was an activation of the extensor carpi radialis, ECR, and then, later, of the extensor digitorum communis, EDC. Note that in the EDC there were two periods of activity, an early one that occurred shortly after the onset of the reach and a later one that occurred at the end of the reach. As the early burst was facilitative in that it was not observed in all trials, we treat only the larger and later burst in the analysis that follows. The elbow extensor, the lateral head of the triceps
brachii (TriL), was active prior to the onset of the activity in the CIB to produce the anticipatory postural activity that precedes the movement (Schepens and Drew 2003; Yakovenko and Drew 2009). Both the TriL and the palmaris longus, PaL, were then activated strongly at the end of the reach to contribute to the limb extension prior to the lever press.

In the presence of the proximal obstacle (placed closest to the body), there was a more pronounced initial retraction of the shoulder as the limb was brought backward away from the obstacle and an increased elbow flexion as the limb was brought above the obstacle. In the EMGs, there was a pronounced increase in the magnitude of the shoulder retractors, e.g., the LtD, an increase in the duration of the Br and the CIB, and a noticeable delay in the peak of the activity of the ECR and the onset of the later period of activity in the EDC. The distal obstacle, placed closer to the lever caused similar but smaller effects. Noticeably the duration of the LtD was not as prolonged as for reaches over the proximal obstacle.

Neuronal database

This manuscript is based on recordings of 151 PTNs, which had a receptive field on the forelimbs or were recorded adjacent to other cells with forelimb receptive fields and which were recorded during at least five reaches with the contralateral limb. These neurons include all of those PTNs used in a previous report (Yakovenko and Drew 2009) that had receptive fields on the forelimb plus additional neurons that had a receptive field on the forelimb but that were not recorded during reaches with both limbs and were, therefore, not included in the previous report. All 151 PTNs were recorded in area 4 of the pericruciate cortex, mostly in the anterior and lateral sigmoid gyri; they are included in the trajectories illustrated in Fig. 5 of Yakovenko and Drew (2009). The mean conduction velocity of the population of 151 PTNs, based on a conduction distance of 44 mm (Armstrong and Drew 1984), was 30.3 ± 14.3 m.s⁻¹. Note that these values underestimate the true conduction velocity because we take no account of the time taken to initiate the action potential (utilization time).

Motor cortical activity during reaching

GENERAL CHARACTERISTICS. In the first stage of our analyses, we examined the general characteristics of the neuronal discharge patterns on the basis of the averaged activity. This analysis was performed on the entire database of 151 PTNs.

During reaches in the unobstructed condition, a total of 134/151 (89%) neurons showed a significant deviation from the control activity (i.e., the 500 ms period of activity immediately prior to the GO signal) during at least one of the three periods of the reach as defined in METHODS. Of the total population, 111/151 (74%) showed a significant change in activity during the transport period of the reach, i.e., between the onset of activity in the coCIB and the onset of the lever press (Table 1, All Cells, unobstructed trials). In a majority of these cells 62/111 (56%), this significant change was defined as a peak (44/111) or a trough (18/111) of activity according to the definitions provided in METHODS (2 cells showed both a significant peak and trough). In the other 49/111 PTNs showing modified activity during the transport period, the discharge was more tonic and continued into the following period when

<table>
<thead>
<tr>
<th>TABLE 1. Forelimb-related PTNs recorded during contralateral reach</th>
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<tbody>
<tr>
<td>Transport</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>All Cells, unobstructed trials (n = 151 cells)</td>
</tr>
<tr>
<td>Modified</td>
</tr>
<tr>
<td>Increases</td>
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<tr>
<td>Decreases</td>
</tr>
<tr>
<td>Peaks</td>
</tr>
<tr>
<td>Troughs</td>
</tr>
<tr>
<td>Cells with obstacles, all trials (n = 98 cells)</td>
</tr>
<tr>
<td>Modified</td>
</tr>
<tr>
<td>Increases</td>
</tr>
<tr>
<td>Decreases</td>
</tr>
<tr>
<td>Peaks</td>
</tr>
<tr>
<td>Troughs</td>
</tr>
<tr>
<td>All Cells, all trials (n = 151 cells)</td>
</tr>
<tr>
<td>Modified</td>
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<tr>
<td>Increases</td>
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<tr>
<td>Decreases</td>
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<tr>
<td>Peaks</td>
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<tr>
<td>Troughs</td>
</tr>
<tr>
<td>Cells included in quantitative, trial by trial analysis</td>
</tr>
<tr>
<td>Peaks</td>
</tr>
<tr>
<td>Troughs</td>
</tr>
</tbody>
</table>

The table shows the number and percentage of cells (in parentheses) with significant increases or decreases of activity during the transport, lever-press, or retraction periods of the movement. Modified indicates cells that showed any significant change according to the threshold crossing analysis. Increases and Decreases include all significant changes, including both phasic and tonic changes. Peaks and Troughs indicate cells that showed significant phasic increases or decreases in activity (see METHODS and text). The two rows in bold indicate the data that are graphed in Fig. 3. The nominator in the last section identifies those cells with significant peaks and troughs that we were also able to analyze on a trial by trial basis.

The cat pressed on the lever. Similar proportions of cells also showed discrete, phasic periods of activity during the lever-press stage and in the retraction stage that brought the limb back to the force platform (Table 1). A comparison of those cells that showed an initial period of activity time-locked to the GO stimulus (Yakovenko and Drew 2009) and those that showed activity only related to the movement showed no differences in the basic properties of these neurons during the reach and they are therefore included together in these analyses.

A total of 98 PTNs were recorded during reaches made with an obstacle placed either in the proximal or distal location or both. Considering together all trials in these 98 PTNs (with and without obstacles), we found that similar but slightly increased proportions of cells showed significant changes during the three periods of the reach (Table 1, Cells with obstacles, all trials) as when considering only the unobstructed trials. Similar proportions were equally observed when examining all trials for all cells, including those with and without obstacles (Table 1, All cells, all trials). Given the similarity in the proportion of cells showing peaks and troughs in the three conditions, in the analysis that follows, we use data from all 151 cells including trials with and without obstacles.

Examples of cells showing increased activity during the transport or lever-press period of the movement are shown in
Fig. 2. The three cells illustrated in Fig. 2, A–C, all showed a significant peak of activity during the transport period (Tr) of the behavior. In the cells illustrated in Fig. 2, A and C, there was a subsequent significant decrease in the discharge activity during the lever-press (LP) period (with respect to control), whereas in the cell in Fig. 2B, the cell discharge remained significantly greater during the lever-press period than in control. In the cells illustrated in Fig. 2, B and C, there was a secondary increase in activity with respect to control as the arm was retracted (Retr; 2nd vertical dotted line) and replaced on the platform. The values above each plot (+1, −1) indicate, respectively, whether there was a significant peak or trough during the indicated period. Note that a cell might show a significant change in activity but no peak according to the definitions in METHODS. For example, the decrease in activity in the cell in Fig. 2A following the initial increase was classified as a tonic change. The cell illustrated in Fig. 2D had a significant peak of activity occurring just subsequent to the lever press and had a small but significant decrease in its activity (trough) during the preceding transport phase. The decrease in discharge in the transport period in this cell is particularly clear in the raster display. The cells in Fig. 2, A and B, were passively activated by light touching of the paw pads and the ventral surface of the forearm (see insets), whereas the cell in Fig. 2C was activated only by passive dorsiflexion of the digits. No receptive field was identified for the cell in Fig. 2D.

As is clear from Fig. 2, different cells discharged maximally during different periods of the behavior (transport, lever press, retraction) and also discharged at different phases within any one given period. For example, the cells in Fig. 2, A–C, all...
discharged maximally at slightly different phases (0.29, 0.39, and 0.5, respectively) during the transport period of the movement. The phase of activity of all of those neurons showing a significant peak or trough of activity during at least one of the three periods of the reach is illustrated in Fig. 3. In brief, both peaks (black lines and filled circles) and troughs (gray lines and gray circles) were distributed throughout each of the three periods in a continuous manner. Peak frequencies for cells with significant peaks ranged ≤150 Hz.

When considering all trials together (Table 1, All cells, all trials), 52 PTNs had a significant peak during the transport period and 32 a significant trough. Moreover, 34/52 of the cells with a significant peak also had a significant peak (19/52) or trough (15/52) during either the lever-press or the retraction period. Similarly, 24/32 of the cells with a significant trough during the transport period had a significant peak (19/32) or trough (5/32) in one of the other two periods.

**EFFECT OF OBSTACLES.** The presence and the location of the obstacle sometimes modified either the phase and/or the magnitude of the PTN response. For example, in Fig. 2, B and C, the averaged discharge activity in the presence of either obstacle (blue and red superimposed traces) was significantly greater than in the unobstructed condition. In contrast, in the example of Fig. 2A, there was little difference between the activity in the unobstructed condition and that observed in the presence of the two obstacles.

Overall, 37 cells showing a significant peak of activity during the transport period were recorded during reaches over the distal obstacle and 23 during reaches over the proximal obstacle (Table 2). Of these, 10/37 (27%) of the cells showed a significant difference in the presence of the distal obstacle with respect to the unobstructed condition, and 7/23 (30%) in the presence of the proximal obstacle. Similar proportions were observed for the lever-press period but were lower for the retraction period (Table 2). The 23 cells recorded in the presence of the proximal obstacle were all also recorded in the presence of the distal obstacle. A comparison of these two conditions showed that 5/23 (22%) showed a significant difference in discharge frequency (Table 2). There were no substantial changes in the time of peak activity with the different obstacles.

**RELATIONSHIP TO MUSCLE ACTIVITY.** Figures 2 and 3 clearly show that different populations of cells were active at different times during the reaching movement with some cells being most active during the transport period, others during the lever press, and still others during the subsequent retraction. Moreover, even during transport, the phase of the peak discharge of the cells was distributed throughout the period. This sequential activation is similar to that observed during voluntary gait modifications (Drew 1993) and suggests that PTNs might contribute to the control of the activity of different groups of synergistic muscles during the reach in much the same way as suggested for locomotion (Drew et al. 2008b; Krouchev et al. 2006). This viewpoint is supported by more detailed examination of the relationship between the phase and magnitude of the cell and muscle activity.

**TABLE 2. Number of identified peaks or troughs that had significant differences when comparing response magnitude**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Transport</th>
<th>Lever Press</th>
<th>Retraction</th>
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<tbody>
<tr>
<td>Unobstructed vs. distal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peaks</td>
<td>10/37 (27)</td>
<td>5/20 (25)</td>
<td>2/23 (9)</td>
</tr>
<tr>
<td>Troughs</td>
<td>2/26 (8)</td>
<td>0/12 (0)</td>
<td>2/7 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>12/63 (19)</td>
<td>5/32 (16)</td>
<td>4/30 (13)</td>
</tr>
<tr>
<td>Unobstructed vs. proximal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peaks</td>
<td>7/23 (30)</td>
<td>3/15 (20)</td>
<td>1/13 (8)</td>
</tr>
<tr>
<td>Troughs</td>
<td>0/15 (0)</td>
<td>0/9 (0)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>7/38 (18)</td>
<td>3/24 (13)</td>
<td>2/18 (11)</td>
</tr>
<tr>
<td>Distal vs. proximal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peaks</td>
<td>5/23 (22)</td>
<td>4/15 (27)</td>
<td>3/13 (23)</td>
</tr>
<tr>
<td>Troughs</td>
<td>0/15 (0)</td>
<td>0/9 (0)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>5/38 (13)</td>
<td>4/24 (17)</td>
<td>3/18 (17)</td>
</tr>
</tbody>
</table>

For unobstructed vs. distal and unobstructed vs. proximal, the discharge activity with either the distally or proximally located obstacle is compared to the discharge activity in the absence of an obstacle. The last condition (Distal vs. proximal) compares the discharge activity during a reach over the distally located obstacle to reaches over the proximally located obstacle. We compare only data from cells identified as having a significant peak or trough in both conditions. As an example, 37 cells had significant peaks in the transport period during both the unobstructed condition and during reaches over the distal obstacle. In 10/37 (27) of these cells the discharge activity in the two conditions was significantly different.
IDENTIFICATION OF SYNERGIES. We plotted the activity of the recorded muscles in phase space and used an associative cluster analysis to identify those muscles that discharged coincidentally (Krouchev et al. 2006). For comparison with the synergies identified during unobstructed locomotion in the study of Krouchev et al. (2006), we used only trials from unobstructed reaches. The results of this analysis for cats MC29 and MC28 are shown in Fig. 4A and B, respectively. In both cats, the recorded muscles clustered in four clearly separate areas of the phase space. In MC29, the largest group of points (in the bottom left of the plot) was subdivided into three by the cluster analysis providing a total of five clusters in which both onset and offset occurred during the transport period (phases 0.0–1.0), together with a sixth cluster related to extensor activity associated with the lever press. In cat MC28, a similar organization was observed with the exception that the absence of recordings from the Br in this cat precluded the identification of a cluster related to elbow flexion. Moreover additional recordings from the acromiodeltoideus muscle (AcD) led to an expansion of the cluster (4) related to limb transport. The muscles included in the different synergies are illustrated in Fig. 4C. The largest of these clusters (1) contained several muscles acting primarily to retract the shoulder but also included a distal muscle, the pronator teres (PrT). Clusters 2 and 3 were represented by single muscles in this plot, namely the Br acting to flex the elbow and the ECR acting to dorsiflex the wrist. Cluster 4 was also represented by a single muscle, the CIB, acting to transport the limb forward, whereas cluster 5 was represented by both wrist and shoulder muscles active just prior to contact with the lever. The sixth cluster included extensor muscles acting at the elbow and wrist that served to depress the lever.

Based on this cluster analysis, we selected five muscles to represent the synergies identified in cat MC28. We use the LtD, a shoulder retractor to represent synergy 1; the ECR, a wrist dorsiflexor, to represent synergy 3; the EDC, a wrist and digit dorsiflexor, to represent synergy 4; the CIB, a shoulder protractor, to represent synergy 5; and the PaL, a wrist and digit ventralflexor, to represent synergy 6. Because we did not record the Br in cat MC28, this synergy (2) was not included in the analysis.

The temporal distribution of the onset and offset of these selected muscles as measured from the ensemble of the recordings made from both cats MC28 and MC29 is shown in Fig. 4D. The figure shows that the relative phase of activity in each muscle was very stable and occupied only a small portion of the total phase space as quantified by the contour on the top layer of the plot which illustrates 3 SD of the mean phase of onset and offset. Moreover, this phase space is limited even though we combine data from all trials (unobstructed and with obstacles) from both cats MC28 and MC29. This emphasizes the stability of the representation in phase space across conditions and across cats. Even combining all of these data, the period of maximal activity in each muscle is clearly distinct from that in the other muscles and the only minimal overlap is between the phase space occupied by the LtD and the ECR.

Because most of the synergies that we identified occur within the transport period, the emphasis on the analysis that follows is placed on this period of the reach.

TEMPORAL RELATIONSHIPS BETWEEN CELLS AND SELECTED MUSCLES. The period of activity of many of the cells that showed significant phasic changes in activity during the transport period covaried with the activity of one or other of the muscles selected to represent the synergies.

For example, Fig. 5 provides details on the discharge activity of the cell illustrated in Fig. 2B. Aligning the cell with respect to the onset of selected muscles, active at different times during the transport period (see Figs. 1 and 5A), shows that both the onset and offset of the discharge activity in this cell was best temporally related to the period of activity of the ECR (Fig. 5A, middle). There was a second period of activity in the cell during the lever press that occurred at the same time as a second, much weaker, increase in activity in the ECR. The striking visual impression of the temporal relationship of the major period of discharge activity to the ECR is supported by scatterplots both of the temporal relationship between the onset of activity in the cell and the muscle (Fig. 5B) and the magnitude of the activity (C). In each case, the best relationship is with the ECR. Note that in this example, the cell discharge activity during the reaches over each of the obstacles was substantially greater than in the situation in which there was no obstacle in the reaching path (compare red and blue circles with the black circles in Fig. 5C, see also Fig. 2B).

Similar well correlated changes with both phase and magnitude were also observed in many of the other PTNs that we recorded. Figure 6 shows four examples of different cells, each best related to muscles active at different times during the reach. The cell illustrated in Fig. 6A showed a tight relationship between the offsets of the period of cell discharge and the end of the period of activity of the LtD (Fig. 6Aii) although their onsets were less well correlated. This was a common feature of those cells whose peak activity occurred during the activity of the LtD (see following text). There was also a significant relationship between the magnitude of the cell discharge and the muscle activity (Fig. 6Aiii). The activity of the cells illustrated in Fig. 6, B and C was phase delayed with respect to the cell in A. The onset and the offset of the period of cell discharge in the cell illustrated in Fig. 6B aligned with the onset and offset of the activity in the CIB. The significant relationship of the offset of activity in cell and CIB is illustrated in the scatterplot of Fig. 6Bii. There was no relationship between the magnitude of cell discharge and CIB activity in this example (Fig. 6Biii). The cell illustrated in Fig. 6C was still more phase-delayed and the onset of the activity preceded, but was time-locked to, the onset of activity in the EDC (Fig. 6Cii). The end of the period of cell activity was also well related to the offset of the period of EDC activity (not illustrated), and the level of the cell discharge frequency was correlated with EDC magnitude (Fig. 6Ciii). Finally, the example in Fig. 6D showed a late burst of activity that was related to the period of the LtD that served to replace the limb on the force platform after pressing the lever (LtD2). Again the onset of the cell discharge was correlated to the onset of activity in the muscle (Fig. 6Dii), and there was a highly significant relationship with EMG magnitude with the discharge (Diii) being greatest for the proximal obstacle (red symbols).
Fig. 4. Activity of selected muscles in phase space. A and B: phase of offset of selected muscles plotted against the phase of the onset for cats MC29 (A) and MC28 (B). Each muscle is identified by a combination of its color and symbol (see key). Data are taken from unobstructed trials in 5 (MC29) or 6 (MC28) series of recordings. Muscles identified as belonging to different clusters are identified in the key. The thick ovals identify the centroids of the identified clusters. Note that only a part of cluster 6 is illustrated in A. In cat MC28, Br was not well recorded and synergy 2 is absent. Synergy 6 was identified but is not displayed. C: anatomical location of the illustrated muscles, organized according to the identified clusters. Note that in A and C, we identify the behavioral correlate of each cluster of muscles. D: the offset of the period of muscle activity is plotted against the phase of onset of that activity for 5 selected muscles. Data are taken from all measured EMG bursts, with and without obstacles, from both cats MC28 and MC29 (n > 1,000 bursts of activity for all muscles). Data are plotted in red for LtD, blue for ECR, mauve for CIB, cyan for EDC, and red for PaL; brown symbols represent points that were >3 SD of the mean. The bottom plane plots these same data in 3-dimensions (3D) where the height of the peak is proportional to the density of the points. The top plane plots contours equal to 3 SD of the mean of the activity within the phase space. Values connected by arrows to the contours on the top plane indicate the synergy to which the muscles belong.
QUANTITATIVE RELATIONSHIPS BETWEEN CELL AND MUSCLE ACTIVITY. In the second stage of the analysis, we concentrated on the quantitative relationship between cell and muscle activity. This analysis was confined to a subset of PTNs (Table 1, bottom 2 rows), each of which showed at least one significant peak of activity during one of the defined periods of activity (transport, lever press, or retraction), and each of which showed an abrupt change in discharge activity allowing the measurement of the time of onset on a trial by trial basis (see METHODS). This analysis was performed on a total of 105 cells of which 68 exhibited significant peaks and 37 significant troughs (Table 3). We initially present data from our phase space analysis and then compare that with the results from our linear regression analysis.

Phase space analysis. We propose that the periods of maximal (or minimal) activity of cells temporally related to the activity in these representative muscles (or synergistic muscles with similar patterns of activity) should closely overlap the period of activity of the muscles representing each synergy. To determine the extent to which this was true, we performed two complementary analyses as detailed in METHODS. First, we determined if there was overlap between the onset and offset of the period of cell activity and similar measures for muscle activity. Subsequently, we determined the Euclidean distance between the period of cell discharge and the discharge of each of the representative muscles in phase space as detailed in METHODS. These calculations were made using all individual trials, with and without obstacles, to take advantage of the...
variability in the phase of activation produced by the obstacles. As shown in Fig. 1, the presence of obstacles modifies the relative timing of muscles with respect to the onset of CIB. For example, during reaching over obstacles, the phase of the main

TABLE 3. Principal database used for the trial by trial analyses

<table>
<thead>
<tr>
<th>All cells, all trials (trial by trial analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells in this database = 105 (all 3 periods)</td>
</tr>
<tr>
<td>Significant peaks = 68</td>
</tr>
<tr>
<td>Significant troughs = 37</td>
</tr>
<tr>
<td>Cells significantly related to a muscle (all 3 periods) = 71</td>
</tr>
<tr>
<td>Peaks = 53</td>
</tr>
<tr>
<td>Troughs = 18</td>
</tr>
<tr>
<td>Total cells with peak or trough in transport period = 62</td>
</tr>
<tr>
<td>Significant Peaks = 35</td>
</tr>
<tr>
<td>Significant Troughs = 27</td>
</tr>
<tr>
<td>Cells significantly related to a muscle = 41</td>
</tr>
<tr>
<td>Peaks = 27</td>
</tr>
<tr>
<td>Troughs = 14</td>
</tr>
</tbody>
</table>

The table identifies the number of cells used for the analysis of the trial by trial analysis. It shows both the total number of cells and those analyses in the transport period. For each group of cells, we identify the total number of cells showing a significant peak or trough together the number of peaks or troughs that were significantly related to one of the representative muscles.

EDC burst is phase-delayed with respect to the phase of activity of muscles active earlier in the transport period. This variation in timing helps to differentiate cells related to different muscle groups. For example, in Fig. 5, the cell maintains a constant relationship with the ECR for both the proximal (red traces in the raster plots) and the distal (blue traces) obstacle while the relationship with the LtD is clearly different for the two obstacle conditions.

The plots in Fig. 7, A–C, show the phase plots of three representative cells. The activity in each cell is compared with the phase plots of three muscles (LtD, ECR, and EDC), each representing a different synergy. The cell illustrated in Fig. 7A, is the same as that illustrated in Fig. 6A, the cell in Fig. 7B is the same as in Fig. 5, and that in Fig. 7C is the same as in Fig. 6C. As illustrated by the 2 SD ellipses centered on the mean of the cell and muscle activity, the activity of the cell in Fig. 7A overlapped with the period of activity of both the LtD and the ECR, that of the cell in B overlapped only with the activity of the ECR, and that in C overlapped only with the activity of the EDC. These overlaps were used as the initial indication of a relationship between cell and muscle activity. In the second stage of this analysis, we used the Euclidean distance analysis...
to determine whether the cell was preferentially related to a single muscle. For the cells illustrated in Fig. 7, A–C, the average distance between a given cell and each of the three muscles is shown on each plot. In the cell illustrated in Fig. 7A, the Euclidean distance to the LtD (0.31) was only slightly shorter than that to the ECR (0.36), but a paired _t_-test showed that the distance to the LtD was significantly shorter \( (P < 0.05) \) than that to the ECR. Although the cell activity overlapped both the LtD and the ECR, it was, therefore, classified as being best related to the LtD. In the other two cases, the cell overlapped with only one of the representative muscles and the Euclidean distance analysis, calculated on a trial by trial basis, showed that the best relationship (shortest distance) was significantly different \( (P < 0.05) \) from the next lowest distance.

Overall, a total of 71/105 cells (53/68 peaks and 18/37 troughs) showed a significant relationship with one of the selected muscles (Tables 1 and 3). Overall, from the 41 significant bursts during this period, 10 were best related to the LtD, 8 to the ECRL, 10 to the EDC, and 13 to the CIB. Considering only the 27 peaks, 6 each were related to the LtD, ECR, and CIB and 9 to the EDC (Table 4, All). For the 23 bursts of cell activity with a peak \( (n = 16) \) or trough \( (n = 7) \) during the lever-press period, the majority of the cells with a significant relationship (15/23) 

![Fig. 7. Activity of 3 selected cells in phase space. A–C: for 3 cells (from left to right, those illustrated in Figs. 6A, 5, and 6C), we illustrate the phase of activity of the cell (black filled circles) and 3 selected muscles (LtD, open black circles; ECR, open red circles, and EDC, open cyan circles) in phase space. Each circle indicates the activity of the cell or muscle during a single trial. The larger filled circles indicate the mean activity of the cell and each muscle, and the solid lines illustrate the Euclidean distance between the mean phase of the cell activity and that of each muscle. Numbers to the side indicate distance as a phase of the normalized transport period. The large open ovals indicate the 2 SD ellipses drawn around the mean of the periods of cell and muscle activity. D: the mean phase of 62 cells showing a significant peak or trough of activity and for which we were able to measure the onset and offset of the activity on a trial by trial basis is plotted in phase space. Each cell is represented by a filled circle and cells showing significant relationships to a given muscle are connected to the mean phase of the muscle to which they are related by a solid line. Red circles and lines indicate cells showing a peak; blue circles and lines indicate troughs and gray circles indicate cells showing no significant relationship. Red, dotted contours link the cells showing a significant relationship to a given muscle. Cells illustrated in Figs. 6 and 7, A–C, are identified on the plot.](http://jn.physiology.org/doi/abs/10.1152/jn.00110.2010)
were related to the PaL (10 peaks and 4 troughs). Similarly, for the retraction period, 15/20 cells showed a significant relationship, and these were all best related to the second period of activity in the LtD.

In the analysis described in the preceding paragraphs, we used all trials from all conditions with and without obstacle and with both proximally and distally located obstacle. In addition, we also applied the identical analysis to each condition separately. The results from this analysis are illustrated in Table 4 for those 52 cells with significant peaks of activity that could be best related to one of the representative muscles (Table 4, All). In general, fewer cells were related to the indicated muscles in the other three conditions (unobstructed, distal, proximal) than when all trials were considered (all). However, this is probably more due to the small number of trials in each separate condition reducing the significant power of the analysis than to any difference in the relationship between cell and muscle in the different conditions. This suggestion is particularly supported by the fact that for all cells in all conditions, when there was a significant relationship it was always with the same muscle as when all trials were considered. A similar finding was obtained when considering those cells that showed a significant trough of activity.

**Relationship between cell discharge frequency and EMG timing and magnitude.** For those 27 cells showing peaks of activity during the transport phase and that were significantly related to one of the selected muscles on the basis of the phase space analysis, we examined the relationship between the intensity of the cell discharge and both the timing and magnitude of the EMG activity as in Figs. 5, B and C, and 6, A–D (ii and iii). These same relationships were also examined for the 10 cells showing a peak related to the PaL and 15 cells showing a peak related to the second burst of activity in the LtD, during the retraction. These relationships are summarized in Table 5.

With respect to the timing relationships, the linear regressions showed that 11/21 (52%) of the cells related to one of the muscles active during the transport phase showed a significant relationship between the time of onset of cell activity and the time of onset of muscle activity (Table 5, peak onset). This value increased to 16/27 (59%) for the relationship between the offset of cell and muscle activity (peak offset) and to 19/27 (70%) when considering whether there was a relationship between *either* the onset or the offset. We also examined whether the least significant coefficient obtained with the best muscle identified on the basis of the Euclidean distance analysis was greater than with the other five muscles included in the analysis. This was the situation for the relationship with the EMG onset for 15/21 (71%) of the cells active during the transport period and for 15/27 (55%) of the offsets. When considering only those relationships that were significant, the values increased to 11/11 (100%) for the onsets, 13/16 (81%) for the offsets, and 16/19 (84%) when considering either onsets or offsets. Extending the analysis to include also those cells active related to the PaL and to the LtD2 showed that 38/52 (73%) of all cells included in this analysis showed a significant relationship between either the onset or offset of activity. Further, 33/38 (87%) of these significant relationships had a regression coefficient that was greater than with the other five muscles.

With respect to the relationships between cell discharge frequency and cell magnitude, 10/27 (37%) of the cells active during the transport period showed a significant relationship between these two measures (Table 5, peak int vals). In 7/10 (70%) of these cells showing significant relationships and in 15/27 (55%) of the total, the regression coefficient was highest for the muscle showing the best relationship in the Euclidean distance analysis. However, overall, only 6/27 (22%) of the cells showed a coefficient of determination ($R^2$) of >0.3 between cell discharge and the magnitude of the muscle with which it was best related.

**Timing of peak discharge**

One question that arises is how the partition of the muscles into phase space on the basis of the analyses illustrated in Fig. 7 relates to the continuum of peaks illustrated in Fig. 3. This is addressed in Fig. 8, A and B, which uses a color code (see legend) to identify cells classified as being significantly related to one or other of the selected muscles on the basis of the analysis illustrated in Fig. 7. Figure 8A shows the entire database of 84 cells showing significant peaks of activity. From this figure, it is clear that the peak phases of cells significantly related to a given muscle are largely distinct from each other with minimal overlap. At the same time, cells within each group show a relatively wide range of peak phase values. Examples of cells belonging to different groups but showing peak discharges close to the beginning and end of the continuum of each cluster are illustrated in Fig. 8C. The two cells identified as being related to the EDC show the largest separation in peak values although it is clear that discharge in both cells completely overlaps the burst in the EDC muscle (offset indicated by small tick on each raster line). In the case of the cell with peak discharge at phase $\phi_1 = 0.57$, the cell began to discharge prior to the onset of activity in the EDC; in the case of the cell with peak discharge at $\phi_2 = 0.98$, the cell discharge is greatest at the end of the period of activity in the EDC. The six cells illustrated in Fig. 8C are represented in phase space in D. Cells that could not be significantly related to one of the select muscles (open circles in Fig. 8A) were found intermingled among those that were. Note that in many cases the phase of the peak discharge of cells defined as being best related to a given muscle on the basis of the phase space analysis overlaps with the period of activity of several muscles. The peak

**TABLE 4. Results of the phase space analysis for the significant peaks in different conditions**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>All</th>
<th>Unobstructed</th>
<th>Distal</th>
<th>Proximal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LtD</td>
<td>6</td>
<td>3/6</td>
<td>3/4</td>
<td>1/2</td>
</tr>
<tr>
<td>CIB</td>
<td>6</td>
<td>6/6</td>
<td>2/5</td>
<td>1/2</td>
</tr>
<tr>
<td>ECR</td>
<td>6</td>
<td>2/6</td>
<td>5/6</td>
<td>3/5</td>
</tr>
<tr>
<td>EDC</td>
<td>9</td>
<td>5/9</td>
<td>4/6</td>
<td>1/3</td>
</tr>
<tr>
<td>PaL</td>
<td>10</td>
<td>9/10</td>
<td>9/9</td>
<td>4/5</td>
</tr>
<tr>
<td>LtD2</td>
<td>15</td>
<td>15/15</td>
<td>13/15</td>
<td>6/7</td>
</tr>
</tbody>
</table>

For each representative muscle, we show the number of cells related to the indicated muscle when considering all trials (All) as in Fig. 7 together with the number of cells related when considering only obstructed trials and reaches made with either the distal or proximal obstacles. In the last three columns, the first value indicates the number of significantly related cells and the second the number of cells tested in that condition (note that data were not always available for all conditions for all cells). LtD, latissimus dorsi; CIB, clidobrachialis; ECR, extensor carpi radialis; EDC, extensor digitorum communis; PaL, palmasis longus; LtD2, burst activity in the LtD occurring just prior to limb retraction.
The table provides the number of cells showing significant relationships ($P < 0.05$) between cell activity and different measures of timing and magnitude of electromyographic (EMG) activity as determined by linear regression analysis. All cells included in the analysis were classified as having significant peaks of activity that were related to one of the representative muscles on the basis of the phase space analysis. Linear regressions were made between different parameters of the cell discharge and the EMG activity of that muscle with which the cell was best related. For peak onset and peak offset, we show the number of cells showing a significant relationship between the phase of onset or offset of activity with respect to CIB onset. For example, 6 cells had significant peaks of activity that were related to the LtD on the basis of the phase space analysis, and 3 of them showed significant relationships between the onset of the cell discharge in individual trials and the onset of the bursts of muscle activity. Note that there are no data for CIB onset as this was the muscle used for synchronization and the phase of onset was therefore always 0.0. Rows with Best $R^2$ indicate how many of the linear regressions for all cells related on the basis of the Euclidean distance (1st pair) and those also showing significant regressions (2nd pair) were greater for the indicated muscle. Again, for the 6 cells related to LtD, 5 of them showed a higher regression coefficient for the regression of cell onset vs LtD onset then for cell onset vs. any other muscle. Similarly of the 3 cells showing significant regressions, all 3 were higher than with any other muscle. The row labeled Peak offset OR offset indicates how many cells showed a relationship with either the onset or the offset of the EMG activity. For the two rows examining the integrated values (Int Vals), the table indicates the number of cells showing significant relationships between the onset of the movement (ClB onset) and the onset of each of the groups parallels very closely the averaged activity of those neurons (red lines) identified both as having a significant peak of activity during either the transport or the lever-press period and as being best related to a given muscle on the basis of the phase space analysis (i.e., those illustrated by the red lines in Fig. 7D). Averaging together each of these groups of neurons emphasizes two points. First, there is a progressive shift in the phase of the peak discharge of the averaged activity of each group of neurons. Second, the initial peak of activity in each of the groups parallels very closely the averaged activity of the representative muscle with which they are best related (black lines).

### Timing of onset

The timing of the onset of the cell discharge with respect to both the onset of the movement (CIB onset) and the onset of the muscle to which the cell was significantly related on the basis of the phase space analysis is illustrated in Fig. 10, A and B, respectively. The figure includes data from cells showing either a peak or trough during the transport or lever-press period and is based on measures made from individual trials. As would be expected on the basis of the sequential activation of these cells (see Fig. 9), there was a progressive increase in the timing of cell onset relative to the LID discharged earliest (Fig. 10A, top), whereas those significantly related to the EDC and the PaL discharged latest (Fig. 10A, bottom). On average, cell discharge preceded CIB onset by 123 ms for the LID population while it followed CIB onset by 117 ms for the EDC populations and by 282 ms for the PaL population. When the onset of cell discharge was compared with the onset of the muscle with which it was best related (Fig. 10B), cell and muscle onset occurred, on average, simultaneously (range: 3–32 ms) for most muscles. This suggests that, on average, the cell discharge precedes only slightly the onset of the muscle activity. In contrast, the LID population was clearly different from the others in that cell onset preceded muscle onset by an average of 92 ms. For comparison, the timing of the onset of muscle activity with respect to the onset of the CIB is illustrated in Fig. 10C. The average onset of a given muscle with respect to CIB onset (Fig. 10C) is similar to the values for the time of cell onset (A) for all muscles except the LID.

### Microstimulation and localization

Microstimulation with the cat at rest was applied at all sites from which task-related cells were recorded. As reported in a previous publication (Yakovenko and Drew 2009) for the same dataset, microstimulation in many of these sites evoked a combination of effects acting around the shoulder, elbow, and wrist. We were unable to find any simple relationship between the nature of the responses evoked at a given site and the classification of a cell as being related to a given synergy. However, at a stimulus strength of 25 $\mu$A, the stimulus-evoked effects, either a facilitation or a depression, always included the muscle with which the cell was significantly related on the basis of the phase space analysis.

We were equally unable to find any relationship between the muscle with which a cell was best related and the location at which that cell was recorded. Cells significantly related to any one of the selected muscles were scattered throughout the mediolateral extent of the region from which we recorded (not illustrated).
DISCUSSION

This study examined the discharge activity of identified PTNs recorded in the motor cortex of the cat during reaching movements made to depress a lever. In agreement with other studies in cats and primates (see next paragraph), the results show that neurons in the cat motor cortex show substantial increases in discharge frequency at different periods of this movement. The results extend previous findings by showing that PTNs may be organized in functional groupings that are activated sequentially during the reach. Moreover, in agreement with our previous studies during locomotion (Drew 1993; Drew et al. 2008a,b; Krouchev et al. 2006) and with some previous studies in primates (e.g., Holdefer and Miller 2002; Morrow et al. 2009), our results support a contribution of motor cortical neurons to the control of intralimb coordination by the activation and/or modulation of muscle synergies.
prised of small groups of muscles. We suggest that this organization would provide a flexible environment that would allow relatively small changes in cortical activity to produce the coordinated changes in muscle activity patterns required to modify the limb trajectory, for example, to circumvent obstacles in the path of the reach.

Sequential activity, phase space, and synergies

The general discharge characteristics of the cells observed in this task were similar to those described in many other papers examining motor cortical activity in cats (Perfiliev 2005; Vicario et al. 1983) and primates (Griffin et al. 2008; Holdefer and Miller 2002; Lemon 1981a,b; McKiernan et al. 2000; Murphy et al. 1985) during reaching or reach to grasp movements. For most cells, discharge activity consisted of a single peak of activity that occurred either during the transport, lever-press, or retraction period, although in a number of cells there were two peaks of activity, one during transport and one during retraction.

Beyond the basic distinction between cells active in the three periods of the reach (transport, lever press, and retraction), we also found clear evidence for a discrete, sequential activation of PTNs within a period with different cells being activated phasically for brief periods at different times during the transport. We suggest that this sequential activation of different populations of PTNs contributes to the sequential activation of different synergies that is necessary to ensure intralimb coordination during the reach. This organization is conceptually similar to that previously proposed for the cortical contribution to the control of the swing phase of locomotion (Drew et al. 2008b; Krouchev et al. 2006).

Muscle synergies

The idea that muscle synergies may provide a substrate for the control of multiarticular movements is popular (Bizzi et al. 2000; Cheung et al. 2009; Giszter et al. 2007; Hart and Giszter 2010; Tresch and Jarc 2009; Tresch et al. 2002) but not without controversy (see e.g., Kurtzer et al. 2006). As summarized in a recent review (Tresch and Jarc 2009), there is a great deal of evidence to suggest that muscle synergies may provide a substrate to facilitate the control of complex, multiarticular movements. At the same time, there is much less evidence to show how these synergies might be used by the CNS to facilitate the control of such movements. Moreover the definition of a synergy also varies in the literature with most studies describing muscle activity patterns in terms of time-invariant synergies in which all muscles in a synergy are activated simultaneously, whereas others define time-varying synergies in which the activity of component muscles may be varied in both time and amplitude with respect to other muscles in a synergy (see d’Avella et al. 2003; Tresch and Jarc 2009). In addition, the vast majority of studies use mathematical decomposition methods to define synergies in which it is the relative weights of each muscle that define the final activation period.

In contrast to the more commonly used decomposition methods, in a previous publication (Krouchev et al. 2006), we used an associative cluster analysis to examine the organization of muscle synergies during unobstructed locomotion in the intact cat. In that study, we used a much more restricted definition of a synergy as a group of muscles that are temporally co-activated and in which muscle activity begins and ends synchronously. On this basis, we identified nine synergies during the swing phase of locomotion and suggested that these synergies might form the basis by which the motor cortex modifies gait modifications during locomotion (Drew et al. 2008b). Many of these synergies were multiarticular in that their activation would produce changes around more than one of the major joints of the forelimb. Moreover the synergies were sparse in that each synergy contained only a small subset of the muscles in the total dataset. In the current study, we identified five synergies during the transport phase of the limb. However, because Br was well recorded in only one cat, we implicitly included the Br and the ECR together, reducing the number of synergies used in the analysis to four during the transport period. As during locomotion, each of these synergies was related to distinct behavioral events during the transport period, namely, 1) shoulder retraction, 2 and 3) elbow flexion and wrist doriflexion, 4) limb transport, and 5) extension of the limb prior to contact with the lever. There was also a further synergy (6) activated later in the movement that grouped together muscles active during the limb extension preceding but also continuing throughout the lever press. These behavioral periods are analogous to those identified in our locomotion study (Krouchev et al. 2006).

The smaller number of synergies identified in this study compared with locomotion can be explained primarily by the formation of one large cluster (1) at the initiation of the reaching movement in this study (Fig. 4) compared with the presence of three clusters during the same analogous moment during locomotion. It is not clear whether this difference is methodological or functional. For example, it is possible that the difference may reflect a greater variability.
in activity patterns during reaching compared with treadmill locomotion where cycle duration is constrained by the treadmill speed. Equally, it might be related to poor recordings from one or two muscles in this study given that we do not have examples from multiple cats during reach for the purposes of comparison as we did during locomotion. Al-
ternatively, it may reflect a real overlap of activity in muscles during the transport phase of reach that were temporally distinct during the swing phase of locomotion. It will need additional studies to determine whether the cluster of activity of different muscles at the onset of reach is the result of a limited database or a true reflection of the biomechanical constraints and the differences in neural control required to initiate reaching from a stationary support as opposed to regulating the dynamic transition between stance and swing.

It should also be noted that some of the synergies are composed of a single muscle. As discussed previously (Krouchev et al. 2006), this probably reflects the limited number of muscles from which EMG activity was recorded. For example, recordings from multiple wrist dorsiflexors would undoubtedly show additional muscles active in phase with the ECR and thus incorporated into cluster 3 (Fig. 4A) containing this muscle. Similarly, there are other muscles such as the cleidotrapezius and the levator scapularis that are involved in transporting the limb forward that were in the same cluster as the CIB during locomotion but that were not recorded in this study. At the same time, we do not believe that additional recordings would substantially increase the number of synergies (with the possible exception of those at reach onset) as the synergies identified already define the major biomechanical elements of the transport period.

Sequential activation of PTNs and phase space

On the basis of our analysis of the temporal relationships between cell and muscle activity in phase space, we identified four groups of PTNs during transport. Each of the PTNs in these groups was significantly related to one of the muscles chosen to represent the synergies active during the transport phase. We identified one additional group related to the activity of the extensor muscles related to the lever press. Synergies during retraction were not examined in any detail. The ensemble averaged activity of the cells in each of the five groups of PTNs together with the averaged activity of simultaneously recorded EMGs was summarized in Fig. 9. We propose that each of these groups of PTNs contributes to the regulation of the activity of one of the synergies. In this view, different populations of motor cortical cells modulate the activity of groups of muscles controlling multiple joints and active at different times during the reach. Intralimb coordination is then assured, in part, by the appropriate sequential activation of the appropriate groups of motor cortical cells. Although arguments based only on temporal coincidence of discharge are open to criticism (Fetz and Finocchio 1975), it bears emphasizing that it is also clear that cells that do discharge coincidentally with muscles are those most likely to directly influence their activity. Ideally, one would identify the muscle field (the muscles directly activated by a given cortical output cell) of a recorded cortical cell by spike triggered averaging (STA) as in the primate to support claims of causality. However, this has not proven possible in the cat (Drew 1991; Yakovenko and Drew 2009) in large part because of the absence of direct corticomotoneuronal projections in this species. We are therefore limited to the more indirect methods that we used.

It should be emphasized that our criteria to show a relationship between a cell and a muscle were very strict and the phase space analysis required a close overlap between the entire period of cell and muscle activity. Moreover, this relationship needed to be maintained both in the presence of the natural variations in the spatiotemporal activity patterns of the recorded muscles as well as during the larger changes in activity observed in the presence of the obstacles. Cells were only classified as being significantly related to a muscle if their onset and their offset covaried with one muscle, independently of that of other muscles. The tight relationship between cell and muscle activity is well represented in Figs. 5 and 6. This strict requirement is more likely to introduce false negatives (cells that were inaccurately classified as not being related to a given muscle and its related synergy) than false positives. For example, the cell illustrated in Fig. 2C showed an excellent temporal relationship with the activity of the EDC muscle, but the activity sufficiently overlapped the period of activity of the CIB that it was not significantly better related to the EDC than to the CIB. Moreover this strict requirement removes the ambiguity that occurs when the period of activity of muscles overlaps but has different durations (e.g., CIB and ECR). It is also unlikely that these relationships are the result of false positives, i.e., by chance. Although there is a clear continuum of peak activity, there is no a priori reason why the on- and offset of a given burst would closely overlap the onset and offset of a given muscle representing one of the synergies. Indeed, if the onset and offset of a given burst were truly random, one might expect a majority of cells to show no relationship to any given muscle. As Fig. 7D clearly shows, this was not the case. The most parsimonious reason for the overlap that we observed would seem to be a true relationship between the phase of activity of the cells and the phase of activity of the muscles in the respective synergy.

In addition to the phase space analysis, we also examined the relationships of PTNs to muscle activity using more conventional linear regression analyses (Table 5). This analysis was not a replacement for the phase space analysis but rather a complement to determine the extent to which individual cell and muscle parameters were correlated. In general, the results from this analysis were complementary to those obtained from the phase space analysis. Thus 70% of the cells identified as being related to a given muscle on the basis of the phase space analysis showed a significant correlation between either the onset or offset of the cell activity and the onset or offset of the period of activity of the muscle to which it was related. This suggests that not only do the periods of the cell and muscle activity overlap, but, in the majority of the cases, there is a strict relationship between the onset and or offset. This very striking match between the results from the phase space analysis and those from the linear regression analysis strongly supports our arguments that these cells contribute to the modification of muscle synergies.

Many fewer cells (37%), however, showed a significant relationship between the magnitude of the cell discharge and the magnitude of the EMG activity of the muscle to which they were best related. This is similar to the findings of other studies that have examined this relationship and is probably related to
the fact that multiple cells contribute to the level of activity of most muscles (see following text).

Although our analysis identified populations of PTNs activity whose covaried with a given synergy, it is unlikely that each population influenced only the muscles in a given synergy. Microstimulation studies, for example, emphasize that even relatively low intensity current at a single motor cortical site may activate multiple muscles, especially during locomotion (Armstrong and Drew 1985). Indeed at some sites, stimulation during locomotion may activate muscles active in different synergies, for example Br, EDC, and TrM or LtD. Similarly, the more specific spike triggered averaging technique in primates frequently shows that a broad range of muscles receive postspike facilitation from a single corticomotoneuronal cell (McKiernan et al. 1998). Further, anatomical tracing techniques show that individual axons branch to innervate multiple motoneuron pools (Futami et al. 1979) and interneurons contacted by corticospinal fibers likewise branch to multiple motoneurons (Alstermark et al. 1990, 1991). Therefore despite the fact that the activity of these PTNs covaries with the activity of muscles restricted to a single synergy, it is probable that they also influence muscles active in other synergies at different times during the transport period. In other words, it is probable that the activity in the PTN covaries with the activity of only some of the muscles whose activity may be influenced by that cell. Indeed this is exactly what was shown by Bennet and Lemon (1996; see also Fetz and Finocchio 1975; Griffin et al. 2008; Lemon 1993) in the primate. Bennet and Lemon (1996) demonstrated that in situations in which two muscles receiving input from a given corticospinal cell discharged differentially (were fractionated), the cell activity preferentially covaried with the activity of only one of these. In our task, it is possible that collateral branches influencing muscles outside the targeted synergy may facilitate intralimb coordination by priming the interneuronal circuits that are the next to be activated in the sequence (see also Drew 1991).

Comparison with other studies

Our view of the contribution of the motor cortex to the control of intralimb coordination via multijarticular synergies is clearly different from that proposed by Murphy et al. (1985) in which cells controlling more proximal and more distal muscles were activated sequentially according to the nested-zone hypothesis (Murphy et al. 1978). In particular, several of our synergies contain both proximal and distal muscles that are activated simultaneously not sequentially as in the study of Murphy et al. (1985). Rather, in our view, the intralimb coordination is specified by the sequential activation of these mixed synergies. Our results are, however, compatible with two other studies that have examined motor cortical cells related to different joints during reaching. In the study of Scott (1997), motor cortical cells related to the shoulder or the elbow were recorded during reaching movements in different directions. He found that changes in onset time and magnitude of the two groups of cells showed similar changes to the changes of these parameters in the two groups of muscles, suggesting a specific contribution to muscles acting around these two different joints. Our results are particularly compatible with those of Holdefer and Miller (2002), who used a cross-correlation analysis to determine the strength of the relationship between a given cell and different limb muscles during reaching in the primate. Despite the very different methods used in their study and the difference in species, they also concluded that groups of cells served to regulate the activity of different groups of muscles active at different times of the movement. Although they did not try to determine synergies they did identify groups of cells related to functional groupings of muscles required to extend the limb, open the hand, and press on a button. In more recent work (Morrow et al. 2007, 2009), the authors used a vector analysis to define the muscle space in which cortical neurons were active. They found that the vectors from individual cells clustered in different parts of their muscle space and suggested that each cluster of neurons might contribute to the control of different groups of synergistic muscles.

Our results are also compatible with those of a more recent study by Griffin et al. (2008), who used STA to directly identify the muscle field of the corticomotoneuronal cells that they recorded. They recorded neuronal activity during a reach and grasp task, which they divided into 10 segments and then determined whether the peak of activity in a given CM cell occurred in the same segment as the peak activity of the muscle showing the largest postspike facilitation during STA. Somewhat surprisingly only 20% of CM cells showed this level of overlap. However, fully 95% of cells showed an overlap between one period of activity in cell and muscle, supporting the authors’ conclusion that the motor cortex encodes movement in a muscle-based framework (see also McKiernan et al. 2000). However, as also emphasized in the preceding text, it must also be realized that not all cells show such a simple relationship, and even for corticomotoneuronal cells, there is not always a simple relationship between the pattern of cell discharge and the activity of muscles showing postspike facilitation or depression (Bennet and Lemon 1996; Griffin et al. 2008; McKiernan et al. 2000).

A contribution of the motor cortex to the regulation of muscle synergies during reach is also supported by the recent work of Cheung et al. (2009), who demonstrated that while the composition of synergies during arm movements was not affected by cortical stroke, the level of the activation of synergies was modified. The authors took this as support for a cortical modulation of spinal synergies. A similar conclusion was also reached by a study that examined modular organization during locomotion following stroke (Clark et al. 2010).

Other cells

In the preceding discussion, we have placed an emphasis on those cells for which we were able to show a significant relationship with the activity of single muscles representing the various synergies active during the movement. Although these cells form a majority of those cells that we were able to test by using the phase space analysis (41/62, 66%), they are a minority of the overall database of the modulated cells (41/123, 33%). The cells that could not be related to specific synergies may be considered separately according to whether they were tested using the phase space analysis or
not. First, for those cells that were analyzed with this method, the lack of a significant relationship may reflect a false negative (as suggested in the preceding text), a relationship to unidentified synergies, or a more complex relationship to multiple synergies or kinematic relationships. We are unable to distinguish between these possibilities. Second, a large number of cells that showed phasic activity were not analyzed with the Euclidean distance methods because we were unable to accurately detect on- and offset of activity from single trials. Such cells may include both those that were related to the synergies as well as those that were unrelated for the same reasons as for the population treated in the preceding text. Last, some cells (26/123) showed a tonic change of activity throughout the entire reach. Overall, the data suggest that a majority of cells show a relatively discrete phasic discharge related to specific events during the reach.

Timing of cell activity

It has frequently been reported that the onset of cell discharge in the motor cortex precedes the onset of movement by \( \leq 150 \) ms (see e.g., Cheney and Fetz 1980). This is similar to the average value of 92 ms that we observed for those cells best related to the onset of activity in LtD, the muscle representing the earliest synergy to be activated. However, the timing relationships between cell and muscle for those cells activated sequentially later in the reach were substantially less, ranging from an average value of 3 ms for the cells best related to ECR to values of 25 and 32 ms for those cells best related to the EDC and PaL, respectively. We suggest that these differences in relative timing between the cells related to the LtD and the other populations reflect the sequential nature of the reach. At the onset of the reach, the level of depolarization in the flexor motoneurons is relatively low, and the cat needs to produce sufficient activity to depolarize them sufficiently to initiate the movement. This situation is similar to that in most studies in which timing information in cells is related to the time of the first change in EMG activity or movement. However, as we argue in the preceding text, because of their widely branching axons, the cells responsible for activating the muscles included in synergy 1 are also likely to depolarize, or prime, the activity of the muscles that will subsequently be activated in the sequence. As a consequence, one would expect, as observed, that such primed motoneurons would reach threshold substantially faster than those of the unprimed LtD. These relatively short time lags are similar to those observed by Griffin et al. (2008) for their populations of cells, which included many that were activated after the onset of the reach.

Conclusions

During a reach toward a target, muscles have to be activated in the appropriate order and at the appropriate level to both control the trajectory of the limb and the endpoint of the reach. This control is particularly important when the trajectory has to be modified to avoid obstacles in the direct path. Our analysis of the averaged EMG activity of forelimb muscles showed that different groups of muscles were activated in a sequential pattern of activity and that both the relative timing and the magnitude of EMG activity was modulated by the obstacles. More interestingly our cluster analysis showed that different groups of muscles were activated as synergies at different times during the reach. As we have suggested for locomotion (Drew et al. 2008b; Krouchev et al. 2006), the ordered activation of these multiarticular, synergistic groups of muscles may provide a means to regulate interlimb coordination and modify limb trajectory according to the specific goals of the reach.

The results from our unit recording study show that the activity of PTNs in the forelimb representation of the motor cortex are compatible with a control system whereby subpopulations of PTNs regulate the activity of different muscle synergies. We suggest that PTNs in each subpopulation activate some or all of the muscles included in a given synergy. As there are no monosynaptic corticospinal connections with motoneurons in the cat (Illert et al. 1976), this projection is via interneurons that may provide an anatomical substrate for activating synergistic groups of muscles. As each of the synergies in this study defines different biomechanical actions, slight modifications in activity in the different subpopulations of neurons would provide substantial flexibility in modifying limb trajectory. However, it also needs to be emphasized that the results from the present study have the limitation that we examined only reaching movements in a sagittal plane. Whether such an organization extends to control at the shoulder, which can produce a wider range of movements, is open to question, although the recent studies of d’Avella et al. (2008) suggest that such is the case.

If the corticospinal system projects onto spinal circuits that originally evolved to control locomotion, as suggested by Georgopoulos and Grillner (1989), it is possible that the same corticospinal organization and synergies may be used both for locomotion and reaching. The wide range of patterns of cortical activity that are then observed during reaching may reflect a dual system: one that acts through evolutionary old circuits and takes advantage of the possibility of controlling intralimb coordination via these circuits and a second that needs to circumvent these circuits to produce more fractionated movements. The complexity of cortical circuits may, in part, reflect the difficulties of producing complex patterns of fractionated activity via spinal circuits that conserve a capacity to coordinate more evolutionarily simpler patterns of movement.

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Disclosures

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

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