Do Corticomotoneuronal Cells Predict Target Muscle EMG Activity?

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Abstract

Data from two rhesus macaques was used to investigate the pattern of cortical cell activation during reach-to-grasp movements in relation to the corresponding activation pattern of the cell’s facilitated target muscles. The presence of postspike facilitation (PSpF) in spike-triggered averages (SpTA) of electromyographic (EMG) activity was used to identify cortical neurons with excitatory synaptic linkages with motoneurons. EMG activity from 22-24 muscles of the forelimb was recorded together with the activity of M1 cortical neurons. The extent of covariation was characterized by: 1) identifying the task segment containing the cell and target muscle activity peaks, 2) quantifying the timing and overlap between CM cell and EMG peaks, and 3) applying Pearson correlation analysis to plots of CM cell firing rate versus EMG activity of the cell’s facilitated muscles. At least one firing rate peak, for nearly all (95%) CM cells tested matched a corresponding peak in the EMG activity of the cell’s target muscles. Although some individual CM cells had very strong correlations with target muscles, overall, substantial disparities were common. We also investigated correlations for ensembles of CM cells sharing the same target muscle. The ensemble population activity of even a small number of CM cells influencing the same target muscle produced a relatively good match ($r \geq 0.8$) to target muscle EMG activity. Our results provide evidence in support of the notion that corticomotoneuronal output from primary motor cortex encodes movement in a framework of muscle based parameters, specifically, muscle activation patterns as reflected in EMG activity.
Introduction

The presence of PSpF in spike-triggered averages of EMG activity provides a means of identifying cortical neurons with demonstrable excitatory synaptic linkages to motoneurons (Buys et al. 1986; Fetz and Cheney 1980; Schieber and Rivlis 2005). Similarly, postspike suppression (PSpS) identifies the presence of underlying inhibitory synaptic linkages (Kasser and Cheney 1985). Muscles with PSpF or PSpS are defined as the cell’s target muscles. PSpF effects with durations at half magnitude of 9 ms or less can be attributed to underlying monosynaptic connections (Baker and Lemon 1998; Schieber and Rivlis 2005). Accordingly, cells producing these PSpF effects can be more confidently categorized as corticomotoneuronal (CM) cells.

Given that the presence of PSpF is evidence of an underlying synaptic linkage and that neurons producing PSpF represent the output signal from motor cortex to spinal motoneurons, a fundamental issue concerns the extent to which the activity of these cortical cells predicts or even encodes target muscle EMG activity (Schieber and Rivlis 2007; Towsend et al. 2006; Hamed et al. 2007). There is an underlying assumption that if postspike effects on muscle activity are functionally meaningful, then the cells producing the effects and their target muscles should show some level of covarying activity during task performance. Our previous work (McKiernan et al. 2000), using long duration cross-correlations of continuous data (Houk et al. 1987), suggests this is true for identified CM cells. Taking this analysis a step further, one might also expect that the temporal pattern of activity of an individual CM cell might closely resemble the temporal
pattern of target muscle EMG activity. However, these expectations must be tempered by the fact that muscle activation reflects the summation of converging EPSPs from many cells terminating within the motoneuron pool. A single cell will only make a small contribution to overall motor unit activation so its relationship to the pattern of target muscle activity may be weak and variable. In view of this, one minimal expectation might be that the activity of the majority of CM cells and their facilitated muscles should at least show coactivation during the same segment of a movement task and that their peaks of activity should exhibit overlap. Although individual CM cells might have temporal patterns of activation that closely match the pattern of target muscle EMG activity, this is not essential. However, it is true that the ensemble activity of an identified population of CM cells sharing a common target muscle should have a temporal pattern of activity during movement that closely resembles the pattern of EMG activity, assuming that CM input to the motoneuron pool is a major factor driving motoneuron depolarization underlying an EMG peak and assuming a relatively linear transformation of cortical spike trains into EMG activity.

To further investigate the extent of covariation between CM cells and their target muscles, we have identified where peaks in their activity occur during a forelimb reach-to-grasp task and have quantified the extent of overlap between them. The results show that 71% of CM cell peaks match a target muscle peak in the same task segment. CM cell peaks show an average of 74% overlap with peaks in their target muscles. We also report significantly improved correlations between the ensemble activity of a population of CM cells influencing the same
target muscle and that muscle’s EMG activity compared to the individual CM cell correlations.

**Methods**

*Behavioral task*

Two male rhesus macaques were trained to perform a reach-to-grasp task as described previously (McKiernan et al. 1998). Inside a sound attenuating chamber, the monkey was seated in a custom built primate chair facing a computer monitor providing audiovisual cues. The monkey’s left arm was comfortably restrained and the task was performed with the right arm. The task was initiated when the monkey placed his right hand, palm down, on a pressure detecting plate (home plate) at waist level in front of him on the right side. Holding the plate down for a preprogrammed length of time (1-2 seconds) triggered the release of a food reward into a cylindrical well at arms length from the monkey. The monkey then grasped and brought the food reward to its mouth. This task provided a robust paradigm in which to test relationships between CM cell and target muscle activity. The task broadly coactivated both proximal and distal forelimb muscles while at the same time yielding a relatively high level of fractionation in terms of the detailed structure of the EMG pattern in different muscles.

*Surgical procedures*
After training, a 22 mm diameter stainless steel chamber was centered over the hand area of M1 of the left hemisphere of each monkey and anchored to the skull with 12 vitallium screws and dental acrylic. Threaded stainless steel nuts were also attached over the occipital aspect of the skull using 12 additional vitallium screws and dental acrylic. These nuts provided a point of attachment for a flexible head restraint system during recording (McKiernan et al. 1998, 2000).

EMG activity was recorded with pairs of insulated, multi-stranded stainless steel wires inserted transcutaneously into each of the target muscles (McKiernan et al. 1998, 2000). Electrode locations were confirmed by stimulation through the electrode pair and observation of appropriate muscle twitches. Electrode wires and connector terminals were affixed using medical adhesive tape. Following surgery, the monkeys wore a Kevlar vest and sleeve to protect the implant. EMG activity was recorded simultaneously from 22-24 forelimb muscles (Table 1).

For all surgeries, the monkeys were tranquilized with Ketamine (10 mg/kg) and anesthetized with isoflurane gas. Surgeries were performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) using full sterile procedures. All work involving these monkeys conformed to the procedures outlined in the Guide for the Care and Use of Laboratory Animals published by the U.S. Department of Health and Human Services and the National Institutes of Health.

Cortical recording
Single cells in primary motor cortex (M1) were recorded using glass and mylar insulated platinum-iridium electrodes with typical impedances between 0.7 and 1.5 MΩ. A recording electrode was positioned within the chamber using an X-Y coordinate manipulator and was advanced into the cortex with a manual hydraulic microdrive (FHC Corp.). Electrode orientation was at a right angle to the cortical surface.

Spike-triggered averages

Cortical cell activity, EMG activity and position signals were recorded on analog tape using a 28-channel TEAC instrumentation recorder. Spike-triggered averages and response averages were compiled off-line using a custom software package (Windows Neural Averager, Larry Shupe, University of Washington, Seattle). The action potentials of single cells in M1 served as the triggers for computing SpTAs. Single unit spikes were isolated using an Alpha Omega MSD spike discriminator. EMG activity was routinely filtered from 30 Hz to 1 KHz, digitized at 4 KHz and full-wave rectified. Averages were compiled using an epoch of 60 ms, extending from 20 ms before to 40 ms after the unit spike.

Segments of EMG activity associated with each spike were evaluated by the software and accepted for averaging only if the average of all data points over the entire epoch was ≥ 5% of full scale input. This prevented averaging EMG segments where activity was minimal or absent (McKiernan et al. 1998).

Categorization and quantification of postspike effects and cell firing frequency
The CM cells analyzed here were used in previous studies of postspike effects in forelimb muscles (McKiernan et al. 1998, 2000). For the present analysis, the postspike effects of many of the cells were recomputed from tape playback and enhanced by increasing the number of trigger events.

Categorization of effects in spike triggered averages was based on the latency and width of effects. We estimated the minimum reasonable latency for PSpF of muscles at different joints to be: 3.4 ms for shoulder muscles, 4.2 ms for elbow muscles, and 6.0 ms for intrinsic hand muscles (McKiernan et al. 1998, 2000). Effects with shorter latencies were presumed to have synchrony components. Schieber and Rivlis (2005) evaluated PSpF effects using a criterion developed by Baker and Lemon (1998) derived from a spike-triggered averaging simulation model. This model suggests that pure PSpF effects arising from underlying monosynaptic connections with motoneurons can be identified based on the peak width of PSpF at half magnitude (PWHM). A PWHM of 9 ms or less was suggested as an effective criterion for identifying PSpF effects that are most likely due to underlying monosynaptic PSpF (Baker and Lemon 1998). A PWHM of 9 ms was the criterion applied by Schieber and Rivlis (2005) and we have also adopted this criterion. Taking into account these latency and width factors, in this study we categorized PSpF effects as: 1) pure PSpF if this was the only effect present and its PWHM was 9 ms or less; 2) PSpF on synchrony (PSpF+Sync) if a primary PSpF could be identified based on a discontinuity in the slope of the rising phase of an underlying synchrony facilitation and the primary PSpF effect possessed a latency consistent with a minimum cortex to muscle pathway.
(Flament et al. 1992); 3) late widening PSpF (Schieber and Rivlis 2005) if only a primary effect was present but the PWHM was greater than 9 ms and its latency could be explained without requiring the presence of synchrony; and 4) pure synchrony facilitation (SyncF) if the effect was broad with an onset latency inconsistent with a realistic minimum cortex to muscle pathway and no primary PSpF could be identified as a sharp peak riding on a broad synchrony peak. A similar categorization was used for suppression effects. Although synchrony effects are of interest and may contain a component mediated by a synaptic output linkage between the cortical cell and motoneurons, for the purposes of this study, we have excluded synchrony effects from the analysis. All effects included in this study were either pure PSpF or late widening PSpF effects. For convenience, we will refer to cortical cells producing these effects as CM cells.

All identified postspike effects were assigned a ranking of weak, moderate, or strong based on the magnitude of the effect (Figure 1). Nonstationary, ramping baseline activity was routinely subtracted from SpTAs using our analysis software. The EMG values from a range of bins in the pretrigger period were averaged to arrive at a baseline mean and standard deviation (SD). The baseline typically was determined by averaging a 10 ms segment of each record during the pretrigger period. The onset and offset of each peak were determined as the points where the record crossed a level equivalent to + 2 SD above the mean of the baseline EMG (see McKiernan et al. 1998; Figure 4A). Peaks less than 2 SD of baseline and peaks that remained above 2 SD for less than a 0.75 ms period were considered insignificant, and the
average was categorized as having no effect (Figure 1). The color coding of
effects based on magnitude used in Figure 1 is maintained throughout all figures
of the paper.

The peak of each effect was defined as the highest point of the PSpF. The magnitude of each PSpF was quantified in terms of its peak percent
increase (PPI) above baseline, peak-to-noise ratio (P/N), and P/N normalized to
10,000 trigger events. P/N magnitudes were normalized based on the principle
that signal-to-noise ratios should increase as the square root of the number of
trigger events (Belhaj-Saïf et al. 1998). Ten thousand was approximately equal
to the median of the number of trigger events for all PSpE analyzed (Park et al.
2004). Expressions for these measures are as follows:

\[
PPI = 100 \frac{(\text{Maximum bin value} - \text{baseline mean})}{\text{baseline mean}}
\]

\[
P/N = \frac{(\text{PSpF peak} - \text{baseline mean})}{\text{baseline standard deviation}}
\]

\[
\text{Normalized } P/N = \sqrt{\frac{10,000}{\# \text{ trigger events}}} \times \frac{P}{N}
\]

After normalizing the P/N ratio, the magnitude of PSpF effects were
categorized as follows (Figure 1). Weak PSpF effects had peaks greater than 2
SD of mean baseline activity but less than 4 SD; moderate effects had peaks
equal to or greater than 4 SD of mean baseline activity but less than 7 SD; and
strong PSpF effects had peaks of 7 SD or greater.

The depth of modulation (DOM) in CM cell firing rate (Hz) was measured
for all peaks using response average records referenced to different parts of the
movement sequence. CM cell activity peaks were identified in segments of the record that exceeded 2 SD of the baseline points. Baseline was determined from activity while the monkey’s hand was on home plate (segment #1 in Figure 2) and EMG activity was largely absent. DOM was then calculated by subtracting the cell’s lowest firing rate during baseline activity from its highest firing rate during the peak of activity. Peaks in CM cell activity were then ranked by magnitude as primary (highest peak value), secondary (2nd highest peak value) tertiary (3rd highest), and quaternary (4th highest).

Response averages consisting of unit firing rate, full wave rectified EMG activity for each of 22 to 24 implanted forelimb muscles, the home plate signal and the food well signal were aligned to each of the four segments of the task: leaving home plate, entering target food well, exiting target food well and returning to home plate. Response averages were typically based on 40-60 trials and were four seconds in duration. The bin width for unit spikes was 10 ms and the sampling rate for all analog channels (EMG and movement parameters) was 100 Hz or 10 ms/point. EMG was full-wave rectified and low pass filtered.

**Quantification of cell-muscle covariation**

For each response average, peaks in CM cell and EMG activity were assigned to one of 10 segments of the reach-to-grasp task as illustrated in Figure 2. The details of timing for defining the boundaries of each task segment are given in the legend for Figure 2. These segments were then used as the criterion for determining if peaks in CM cell activity were associated with peaks in target
muscle EMG activity. Peaks in CM cell and target muscle EMG activity were considered “matching” if they both fell within the same segment of the task. The durations of segments 1 (on home plate) and 5 (in the food cylinder) were considerably longer than other segments and potentially could allow non-overlapping peaks in CM cell and muscle activity to be called “matching”. However, the mean peak time difference between CM cell and target muscle EMG activity was not significantly greater for these movement segments compared to other movement segments.

Our goal in segmenting the reach-to-grasp task was not only to identify the location of CM cell firing rate peaks relative to functionally distinct task segments, but also to establish a sufficient number of segments to provide reasonable temporal resolution. The onset and duration of segment 8 (at the mouth) were estimated based on the fact that the monkey’s hand reached his mouth about half way between exiting the food well and depressing home plate.

As noted above, one objective of this approach was to document what phase of the reach-to-grasp task engaged the activity of each CM cell and its target muscles. This approach also provided a measure of the extent to which peaks in CM cell and target muscle activity occurred during the same functionally distinct task segment. Given the fact that a single CM cell is just one of hundreds of cells contributing to the activity of motoneurons belonging to the target muscle, it is unreasonable to expect that the cell and muscle peaks should necessarily be completely overlapping and coincident. However, if the cell is part of a larger neural network causally involved in generating muscle activity, it is reasonable to
expect that the peaks of activity should at least be partially overlapping and would occur during the same functional task segment. To quantify the temporal coupling between CM cell and target muscle EMG activity we measured the time difference between their matching peaks, that is, peaks falling within the same segment of the task, and the extent of overlap between peaks.

Covariation was visualized and quantified by plotting CM cell firing rate in response averages against target muscle EMG point-for-point as a scatter plot (Griffin et al. 2004; Schieber and Rivlis 2007). Pearson correlation coefficients (r) were then calculated for these scatter plots. Four response averages were generated for each CM cell as described above. The analysis period was sufficiently long to contain the entire movement cycle within each average. However, the average producing the highest peak in cell activity revealed the aspect of movement the cell was best related to and this average was used to calculate the correlation coefficient. For example, in Figure 4, all four averages show a peak in CM cell activity corresponding to exiting the food well. However, the peak was most sharply defined in the average triggered from exiting the food well so that average was selected for performing the correlation analysis. However, the correlation coefficients were very similar for all four sets of averages belonging to a particular cell.

**Measurement of EMG cross-talk**

Cross-talk between EMG electrodes was evaluated by constructing EMG triggered averages. This procedure involved using the motor unit potentials from
one muscle as triggers for compiling averages of rectified EMG activity of all other muscles. The criterion established by Buys et al. (1986) was used to eliminate effects with cross-talk. To be accepted as a valid postspike effect; the ratio of PSpF between test and trigger muscle needed to exceed the ratio of their cross-talk peaks by a factor of two or more. One muscle of any muscle pair that did not meet this criterion was eliminated from the data base. Based on this criterion, we eliminated at total of 11 effects from both monkeys over the course of four EMG implants.

Cortical Maps

The procedure used for producing a two-dimensional rendering of the location of cortical sites was described previously (Park et al. 2001). Briefly, the cortex was unfolded and the location of cells were mapped onto a two dimensional cortical sheet based on the cell’s X-Y coordinate, known architectural landmarks and observations noted during the cortical implant surgeries (Figure 3).

Results

Data were collected from the left M1 cortex in two rhesus monkeys. A total of 44 task related CM cells were recorded, 22 in monkey N and 22 in monkey K. CM cells used in this study were derived from the same database used in two previous reports (McKiernan et al. 1998, 2000). Spike-triggered averaging of EMG activity from 22-24 forelimb muscles yielded 187 postspike
and synchrony effects as follows: 135 pure PSpF or late widening PSpF effects, 14 syncF, 7 PSpF+S, and 31 pure PSpS. The total number of pure or late widening PSpF effects obtained by joint was: 18 shoulder, 28 elbow, 27 wrist, 23 intrinsic hand, and 39 digit. Of the total, 13% were strong effects (>7 times the SD of the baseline points), 41% were moderate effects (4-7 times the SD of the baseline points) and 46% were weak effects (2-4 times the SD of the baseline points). Eighty percent of the CM cells facilitated more than one muscle; 61% facilitated three or more muscles.

CM cell-target muscle modulation during reach-to-grasp

Response averages referenced to leaving home plate, entering the food well, leaving the food well and returning to home plate were generated for each CM cell (Figures 2 and 4). The maximum DOM observed among the 44 CM cells during the reach-to-grasp task ranged from 186 Hz for the 1° peak to 12 Hz for the 4° peak. The overall mean DOM for primary peaks was 80 Hz and 56 Hz across all peaks. Figure 4 shows an example of a complete set of four response averages compiled for one CM cell. As noted in the Methods, the analysis period was sufficiently long that all segments of the task are present in each response average. The peak in activity for the cell in Figure 4 was strongest in the response average triggered from exiting the target food well, although the peak of activity actually occurred about midway through segment 5 of the task (digits in the food well). The discharge peaked about 300 ms before leaving the food well with a DOM of 97Hz. All four of the cell’s facilitated target muscles (green and
blue records corresponding to moderate and weak PSpF effects respectively) show a peak in EMG activity within the same segment of the task (gray shading), defined as a “matching” peak. Several non-target muscles also showed matching peaks of activity including ECU, ED2,3, ED4,5, EDC, ECR, FCU, and TLON. The peaks in activity of the cell’s facilitated muscles lagged the CM cell’s peak by 30–140 ms but they all (except FDI) began to rise in advance of the CM cell’s peak. All of the target muscle peaks were present in the same segment of the task and overlapped substantially with the cell’s peak.

For 1 of 3 CM cells with single peaks of activity, the primary EMG peaks in all facilitated target muscles occurred in the same segment of the task (100% matching); the match was 25% (i.e., one of four target muscles) for another cell and for the 3rd cell, the primary target muscle EMG peaks were in different segments of the task. However, most CM cells had multiple peaks of modulation during the task. The total number of activity peaks was as follows: three had one peak, six had two peaks, 14 had three peaks, and 21 had four peaks.

The peaks in CM cell activity were distributed over the entire movement cycle. Figure 5 shows a histogram of all the CM cell peaks associated with each segment of the task coded for whether it was the cell’s primary (strongest) peak or a weaker peak. The majority of firing rate peaks (44%) occurred in segments 4 (entering target food well), 5 (in target food well) and 6 (exiting target food well) of the movement cycle. A substantial number (28%) were also associated with segments 8 (at the mouth) and 9 (in transit back to home plate). It is noteworthy that these are all phases of task that most heavily rely on skilled use of the distal
muscles and correlates with the fact that a majority of cells (52%) facilitated distal muscles exclusively or most strongly. For example, the cell in Figure 4 facilitated digit and wrist flexor muscles and showed a single strong peak in segment five of the task, undoubtedly associated with flexion of the wrist and digits related to grasp of the food pellet. The concentration of peaks in activity associated with activity in the food well and at the mouth reflects the importance of CM cells in controlling distal muscles associated with shaping the hand, grasping the reward and release of the food pellet into the mouth. Relatively few CM cell peaks (3.5%) were associated with segments 10 (depression of home plate) and 1 (hold on home plate). The EMG levels during these segments of the task were also relatively low.

Do peaks in CM cell activity match EMG peaks in their facilitated target muscles?

Peaks in CM cell firing rate and target muscle EMG activity were compared to determine the extent to which they occurred in the same segment of the reach-to-grasp movement task (defined as matching peaks). This approach is based on the rationale that while the timing and duration of peaks in individual CM cells and target muscles would not be expected to correlate perfectly, they should at least be associated with the same functional segment of the task and show some overlap. Figure 6 shows the results obtained using criteria that varied in the level of rigor needed to conclude that the cell’s peaks matched the target muscle’s peaks, with Figure 6A being the most rigorous and 6C the least rigorous. In Figure 6A, we determined the number of cells whose 1° peak was in
the same segment of the task as the 1° EMG peaks of the target muscles. Since most CM cells had multiple target muscles, the percentage given for each cell reflects the fraction of target muscles that met the criterion. Secondary firing rate peaks were ignored. For 64% of CM cells (numbers 1-28) none of the target muscle primary peaks matched the cell’s primary peak. For this strictest criterion, the mean CM cell-target muscle peak match was 20% including the cells with zero matches. Some CM cells (7.0%) showed a 100% match, that is, all the cell’s target muscles had their primary peak in the same segment of the task as the CM cell. The outcome did not correlate with either the number of facilitated muscles or the number of muscle peaks. We then relaxed the criterion and determined for each CM cell whether its 1° peak was in the same segment of the task as any peak in the target muscle EMG (Figure 6B). Once again the percentage given for each cell reflects the number of target muscles that met this criterion. This yielded a mean CM cell-target muscle peak match of 45%, that is, 45% of target muscles had a peak of some magnitude in the same segment of the task as the primary peak of the CM cell. In Figure 6C, we determined for each CM cell the percent of target muscles that had a peak of any magnitude that matched a CM cell peak of any magnitude. This yielded an average match of 85%. Overall, 71% of CM cell firing rate peaks had a matching target muscle EMG peak. Nearly all CM cells (95%) had at least one peak that matched a peak in a target muscle.

Finally, for each CM cell, we determined the percent of CM cell firing rate peaks with matching EMG peaks relative to the number of total possible matches
(Figure 6D). For example, if a CM cell had two peaks of activity, each of its target muscles would need to show two corresponding peaks in the same segments of the task for a 100% match. If the same CM cell facilitated four target muscles, the total possible chances for matching peaks would be eight. Therefore a 50% match for this cell would reflect any combination of cell and target muscle peak matches where there were four EMG peaks that matched a CM cell peak. This yielded an average CM cell-target muscle peak match of 45% (Figure 6D). In three cases, all the peaks in CM cell activity were matched by corresponding peaks in target muscle EMG activity.

Figure 7 shows two examples of identifying matching peaks in CM cell firing rate and EMG activity (peaks occurring in the same segment of the task) and how this data was used to construct the plots in Figure 6. A subset of task segments are color coded and labeled 4-9 at the bottom of the figure. CM cell105N6, represented by the black bars in Figure 6, and CM cell 65N6, represented by the grey bars in Figure 6, both show four peaks of activity. Both cells have a primary peak (highest firing rate) associated with segment 6 (exiting the target food well) of the reach-to-grasp task. Only 105N6 has a primary peak that matches a primary peak of EMG activity in one of its facilitated muscles (APB). Since 105N6 had seven target muscles, 14% of all target muscles had primary peaks that matched the cell’s primary peak. Similarly, 65N6 showed a 0% match (0/3) to primary peaks in its target muscles. However, 105N6’s primary peak matches three of the nonprimary peaks in its muscles (the tertiary peak of TLAT and the secondary peak of both BRA and BR). This yields a 57%
match between the cell’s primary peak and any target muscle EMG peak (Figure 6B). 65N6 shows only one target muscle peak match with its primary peak (secondary peak of ED45) yielding a 33% match based on the criterion of Figure 6B. Taking this analysis further, all seven of 105N6’s target muscles show at least one peak that matched one of the cell’s peaks yielding a value of 100% in Figure 6C. By this same criterion, 65N6 had two target muscles with peaks that matched one of the cell’s peaks yielding a 67% match in Figure 6C. Since 105N6 has 4 peaks of activity and facilitates 7 muscles, the total possible matches would be 28. However, only 14 peaks in EMG activity actually match peaks in CM cell activity - a 50% match in Figure 6D. 65N6 has 4 peaks of activity and facilitates 3 muscles yielding 12 total possible matches. However, only 2 actual matches were observed for this cell and its target muscles - a 17% match in Figure 6D.

**Timing between peaks in CM cell and target muscle activity**

To provide detailed information on timing, we measured the time lag between matching peaks in CM cell and target muscle EMG activity. Figures 8A and B show the distribution of time lags plotted according to the strength of synaptic connection (magnitude of PSpF; Figure 1) and cell firing rate modulation (DOM). Cell-muscle peak time difference was determined using the time corresponding to the highest point in the peak for both unit and EMG activity. Fifty-six percent of peaks were within ±100ms of each other. Based on analysis of 190 matching activity peaks, the CM cell peak led the target muscle EMG peak
by an average of 23 ms ±150 (Table 2). The median CM cell to EMG peak time differences were not statistically significant for the different distributions based on magnitude of PSpF (P = 0.68, Kruskal-Wallis) or DOM (P = 0.18, Mann-Whitney). The peaks in the timing distributions for different strengths of PSpF were similar. However, the tightest coupling (smallest range) between peak time in CM cell activity and target muscle EMG activity occurred for cell-muscle pairs exhibiting strong and moderate PSpF (P < .01, Levene median test). A similar result was obtained for DOM. The distribution of timing between CM cell and target muscle EMG peaks was narrower (less variability) for CM cells with high DOM, greater than 75 Hz, compared to those with DOM less than 75 Hz (P < .05, Levene median test). The same result was obtained with a DOM cutoff of 50 Hz. Note that DOM and strength of PSpF were not significantly correlated (r = 0.01, P = 0.95).

Figures 8C and D quantify the extent of overlap in matching peaks of cell and target muscle activity. Peak width was evaluated in terms of the percent of overlap with respect to both muscle activity and CM cell activity. Figure 8C shows the percent of each CM cell peak that was overlapped by matching, facilitated target muscle EMG peaks while Figure 8D shows the percent of the target muscle EMG peak that was overlapped by the CM cell peak. Note that the distribution is narrower with more pairs toward the greater overlap end of the distribution for strong PSpF effects. This was true for both the extent of overlap of the CM cell peak by the muscle peak and the overlap of the target muscle EMG peak by the CM cell peak. On average, 74% of the CM cell peak was
overlapped by the facilitated target muscle peak and this rose to 90% for muscles with strong PSpF (Table 2, P < .05, Kruskal-Wallis). Conversely, 57% of the target muscle peak was overlapped by the CM cell peak and this was also higher for muscles with strong PSpF. We quantified the number of CM cell – target muscle EMG peaks with 50% or greater overlap: 81% of CM cell peaks showed 50% or more overlap by one or more individual target muscles.

**Correlations between CM cell activity and target muscle EMG activity**

Cell-target muscle covariation during the reach-to-grasp task was quantified by plotting the average CM cell firing rate during reach-to-grasp against target muscle EMG (Figure 9). Scatter plots were generated from this procedure and subjected to correlation analysis. Pearson’s correlation coefficient (r) was used to quantify the covariation of CM cell and target muscle activity during the reach-to-grasp movement cycle. Firing rate was plotted against EMG activity with no time shift based on the rationale that the time delay between the firing of a CM cell and its effect on muscle activity should be roughly equal to the conduction time through the CM pathway to muscles and should be approximated by the peak latency of PSpF (see Discussion). This latency is in the range of 8-14 ms (Park et al. 2004) depending on the muscle and can be ignored for this analysis because it is close to our sampling rate, that is, one sample point. Firing rates and corresponding target muscle EMGs that have the same temporal profile with no phase shift should have correlations close to one. In Figure 9, the bulk of points in the > 60Hz firing rate range are from a part of the
response average record containing the cell’s primary activity peak. During much of this time, ECR’s EMG activity was relatively flat. This generates a group of points that are relatively constant on the EMG axis but vary over the range from 60–100Hz on the CM cell firing rate axis. These points tend to diminish the overall correlation since throughout most of the remainder of the record, CM cell and muscle activity covary more closely. The broader, slower trends in firing rate and EMG activity contribute significantly to the overall strength of the correlation.

The distribution of correlation coefficients for all CM cell-target muscle pairs with matching peaks of activity is given in Figure 10. The median correlation coefficient was 0.46 with a peak between 0.5 and 0.6. Eighty-four percent of the correlations were positive and 16% were negative despite the presence of PSpF. However, PSpF was weak for 50% of the muscles with negative correlations; none of these muscles had strong PSpF.

**PSpF magnitude relationships**

The magnitude of pure PSpF (PPI and normalized P/N ratio) was plotted against the Pearson correlation coefficient (r) for all 135 cell-target muscle pairs which showed PSpF and had 2,000 or more sweeps in the SpTA. Although the correlations were weak, PSpF magnitude measured as P/N showed a significant positive relationship with CM cell-target muscle covariation (r = 0.25, P < 0.01), but this weakened to only a trend toward significance when P/N was normalized (r = 0.13, P = 0.14). Using PPI as a measure of PSpF yielded no significance or trend (r = 0.03, P = 0.72). It is worth noting that differences in baseline
magnitude can potentially distort the true strength of PSpF based on PPI measurements.

**DOM relationships**

DOM of individual CM cell firing rate peaks were plotted against Pearson’s correlation of the covariation between the cell firing rate and target muscle EMG activity. One-hundred fifteen cell-muscle pairs had at least one “matching” peak of activity. In the case of multiple “matching” peaks, the values used were based on the response average with the highest DOM peak. There was no statistically significant tendency for r to be higher for greater DOMs. There was no relationship between DOM and any measure of PSpF magnitude.

**Covariation and PSpS**

For 31 cell-target muscle pairs which exhibited PSpS, 29% (9/31) had a negative r (compared to 16% of cell-target muscle pairs producing PSpF effects). The magnitudes of pure PSpS effects (PPI and normalized P/N) were plotted against r for all 31 cell-target muscle pairs which showed PSpS and had 2,000 or more sweeps in the SpTA. PSpS magnitude did not show a statistically significant relationship with r nor did the relationship change when the analysis was limited to moderate and strong effects.

**Covariation and synchrony effects**
The analysis thus far was limited to PSpF or PSpS without evidence of early onset synchrony. However, we did identify synchrony effects and test their relationship to the strength of covariation. The magnitude of synchrony effects expressed as PPI or normalized P/N was plotted against r for all 21 cell-target muscle pairs which showed either SyncF (n = 14) or PSpF+Sync (n = 7) and had 2,000 or more sweeps in the SpTA. The strength of covariation between CM cell and target muscle activity based on r was not significantly correlated with SyncF magnitude. This was also true for effects rated as moderate or strong.

Correlations with a CM cell’s full muscle field

One factor that might contribute to weak covariation between CM cells and their target muscles is the fact that the output from most CM cells is not limited to one muscle but rather diverges to influence multiple muscles. This raises the possibility that the activity of a CM cell might covary more closely with the summed activity of all of its target muscles rather than with any one muscle. To test this hypothesis, the response averages of all target muscles for 12 representative CM cells were summed together after weighting by the magnitude of PSpF for each muscle. CM cells were selected using the criteria that the PSpF in at least one muscle had to be strong or moderate. Scatter plots were generated by plotting each CM cell’s firing rate record against the summed EMG activity of all of its facilitated target muscles. The resulting correlation coefficient was then evaluated for improvement compared to that of the individual cell-target muscle pairs. Only 3 of 12 CM cells showed stronger correlations with the
summed target muscle EMG record compared to the best correlation with an individual muscle. All three of these were CM cells with a distal only or proximal only muscle field. Also, the mean of correlations between the CM cell’s firing rate record and the summed EMG of all its target muscles was not significantly different from the corresponding mean of all the individual CM cell – target muscle EMG correlations (P=0.41).

**Populations of CM cells converging on a common target muscle**

A major contributor to disparities evident above between CM cell and target muscle covariation is undoubtedly the fact that the activation of muscles is the result of synaptic input from many CM cells (and other cells), not just the recorded cell. Clearly, the input from one cell alone will have only a weak effect on the firing of motoneurons and based on that it is perhaps unrealistic to expect that the activity of one CM cell should correlate closely with the activity of a particular muscle, even though the cell directly facilitates that muscle. However, it is reasonable to expect that a population of CM cells influencing the same muscle should be a much better predictor of the pattern of EMG activity (Fetz et al. 1989; Griffin et al. 2004; Schieber and Rivlis 2007). To test this hypothesis, we identified populations of CM cells influencing the same target muscle and correlated the summed population activity to the muscle’s EMG activity. Of course, the optimal way to perform this experiment would be to simultaneously record from many CM cells that all have at least one target muscle in common. However, lacking this type of data, which would undoubtedly be very difficult to
obtain, we have tried to take advantage of our existing data from sequentially recorded individual CM cells. To simulate the conditions that would exist with simultaneously recorded CM cells, we have only selected cells for which the temporal pattern of EMG activity in the muscle of interest was very similar. For example, Figure 11B shows the EMG records for ED2,3 recorded with three different CM cells (Figure 11A) aligned on entering the food well (Figure 11E). The EMG records for ED2,3 in Figure 11B have the same number of peaks with similar timing and width thereby meeting the criterion for creating a population from the corresponding CM cells. The average firing rate records of these CM cells were then summed together as were their associated EMG records (Table 3). Our data base contained many more individual CM cells that facilitated each of these muscles, but we excluded them based on the dissimilarity in their EMG pattern during the reach-to-grasp task.

Figure 11 is an example of this procedure for ED2,3 population 1 (Table 3). The individual ED2,3 EMG records for each of the three CM cells used to generate the population average are shown in Figure 11B. Note the similarity they have to each other and to the summed EMG record. Figure 11A shows the average firing rate records during the reach-to-grasp task for all three CM cells as well as the population firing rate record. These CM cells produced moderate to strong PSpF of ED2,3 (Figure 11C). Note that the population CM cell firing rate record created by summing together the individual records has a temporal pattern very similar to the EMG record and even shows evidence of the multiple peaks that are clear in the EMG record. The population CM cell firing rate record
was plotted point for point against the summed EMG record for ED2,3 (Figure 11D). The resulting linear correlation coefficient was very strong (r = .90; P < 0.001) demonstrating relatively tight covariation of population CM cell activity with ED2,3 EMG activity. The individual CM cell-ED2,3 EMG correlation coefficients ranged from 0.34 to 0.91 and included one cell-muscle pair with a correlation coefficient that was essentially equal to the population correlation coefficient. However, most noteworthy is the fact that the population CM cell-target muscle correlation of 0.90 is much stronger than the mean of the individual cell-target muscle correlations (0.60).

We were able to apply this analysis to seven muscles in total (Table 3). For four of these muscles, the criterion that the temporal pattern of EMG activity for the muscle of interest had to be similar for each individual CM cell required splitting the CM cells for these muscles into multiple populations. Our final data set consisted of 10 CM cell populations ranging in size from 3 cells to 5 cells. For all but two (Table 3, ED2,3-2, ECR) of these CM cell populations, the population correlation coefficient was either equal to or greater than the correlation coefficient of any individual cell-target muscle pair in the population. However, all but one of the 10 population correlation coefficients were greater than the corresponding means of the individual cell-target muscle correlations. Additionally, the overall mean of the 10 population correlation coefficients was significantly greater than the overall mean of the individual correlation coefficients for each population (r = 0.75 versus 0.58, P = 0.02).
We went to great lengths to select CM cells that had a very similar pattern of EMG activity for the muscle in question. The lowest value of the correlation coefficients between each muscle in a set and the summed EMG for that set ranged from 0.79 – 0.96 (Table 3, column F). Nevertheless, to further test the possibility that improvement in the correlation between the population CM cell firing rate and summed EMG records could have been due to some nonspecific smoothing effect of summing records together, we compared the set of r values obtained from correlating the population CM cell activity and summed EMG, (Table 3, column E) to the mean r values obtained from correlations of the population CM cell activity to the individual EMG records (Table 3, column G). This comparison was not significant (P = 0.43, Mann-Whitney test) supporting the contention of statistical equivalence between the summed and individual EMG records. Finally, we also compared the mean r from the individual CM cell-muscle pair correlations (Table 3, column D) against the mean r derived from correlating the population CM cell activity with each individual muscle EMG (Table 3, column G). The population CM cell activity yielded a stronger correlation although falling slightly below the 0.05 level of statistical significance (r = 0.55 versus 0.44, P = 0.08). Nevertheless, it seems reasonable to conclude that using the population CM cell activity was a major factor contributing to the improvement in CM cell-muscle EMG correlations, not the summing together the EMG records.

Discussion
**Predicting EMG activity from individual CM cells**

The interpretation of data presented in this paper is subject to two points of view. On the one hand it could be argued that for neurons comprising a descending system which is supposedly driving muscle activity, the level of mismatch between the firing rate peaks of individual CM cells and their target muscles seems rather astounding. For example, on average, only 20% of CM cells had their primary peaks in the same segment of the task as the primary peaks in their target muscles. Relaxing the criterion to include any magnitude EMG peak occurring in the same segment of the task as the cell’s primary peak resulted in a match of 45% - better but still surprisingly low.

Alternatively, the similarities in activity between the temporal pattern of activity in CM cells and their target muscles could be emphasized. For example, nearly all CM cells (95%) had a least one firing rate peak that matched (occurred in the same task segment) an EMG peak in at least one of its target muscles. CM cell firing rate peaks also showed substantial overlap (mean = 74%) with peaks in individual target muscle EMG records and the amount of overlap increased to 90% for cell-muscle pairs producing strong PSpF. In this case, it should be noted that even though CM cell and target muscle peaks overlap, the activity of the two may have actually been negatively correlated during part of overlap period with one signal increasing and another decreasing. Nevertheless, these results are evidence in support of the general conclusion that CM cells
exhibit relatively strong and consistent coactivation with their target muscles and this is particularly true of CM cell - target muscle pairs exhibiting strong PSpF.

This conclusion supports the findings of McKiernan and colleagues (2000) which study used long duration cross correlation analysis. Although the long duration cross correlation method has its strengths, for example, it yields a coefficient that describes the correlation and a measure of time lag; it lacks information about where the activity for both the cell and muscle are occurring relative to the task. In the present study, we have been able to describe where CM cell activity peaks occur in relation to their target muscles in a linear non-shifted correlation. This study also demonstrates that the highest peak of cell activity is often not associated with the activity peak of the cell’s target muscle and therefore shifting the EMG signal to match the highest cell activity peak may be imposing an arbitrary association. Also, since the long duration cross correlation method uses single continuous trial records, it is subject to trial by trial variability. The analysis method of this study uses averages of multiple trials which removes the trial by trial variability. We have made the argument that if CM cells are linearly encoding EMG activity, using the present analysis methods, one would expect to see correlation coefficients approaching one without shifting the signals relative to one another.

Correlation studies are an approach to quantifying the extent of linear covariation between CM cells and EMG activity. We plotted the average firing rate records of CM cells against the corresponding target muscle EMG records and subjected the resulting scatter plots to correlation analysis (Figure 9). The
correlation coefficients ranged from -0.69 to 0.91 for individual cell-muscle pairs with PSpF. The median r value was 0.46 with a peak between 0.5 and 0.6 (Figure 10). Overall, the correlations for individual cell-muscle pairs would have to be judged as relatively weak and this result is consistent with the findings of other studies on cortical cells and their facilitated muscles (Schieber and Rivlis 2007). One might expect our results to show even weaker correlation coefficients than those of the Schieber and Rivlis study (2007) since we have used a highly complex multi-joint reaching task which broadly activates forelimb muscles while at the same time fractionating peaks of activity into unique synergies and ultimately providing a robust paradigm with which to test relationships between CM cell and target muscle activity.

What factors might contribute to the existence of major disparities in the location of movement related activity peaks in CM cells compared to their target muscles and to associated weak correlation coefficients? Certainly a major issue is the fact that the depolarization of motoneurons underlying muscle EMG activity results not from the action of just one CM cell but from many CM cells converging on a particular motoneuron pool. In addition, there are numerous additional sources of input to the motoneuron pool that can influence motoneuron activity independent of corticospinal input. At any given time during movement, a single motoneuron is receiving modulated input from hundreds if not thousands of afferent neurons. Another factor that might degrade the fidelity of covariation between a CM cell and its target muscles is the fact that most CM cells do not influence just one muscle; rather they influence multiple muscles as a synergy.
We tested the possibility that correlations might be stronger if a CM cell’s complete muscle field were taken into account. Each muscle of a CM cell’s muscle field was weighted according to the magnitude of PSpF and the resulting EMG records were then summed together. The summed record was correlated with the cell’s firing rate record. However, in most cases, the summed record did not result in significantly stronger correlations than the individual muscle EMG records. Using a similar approach, Schieber and Rivlis (2007) also reported that summing the EMG records of all the target muscles failed to substantially improve the correlations. However, in an interesting modification of this type of analysis, Townsend et al. (2006) recently showed that the EMG activity of all a cell’s target muscles could be used to accurately predict CM cell activity and that the prediction accuracy increased with the size of the muscle field.

In view of the potential sources of disparity, it is only reasonable to predict major dissimilarities in the pattern of activity of any single CM cell and its target muscles. In fact, it might be considered remarkable that the timing of firing rate peaks between single CM cells and target muscle EMG activity are as close as they are and that the correlation coefficients are as strong as they are.

*Predicting EMG from population CM cell activity*

Assume that corticospinal input to motoneurons is the principal driving force under at least some conditions, essentially eliminating multiple sources of synaptic input as a factor contributing to degradation in the strength and quality of covariation between CM cell and EMG activity. In this case, motoneurons
would be depolarized by the actions of multiple CM cells and other corticospinal
neurons. The ensemble firing rate record of a sufficiently large population of CM
cells synaptically coupled to motoneurons of the same muscle might then
approach a perfect correlation with the muscle’s EMG activity. To the extent that
this was possible within our data set, we attempted to test this possibility. We
found that in many cases (6 of 10), the temporal pattern of the ensemble firing
rate record for the CM population closely resembled \((r \geq 0.8)\) the EMG activity of
the common target muscle (Figure 11). Perhaps most noteworthy is the fact that
in all cases except one, the population correlation coefficient was greater than
the corresponding mean of the individual cell-target muscle correlations (Table
3). The one exception was ECR where the population and individual \(r\) values
were essentially the same. Moreover, the mean of the population correlation
coefficients for all 10 muscles tested was significantly greater \((P = 0.02)\) than the
mean of all the individual cell-target muscle correlations. Finally, in all cases
except two (Table 3), the population \(r\) was essentially equal to or greater than the
highest individual cell-muscle correlation.

Some individual cell-target muscle pairs had very strong correlations as
Schieber and Rivlis (2007) have also reported. However, the key issues are
whether the population correlation is better than the individual cell-target muscle
correlations and whether the final population correlation achieves a level
consistent with concluding that the cells as a population could potentially account
for large part of time varying pattern of EMG activity during movement. We
believe our data is consistent with this interpretation and adds further support to
the notion that CM cell output encodes muscle activation (EMG) and should be viewed within the context of a muscle based coordinate system (Hamed et al. 2007; Holdefer and Miller 2002; Morrow et al. 2007; Morrow and Miller 2003; Mussa-Ivaldi 1988; Todorov 2000; Townsend et al. 2006).

Due to the small size of our populations, we could not analyze, in any meaningful way, changes in the population \( r \) with addition of new cells and increase in the size of the population. However, Schieber and Rivlis (2007) were able to do this, using larger populations of CM cells recorded in relation to finger movements. They showed that the pattern of improvement or decline with cell number depended on the order in which cells were added into the population. Using an order that was essentially random, the population \( r \) value fluctuated over a large range with small numbers of cells but then converged toward the final population \( r \). However, despite larger populations of CM cells, the correlations reported by Schieber and Rivlis (2007) were weaker overall than those we have reported in this study. Their strongest population \( r \) value was 0.657 (\( R^2 \) value of 0.431). In contrast, 60% (6 of 10) of our CM cell populations had greater correlations than this and the overall mean \( r \) value was 0.75. The reason for this difference is unclear. The muscles that form our CM cell populations are entirely distal muscles, mostly digit muscles. Although our behavioral task was an unconstrained “free-form” task that might have provided a greater opportunity for yielding a higher level of sculpting of individual muscle EMG activity than the digit flexion/extension task used by Schieber and Rivlis (2007), the fact that their correlations included 12 separate movement conditions
potentially added a much greater opportunity for disparities to occur between cell and muscle activity and this may have contributed to the differences in the strength of correlations between our two studies.

Our results also suggest that cortical input to the motoneuron pool dominates the activity of the motoneurons during the reach-to-grasp movement. If not, other excitatory inputs to the motoneuron pool must show temporal modulation closely matching that of the CM cell input. A significant contributor to the strength of correlations observed in our data is the broader periods of coactivation. We agree with the interpretation of Townsend et al. (2006) that this broad coactivation “accounts for the general correlation between the envelopes of cell and muscle activity”. Superimposed on this broad coactivation are peaks and valleys of activity. Our analysis of these peaks in activity showed a relatively poor correlation between the existence of CM cell primary activity peaks and primary peaks or lesser peaks in the target muscle EMG activity. However, it was true that for 73% of the CM cells, at least one peak in each of the cell's target muscles had a matching peak of some size in CM cell activity. Moreover, the timing of the peaks was relatively tight (25 ms mean with EMG peak lagging, Table 2).

**CM cell effects on motor unit firing: timing issues**

What timing should be expected between peaks in CM cell activity and the effect of that activity on motor unit firing rate? Many studies going back to the original work of Evarts (1968) have demonstrated that cells in motor cortex show
a wide range of timing relationships relative to movement onset with some neurons beginning to fire before the onset of movement and others following the onset of movement. However, nearly all these studies have shown that the mean onset time of the cortical cell population ranges from 60-150 ms before the onset of movement (Porter and Lemon 1993). Extending this analysis to CM cells, Fetz and Cheney (1980) showed that the mean onset of activity relative to the onset of target muscle EMG activity for a simple alternating wrist flexion-extension task was 71 ms (phasic-tonic CM cells). Despite these findings, we agree with Schieber and Rivlis (2007) that logical analysis would suggest that the timing should equal the conduction time through the pathway from cortical cell discharge to motor unit discharge (Morrow and Miller 2003; Towsend et al. 2006). This time can be estimated from the onset latency of PSpF. However, the cell’s peak effect on motor unit firing would more likely correspond to the peak latency of PSpF. It is reasonable to conclude that the timing difference between a CM cell’s firing rate peak and its maximum effect on motor units should also be the peak latency of PSpF. Peak PSpF latencies range from 9-13 ms depending on the muscle (McKiernan et al. 1998). Our sampling rate for response averages was 100 Hz or 10 ms for both unit activity and EMG channels. This means that the time shift expected between a CM cell’s firing rate and its affect on motoneurons is about equal to one sample point, in other words, negligible for our purposes. Accordingly, in plotting CM cell firing rate against EMG activity and performing the Pearson correlation analysis, we did not time shift the records in an effort to achieve stronger correlations. Time shifting
records might have provided stronger correlations in some cases, but we believe that such time shifting does not match the reality of timing that should exist between peaks in CM cell activity and when that activity should exert its maximum excitatory influence over motoneurons (Morrow and Miller 2003; Schieber and Rivlis 2007, Towsend et al. 2006).

Our data provide some support for this view of the timing between CM cell activity and target muscle EMG. Of the 190 cell-target muscle activity peaks occurring during the same segment of the reach-to-grasp task, the peak of CM cell activity led the peak in target muscle EMG by an average of 25 ms ±150 (Table 2). This number is very close to the estimated time of 9-13 ms based on the peak latency of PSpF. Restricting this analysis to peaks occurring during the same segment of the task is justified because other peaks would be unlikely to be causally related. It is also noteworthy that the tightest coupling (smallest range) between peak time in CM cell activity and target muscle EMG activity occurred for cell-muscle pairs exhibiting strong PSpF effects.

The mean EMG peak time lag is notably shorter than the 71 ms reported in a previous study of the timing between CM cell (phasic-tonic cells) and muscle activity (Cheney and Fetz 1980). This difference may be due to differences in the behavioral tasks. The step-tracking task used by Cheney and Fetz (1980) in which wrist movement alternated between flexion and extension position zones engaged the activity of wrist and digit muscles in a heavily reciprocal pattern. While in one zone, the antagonist muscles were generally inactive and their motoneurons were hyperpolarized. Movement toward the opposite target zone
then involved activation of the CM cells for that direction. However, before the appearance of agonist muscle EMG for that direction, the CM cells need to depolarize motoneurons from their hyperpolarized level to firing threshold. The amount of time needed for motoneurons to reach threshold and start firing would contribute to the time delay between the onset of CM cell firing and the onset of target muscle EMG activity. The reach-to-grasp task we have used in the present study differs fundamentally from the reciprocal wrist movement task in that it requires a “free-form”, coordinated, multi-joint reaching movement to a visual target where a food morsel is grasped and carried to the mouth and then the hand is returned to the starting point. EMG activity during this task shows broad coactivation throughout most of the task with specific sculpting of EMG peaks and valleys evident for individual muscles. What is significant about this task is that EMG activity is always present (except on home plate) so peaks in CM cell firing rate should be translated immediately into firing rate changes of the motoneuron without the need to first depolarize the motoneuron to threshold. This fact could have significantly reduced the time difference observed in this study between CM cell firing rate peaks and corresponding target muscle EMG peaks.

Schieber and Rivlis (2007) tested the effect of time shifting the population activity of cortical cells with respect to the cell’s target muscle and found that, in one monkey, the maximum correlation was obtained with the EMG delayed 40-60 ms from the cell activity. The effect of time shifting was not near as dramatic in another monkey. How might this time shift be reconciled with expectations
based on conduction time in the corticospinal pathway? It is tempting to suggest that in the finger flexion/extension task of Schieber and Rivlis (2007), the possible lack of background EMG and need to raise motoneurons to firing threshold might also apply. However, as pointed out by Morrow and Miller (2003), it is difficult to explain the results of correlation studies involving activity over the whole movement cycle, if the delay of 40-60 ms is only present at the onset of movement. They further raise the possibility that persistent inward currents in motoneurons (Lee and Heckman 1998) essentially act as a low-pass filtered amplifier to produce currents that are substantially delayed from and greater than the synaptic currents. While the correct explanation of these timing disparities remains unknown, the findings we have reported in this paper suggest that the disparity may not be as large as previously thought.

**Overall summary and conclusions**

In this paper we report the results of a study of the functional activity patterns of 44 identified CM cells and their target muscles in relation to a free-form reach-to-grasp task. The peaks in activity of individual CM cells were about evenly distributed throughout the movement task, except for the starting position where EMG activity was minimal or absent. CM cell peaks occurred during segments of the task that in general correlated with the occurrence of peaks in target muscle EMG activity. Although many examples of strong correlations between the activity of individual CM cells and their facilitated target muscles were found, overall, the correlations were relatively weak. However, this should
not be surprising given the large number of synaptic inputs driving motoneurons and the relatively small contribution made by any single input neuron. While individual cell-target muscle correlations were relatively weak, the ensemble firing rate records of populations of CM cells sharing a common target muscle produced significantly stronger correlations than the mean of the individual cell-target muscle correlations. The results provide further evidence in support of the notion that cortical output encodes muscle based parameters, specifically, muscle activation as reflected in EMG activity. Morrow and Miller (2003) demonstrated that the ensemble activity of a relatively small number of unidentified cortical cells, time shifted according to the phase differences observed in analog cross-correlations, very closely matched the EMG activity of agonist muscles. Our data extends this to identified CM cells and shows that without any time shifting, the ensemble activity of small populations of CM cells produces a relatively good match ($r \geq 0.8$) to target muscle EMG activity.
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Figure 1. Examples of Spike triggered averages of EMG activity illustrating the criteria used for categorizing the strength of PSpF effects. The zero line corresponds to the action potential of the CM cell used as a trigger for averaging. For this figure and throughout the paper the colors used for each average represent the magnitude of effects as follows: red = strong PSpF, green = moderate PSpF, blue = weak PSpF, black = no effect, yellow = weak PSpS and purple = moderate PSpS. The number of trigger events is given in parentheses.

Figure 2. Segmentation of the reach-to-grasp task and approximate timing of the individual segments: (1) on home plate; ~250 ms duration, (2) leaving home plate; 100 ms flanking the release of home plate, (3) hand in transit to the food well; beginning 100 ms after release of home plate and extending to 50 ms before digit entry into the target food well, (4) entering food well; beginning 50 ms before and extending to 150 ms after digit entry into the food well, (5) in food well; beginning 150 ms after digit entry into the food well and extending up to 100 ms before digit exit from the food well, (6) exiting food well; beginning 100 ms before and extending to 100 ms after digit exit from the food well, (7) hand in transit to the mouth; 100-300 ms after digit exit from the food well, (8) hand at the mouth; beginning 300 ms after digit exit from the food well and extending to 450 ms before depression of home plate, (9) hand in transit back to home plate; beginning 450 ms before and extending to 50 ms before depression of home plate.
plate, (10) contact with home plate; 50 ms before to 150 ms after depression of home plate. The length of the movement cycle and durations of individual components given above represent a typical response. Although total movement durations varied somewhat, the two monkeys used in this study were highly over trained and the responses tended to be consistent and stereotyped. The goal was to assign peaks to the movement segment they were most closely related to functionally. It was not uncommon for the shoulder of a peak to be broad enough to exist in multiple movement segments but assignment was based on the location of the highest point in the peak.

Figure 3. Cortical locations of the 44 CM cells investigated in this study plotted on an unfolded map of the cortex. The solid line is the convexity of the central sulcus and the dotted line is the fundus. Intersection of axes represents the center of the recording chamber.

Figure 4. Example of response averages for cell 110N3 and 22 simultaneously recorded muscles. Four response averages are shown referenced to: A) leaving home plate, B) entering the food well, C) exiting the food well, and D) returning to home plate. Note a single peak in CM cell activity occurs in segment 5 (in the food well) of the reach-to-grasp task. Color coding of EMG records reflects the magnitude of postspike effects (Figure 1).
Figure 5. Histogram showing the segment location in the reach-to-grasp task of the firing rate peaks for the 44 CM cells analyzed in this study.

Figure 6. Analysis of the extent to which peaks in CM cell activity have matching peaks of EMG activity in the cell’s facilitated target muscles. Matching peaks were ones that occurred in the same segment of the task. Each bar represents one of the 44 CM cells studied. A. Most rigorous criterion: the 1\^st peaks of both the CM cell and facilitated target muscle were in the same segment of the reach-to-grasp task. B. Less rigorous: the 1\^st peak of the CM cell was in the same segment as any peak of the facilitated target muscle. C. Least rigorous: any CM cell peak matched any EMG peak in a facilitated target muscle. D. Percent of all possible CM cell peaks that matched facilitated target muscle EMG peaks (see text). Black bars represent data from CM cell 105N6 and grey bars represent data from 65N6 (illustrated in Figure 7).

Figure 7. Identification of matching peaks in CM cell and target muscle activity. An example of two CM cells with multiple peaks of activity during the reach-to-grasp task and associated peaks of activity in the cell’s facilitated target muscles. Task segments 4-9 are color coded. CM cell and muscle peaks were defined as matching if they occurred in the same task segment. In Figure 6, CM cell 105N6 is represented as a black bar and 65N6 as a grey bar.
Figure 8. Timing between all matching CM cell peaks and facilitated target muscle EMG peaks. A: Distribution of peak time differences shaded according to magnitude of effects. B: Distribution of peak time differences shaded according to DOM. C: Percent of CM cell peaks overlapped by matching muscle peaks. D: Percent of muscle peaks overlapped by matching CM cell peaks.

Figure 9. Response average and scatter plot for a cell-target muscle pair with strong PSpF. In the scatter plot, points are color coded according to time. Points at the beginning of the record are red. See color code at the bottom of the response average. Red transitions to purple and then blue representing points at the end of the record.

Figure 10. Distribution of Pearson correlation coefficients (r) for CM cell-target muscle pairs. Correlation coefficients were derived from plotting CM cell firing rate against target muscle EMG activity. Only pairs exhibiting PSpF were included.

Figure 11. Analysis of CM cell populations sharing a common target muscle. A. Individual CM cell firing rate histograms for three CM cells that all facilitated ED2,3. On the right is the population CM cell record obtained by summing the individual records. B. Individual EMG records of ED2,3 recorded with the individual CM cells in A. Note the similarity in the temporal pattern of activity. On the right is the ensemble EMG record obtained by summing the individual
records. C. PSpF for each of the CM cell – target muscle pairs in panels A and B.  D. Scatter plot obtained by plotting the population CM cell firing rate record against the summed EMG record of ED2,3.  E. Population CM cell activity (sum of all three CM cells) and summed EMG activity in relation to task performance.
Table 1. Muscles Recorded

<table>
<thead>
<tr>
<th>Muscle Abbreviation</th>
<th>Proximal muscles</th>
<th>Distal muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHL</td>
<td>Shoulder</td>
<td>WRS</td>
</tr>
<tr>
<td>PEC</td>
<td>Pectoralis Major</td>
<td>Flexor Carpi Radialis</td>
</tr>
<tr>
<td>ADE</td>
<td>Anterior Deltoid</td>
<td>Palmaris Longus</td>
</tr>
<tr>
<td>PDE</td>
<td>Posterior Deltoid</td>
<td>Flexor Carpi Ulnaris</td>
</tr>
<tr>
<td>TMAJ</td>
<td>Teres Major</td>
<td>Extensor Carpi Radialis</td>
</tr>
<tr>
<td>LAT</td>
<td>Latissimus Dorsi</td>
<td>Extensor Carpi Ulnaris</td>
</tr>
<tr>
<td>ELB</td>
<td>Elbow</td>
<td>Intrinsic</td>
</tr>
<tr>
<td>BIS</td>
<td>Short Head of the Biceps</td>
<td>Abductor Pollicis Brevis</td>
</tr>
<tr>
<td>BIL</td>
<td>Long Head of the Biceps</td>
<td>First Dorsal Interosseus</td>
</tr>
<tr>
<td>BRA</td>
<td>Brachialis</td>
<td>Digit</td>
</tr>
<tr>
<td>BR</td>
<td>Brachioradialis</td>
<td>Flexor Digitorum Suprficialis</td>
</tr>
<tr>
<td>TLAT</td>
<td>Lateral Head of the Triceps</td>
<td>Flexor Digitorum Profundus</td>
</tr>
<tr>
<td>TLON</td>
<td>Long Head of the Triceps</td>
<td>Extensor Digitorum Communis</td>
</tr>
<tr>
<td>DE*</td>
<td>Dorsal Epitrochlearis</td>
<td>Extensor Digitorum 2, 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Extensor Digitorum 4, 5</td>
</tr>
</tbody>
</table>

*These muscles were recorded in monkey K but not monkey N.
Table 2. Timing and percentage overlap of peaks in CM cell activity relative to matching peaks in facilitated target muscle EMGs. In all cases, the mean EMG peak lagged the CM cell peak.

<table>
<thead>
<tr>
<th>Peak Time Difference (ms)</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Effects</td>
<td>25 ± 156</td>
<td>30</td>
</tr>
<tr>
<td>Strong Effects</td>
<td>40 ± 127</td>
<td>70</td>
</tr>
<tr>
<td>Moderate Effects</td>
<td>25 ± 111</td>
<td>30</td>
</tr>
<tr>
<td>Weak Effects</td>
<td>22 ± 183</td>
<td>30</td>
</tr>
<tr>
<td>DOM &gt; 75</td>
<td>43 ± 113</td>
<td>30</td>
</tr>
<tr>
<td>DOM &lt; 75</td>
<td>19 ± 167</td>
<td>30</td>
</tr>
<tr>
<td>DOM &gt; 50</td>
<td>37 ± 145</td>
<td>30</td>
</tr>
<tr>
<td>DOM &lt; 50</td>
<td>13 ± 166</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of CM cell peak overlapped by muscle peak</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All Effects</td>
<td>75 ± 28</td>
<td>85</td>
</tr>
<tr>
<td>Strong Effects</td>
<td>89 ± 15</td>
<td>*98</td>
</tr>
<tr>
<td>Moderate Effects</td>
<td>74 ± 28</td>
<td>83</td>
</tr>
<tr>
<td>Weak Effects</td>
<td>73 ± 29</td>
<td>81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of target muscle peak overlapped by CM cell peak</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All Effects</td>
<td>58 ± 31</td>
<td>56</td>
</tr>
<tr>
<td>Strong Effects</td>
<td>64 ± 27</td>
<td>68</td>
</tr>
<tr>
<td>Moderate Effects</td>
<td>59 ± 29</td>
<td>57</td>
</tr>
<tr>
<td>Weak Effects</td>
<td>55 ± 32</td>
<td>53</td>
</tr>
</tbody>
</table>

* There is a statistically significant difference in the median values between strong PSpF and both moderate and weak PSpF (Mann-Whitney, P < 0.05).
Table 3. Analysis of CM cell populations for individual muscles.

<table>
<thead>
<tr>
<th>CM Cell Population</th>
<th># CM Cells</th>
<th>Range of Individual muscle to individual CM cell r values</th>
<th>Mean of individual muscle to individual CM cell r values</th>
<th>Population CM cell to summed muscle r value</th>
<th>Lowest individual muscle to summed muscle r value</th>
<th>Mean of individual muscle to population CM cell r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED2,3 – 1</td>
<td>3</td>
<td>0.342 – 0.912</td>
<td>0.604</td>
<td>0.900</td>
<td>0.891</td>
<td>0.884</td>
</tr>
<tr>
<td>ED2,3 – 2</td>
<td>4</td>
<td>0.069 – 0.813</td>
<td>0.552</td>
<td>0.608</td>
<td>0.918</td>
<td>0.583</td>
</tr>
<tr>
<td>APB – 1</td>
<td>4</td>
<td>0.288 – 0.627</td>
<td>0.457</td>
<td>0.590</td>
<td>0.904</td>
<td>0.567</td>
</tr>
<tr>
<td>APB – 3</td>
<td>4</td>
<td>0.473 – 0.753</td>
<td>0.574</td>
<td>0.847</td>
<td>0.787</td>
<td>0.725</td>
</tr>
<tr>
<td>ECU</td>
<td>3</td>
<td>0.675 – 0.870</td>
<td>0.742</td>
<td>0.867</td>
<td>0.903</td>
<td>0.828</td>
</tr>
<tr>
<td>FDP</td>
<td>4</td>
<td>0.395 – 0.778</td>
<td>0.653</td>
<td>0.876</td>
<td>0.959</td>
<td>0.852</td>
</tr>
<tr>
<td>ECR</td>
<td>4</td>
<td>0.159 – 0.900</td>
<td>0.482</td>
<td>0.471</td>
<td>0.810</td>
<td>0.411</td>
</tr>
<tr>
<td>FDI</td>
<td>5</td>
<td>-0.233 – 0.617</td>
<td>0.299</td>
<td>0.574</td>
<td>0.840</td>
<td>0.516</td>
</tr>
<tr>
<td>ED4,5 – 1</td>
<td>4</td>
<td>0.604 – 0.909</td>
<td>0.786</td>
<td>0.917</td>
<td>0.944</td>
<td>0.911</td>
</tr>
<tr>
<td>ED4,5 – 2</td>
<td>3</td>
<td>0.367 – 0.910</td>
<td>0.620</td>
<td>0.838</td>
<td>0.922</td>
<td>0.797</td>
</tr>
</tbody>
</table>
Standard Deviations From Baseline

- **Post Spike Facilitation**
  - 83K2 APB (31425)
  - 67N7 FDP (3637)
  - 62N1 APB (26930)

- **No Effect**
  - 102N5 TLAT (7420)

- **Post Spike Suppression**
  - 70N1 ED23 (14611)
  - 101N2 FCR (19323)

- Time Scale: 0 - 10 ms
1. On home plate
2. Leaving home plate
3. Hand in transit to the food well
4. Entering food well
5. In food well
6. Exiting food well to the mouth
7. Hand in transit back to home plate
8. At the mouth
9. Hand in transit back to home plate

Segment
Home Plate
Target Food Well
1. On home plate
2. Leaving home plate
3. Hand in transit to the food well
4. Entering food well
5. In food well
6. Exiting food well
7. Hand in transit to the mouth
8. At the mouth
9. Hand in transit back to home plate
10. Back to home plate
A. CM Cell Primary Peak Matches Target Muscle Primary Peak

B. CM Cell Primary Peak Matches any Target Muscle Peak

C. Any CM Cell Peak Matches Any Target Muscle Peak

D. Percent of Possible CM cell Peak-Target Muscle Combinations
A. B. C. D.

Muscle Peak Relative to CM Cell Peak

Muscle Peak Relative to CM Cell Peak

% CM Cell Peak Overlapped by Muscle Peak

% Muscle Peak Overlapped by CM Cell Peak

Strong PSpF Effects
Moderate PSpF Effects
Weak PSpF Effects
Pearson's Correlation Coefficient (r)

Median = 0.46

Number of Cell-Target Muscle Pairs

Median = 0.46

Pearson's Correlation Coefficient (r)
A. Individual CM cell firing rate records

B. Corresponding EMG records

C. Spike triggered averages

D. Pearson's Correlation Coefficient: 0.90 (P < .001)
   Individual cell-target muscle correlation coefficients:
   Mean: 0.60
   Range: 0.34 to 0.91

E. Ensemble firing rate and EMG records