Correlations Between Corticomotoneuronal (CM) Cell Postspike Effects and Cell-Target Muscle Covariation

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McKiernan, Brian J., Joanne K. Marcario, Jennifer Hill Karrer, and Paul D. Cheney. Correlations between corticomotoneuronal (CM) cell postspike effects and cell-target muscle covariation. J. Neurophysiol. 83: 99–115, 2000. The presence of postspike facilitation (PSpF) in spike-triggered averages of electromyographic (EMG) activity provides a useful means of identifying cortical neurons with excitatory synaptic linkages to motoneurons. Similarly, the presence of postspike suppression (PSpS) suggests the presence of underlying inhibitory synaptic linkages. The question we have addressed in this study concerns the extent to which the presence and strength of PSpF and PSpS from corticomotoneuronal (CM) cells correlates with the magnitude of covariation in activity of the CM cell and its target muscles. For this purpose, we have isolated cells during a reach and prehension task during which the activity of 24 individual proximal and distal forelimb muscles was recorded. These muscles show broad coactivation but with a highly fractionated and muscle specific fine structure of peaks and valleys. Covariation was assessed by computing long-term (2 s) cross-correlations between CM cells and forelimb muscles. The magnitude of cross-correlations was greater for muscles with facilitation effects than muscles lacking effects in spike-triggered averages. The results also demonstrate a significant relationship between the sign of the postspike effect (facilitation or suppression) and the presence of a peak or trough in the cross-correlation. Of all the target muscles with facilitation effects in spike-triggered averages (PSpF, PSpF with synchrony, or synchrony facilitation alone), 89.5% were associated with significant cross-correlation peaks, indicating positively covarying muscle and CM cell activity. Seven percent of facilitation effects were not associated with a significant effect in the cross-correlation, whereas only 3.4% of effects were associated with correlation troughs. In contrast, of all the muscles with suppression effects in spike-triggered averages, 38.9% were associated with significant troughs in the cross-correlation, indicating an inverse relation between CM cell and muscle activity consistent with the presence of suppression. Fifty-five percent of suppression effects was associated with correlation peaks, whereas 5.6% was not associated with a significant effect in the cross-correlation. Limiting the analysis to moderate and strong facilitation effects, the magnitude of PSpF was correlated weakly with the magnitude of the cell-muscle cross-correlation peak. Nevertheless, the results show that although many CM cell-target muscle pairs covary during the reach and prehension task in a way consistent with the sign and strength of the CM cell’s synaptic effects on target motoneurons, many exceptions exist. The results are compatible with a model in which control of particular motoneuron pools reflects not only the summation of signals from many CM cells but also signals from additional descending, sensory afferent, and intrinsic spinal cord neurons. Any one neuron will make only a small contribution to the overall activity of the motoneuron pool. In view of this, it is not surprising that relationships between postspike effects and CM cell-target muscle covariation are relatively weak with many apparent incongruities.

INTRODUCTION

Spike-triggered averaging (SpTA) of rectified electromyographic (EMG) activity has proven to be a useful means of identifying motor cortex cells with synaptic linkages to motoneurons. The presence of postspike facilitation (PSpF) or postspike suppression (PSpS) in SpTAs is interpreted as evidence of an underlying synaptic linkage between the cortical cell and motoneurons of its target muscles (Fetz and Cheney 1980; Kasser and Cheney 1985; Lemon et al. 1986). By compiling SpTAs for multiple muscles in a limb, it is possible to determine the “muscle field” of a corticomotoneuronal (CM) cell. Muscle field is defined as the group of agonist and/or antagonist muscles that are facilitated or suppressed by the CM cell during active movement (Buyes et al. 1986; Fetz and Cheney 1979, 1980; Kasser and Cheney 1985). Besides identifying cells possessing a synaptic linkage to motoneurons, the magnitude of PSpF or PSpS can be used as a measure of the strength of the cell’s facilitation or suppression of target motoneurons (Buyes et al. 1986; Fetz and Cheney 1980; Kasser and Cheney 1985).

In contrast to SpTA, which reveals the synaptic linkages between premotor cells and motoneurons, the extent of functional covariation in activity between premotor neurons and muscles can be quantified by computing long-term cross-correlation functions. Houk et al. (1987) and Miller et al. (1992, 1993) have described such a cross-correlation function for estimating the strength and relative timing of covariation between premotor cells and target muscles. Houk et al. (1987) correlated cells of the cat red nucleus and forelimb muscles over a 4-s period during a functional task. Although they found correlational evidence for strong linkages between red nucleus cells and forelimb muscles, they cautioned that other neurophysiological methods need to be combined with cross-correlation data to define specific neuroanatomic relationships between cells and muscles. Miller et al. (1992, 1993) used the cross-correlation technique to examine the relationship between the discharge rate of red nucleus cells in the monkey and the magnitude of rectified, multiunit EMG from forelimb muscles during a reach and prehension task. In addition to quantifying the strength, timing, and dynamics of cross-correlation functions for multiple forelimb muscles, they also calculated SpTAs for the same cell-muscle pairs (Miller et al. 1992). They proposed that large, centrally located peaks in...
the cross-correlation functions suggest the presence of “functional linkages” between a cell and covarying muscle(s). They concluded that there was a tendency for rubromotoneuronal (RM) neurons with strong synaptic linkages (identified by the presence of PSpF in the SpTAs) to also show relatively strong functional linkages.

Using a different methodology, Bennett and Lemon (1994) studied the intrinsic muscles of the hand and found that there was only a weak correlation between the amplitude of a PSpF and the strength of covariation between CM cells and target muscles in the hand. Further, there was no consistent relation between the firing frequency of a CM cell and the amplitude of EMG activity in its target muscles. Although 20 of 48 cell-muscle pairs showed a significant correlation between cell discharge rate and EMG activity, others pairs that were tested showed no correlation or even a negative correlation. However, in a later study, they found that 9 of 13 CM cells discharged more intensely when the target muscle receiving the strongest PSpF was most active (Bennett and Lemon 1996). Fetz and Finnochio (1975) had earlier concluded that coactivation of a cell and muscle is neither necessary nor sufficient evidence for establishing anatomic connections between the two.

We previously described a population of CM cells that produced multiple postspike effects (PSEs) in both proximal and distal muscles of the forelimb of a monkey performing a reach and prehension task (McKieran et al. 1998). Inspection of the patterns of EMG activity revealed broad coactivation of many muscles at different joints throughout task performance but with a high degree of individuality in the fine structure of EMG peaks and troughs. Although the broad patterns of agonist muscle coactivation that occur in relation to simple, single joint, alternating movement tasks we have used in previous work are unlikely to form a useful substrate for testing relations with PSEs, the highly specific fine structure of EMG activity in different muscles during the reach and prehension task was ideal for the purposes of the present study. The goal of this study was to investigate the extent to which the sign and strength of PSEs from CM cells correlates with the sign and magnitude of the covariation pattern between CM cells and their target muscles during a reach and prehension task. We attempted to determine if CM cell-target muscle pairs showing PSpF covary positively and if the strength of PSpF correlates with the magnitude of the cell-target muscle covariation. Similarly, we wanted to determine if CM cells covary inversely with target muscles showing PSpS.

The results of this study show that, during a complex movement task, the presence of strong covariation between a CM cell and its target muscles did not consistently predict the presence of PSpF. On the other hand, PSpFs were consistently associated with significant covariation. Target muscles with PSpF generally covaried positively with the CM cell, whereas many muscles with PSpS covaried negatively. For strong PSpFs, there was weak but positive correlation between the strength of PSpF and the magnitude of cell-target muscle covariation.

METHODS

Training procedures

The data for this project were collected from two male rhesus monkeys (Macaca mulatta) trained for ~9 mo on three different behavioral tasks. Only one of the tasks (reach and prehension) was used to collect the data reported in this paper. Each monkey weighed ~6 kg when data collection began. During each data collection session, the monkey was tested in a sound-attenuating chamber. The monkey’s left forelimb was restrained during task performance in a foam-padded tube that was fitted to the forearm and elbow, while the right forelimb was unrestrained. The monkey was guided in performance of the task by audio and video cues provided by an IBM-compatible computer.

Behavioral task

The task chosen for this project activated multiple proximal and distal forelimb muscles in natural, functional synergies as the monkey actively reached for and retrieved a food reward. The task was self-paced and controlled by a personal computer. The monkey initiated the task by placing its right hand on a pressure plate at wrist height directly in front of him. Pressing on this plate for a preprogrammed length of time triggered the release of a food reward and a go signal. The monkey then reached out to a small well located at shoulder level a little less than an arm’s length away. The monkey used one or two digits to dig the food reward from the well, then grasped it and brought it to his mouth. More details regarding the design of this task and its implementation can be found in McKieran et al. (1998).

Surgical procedures

After training, a 22-mm diam stainless steel chamber was centered over the hand area of the motor cortex of the left hemisphere in each monkey and anchored to the skull with 25–30 vitalium screws and dental acrylic. Threaded nylon nuts also were anchored in dental acrylic over the occipital aspect of the skull to allow for attachment of a flexible head-restraint system during recording sessions. For all implant surgeries, the monkeys were tranquilized with ketamine (10 mg/kg) and anesthetized with isoflurane gas. Surgeries were performed in an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care)-accredited facility using full sterile procedures. Postoperatively, monkeys received prophylactic antibiotic and analgesic medication. All work involving these monkeys conformed with the procedures outlined in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

EMG records from 22–24 different forelimb muscles were recorded with pairs of multistranded stainless steel wires inserted into the target muscles (Table 1). With the monkey under isoflurane anesthesia, pairs of insulated, multistranded stainless steel wires (Cooner AS632) were inserted transcutaneously into each of the target muscles under sterile surgical conditions. Approximately 2 mm of insulation was removed from the end of each wire before insertion. The bared end of each lead wire was inserted “backward” into the cannula of a 21-gauge needle for transcutaneous insertion into the muscle belly. This procedure formed a hook at the end of each wire that tended to anchor the wire in the muscle after the needle was withdrawn. Once inserted, each wire could withstand mild tugging without dislodging. The insertion points for each muscle were identified based on palpation and dissection studies in which optimal insertion points were mapped with reference to external bony landmarks. The ends of each pair of wires were separated by ~5 mm (Loeb and Gans 1986). The placement of each electrode pair was tested for accuracy by electrical stimulation through the electrodes while observing the nature of the resulting movement. In some cases, this also was done midway through the life of the implant to confirm location. Once all electrodes were positioned, the wires were anchored to the monkey’s arm with medical adhesive tape (Johnson & Johnson 5174). This tape is elasticized and highly adhesive. In general, the tape remained firmly anchored to the
TABLE 1. Muscles implanted with EMG electrodes

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>Proximal muscles</td>
<td></td>
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<tr>
<td>Shoulder (SHL)</td>
<td></td>
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<tr>
<td>Pectoralis major</td>
<td>PEC</td>
</tr>
<tr>
<td>Anterior deltoid</td>
<td>ADE</td>
</tr>
<tr>
<td>Posterior deltoid</td>
<td>PDE</td>
</tr>
<tr>
<td>Teres major</td>
<td>TMAJ</td>
</tr>
<tr>
<td>Latissimus dorsi</td>
<td>LAT</td>
</tr>
<tr>
<td>Elbow (ELB)</td>
<td></td>
</tr>
<tr>
<td>Short head of the biceps</td>
<td>BIS</td>
</tr>
<tr>
<td>Long head of the biceps</td>
<td>BIL</td>
</tr>
<tr>
<td>Brachialis</td>
<td>BRA</td>
</tr>
<tr>
<td>Brachioradialis</td>
<td>BR</td>
</tr>
<tr>
<td>Lateral head of the triceps</td>
<td>TLAT</td>
</tr>
<tr>
<td>Long head of the triceps</td>
<td>TLL</td>
</tr>
<tr>
<td>Dorsal epitrochlear</td>
<td>DE</td>
</tr>
<tr>
<td>Distal muscles</td>
<td></td>
</tr>
<tr>
<td>Wrist (WRS)</td>
<td></td>
</tr>
<tr>
<td>Flexor carpi radialis</td>
<td>FCR</td>
</tr>
<tr>
<td>Palmaris longus</td>
<td>PL</td>
</tr>
<tr>
<td>Flexor carpi ulnaris</td>
<td>FCU</td>
</tr>
<tr>
<td>Extensor carpi radialis</td>
<td>ECR</td>
</tr>
<tr>
<td>Extensor carpi ulnaris</td>
<td>ECU</td>
</tr>
<tr>
<td>Intrinsic (INT)</td>
<td></td>
</tr>
<tr>
<td>Abductor pollucis brevis</td>
<td>APB</td>
</tr>
<tr>
<td>First dorsal interosseus</td>
<td>FDI</td>
</tr>
<tr>
<td>Digit (DIG)</td>
<td></td>
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<tr>
<td>Flexor digitorum superficialis</td>
<td>FDS</td>
</tr>
<tr>
<td>Flexor digitorum profundus</td>
<td>FDP</td>
</tr>
<tr>
<td>Extensor digitorum communis</td>
<td>EDC</td>
</tr>
<tr>
<td>Extensor digitorum 2, 3</td>
<td>ED23</td>
</tr>
<tr>
<td>Extensor digitorum 4, 5</td>
<td>ED45</td>
</tr>
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EMG, electromyographic. * The activity of these muscles was recorded in monkey K but not monkey N.

Skin throughout the life of implant. The EMG implants were installed in three independent sections: one for the forearm that included muscles of the wrist and digits and intrinsic muscles of the hand; one for the upper arm that included muscles of the elbow; and one for the shoulder. With this modular approach, specific sections could be replaced, if necessary, without disturbing the entire implant. Each monkey wore a canvas jacket with a full sleeve on the right forelimb and one for the shoulder. With this modular approach, specific sections could be replaced, if necessary, without disturbing the entire implant. Each monkey wore a canvas jacket with a full sleeve on the right forelimb while in its home cage to protect the implanted wires. The implants generally remained functional for 5–8 wk.

Recording procedures

The electrical activity from single motor cortex cells was recorded using a glass insulated platinum-iridium electrode with a typical recording impedance between 0.7 and 1.5 MΩ. The electrode was positioned over the recording area using an X-Y positioner and was advanced into the motor cortex with a manual hydraulic microdrive. Cortical cell and EMG activity were simultaneously recorded on analogue tape along with position signals from the task.

Spike-triggered averaging procedures

During each recording session, cortical cell activity and EMG activity were monitored continuously on oscilloscopes. The action potentials of single cells in the motor cortex served as the triggers for computing SpTAs. Single-unit spikes from the cell of interest were isolated from other cortical cell spikes with a pair of time/amplitude window discriminators connected in series. Two PDP-11/73 computers rectified and digitized the analogue EMG signals before compiling simultaneous SpTAs for all recorded muscles. Some data collection and analysis also was performed with CED (Cambridge Electronics Design) hardware and custom SpTA software for Windows (Neural Averager, Larry Shupe, University of Washington, Seattle). The sampling rate for SpTA was 4 kHz, and the analysis period was 60 ms: 20 ms preceding the unit spike and 40 ms following it. To ensure that sweeps of EMG were only added to the SpTA if there was significant EMG activity present, we implemented a sweep-filtering protocol as previously described for identification and analysis of CM cells (McKiernan et al. 1998).

Quantification of postspike effects

On the basis of criteria established by Flament et al. (1992), we assigned the SpTA for each cell-muscle pair into one of seven possible groups: pure PSpF: postspike facilitation on an underlying synchrony facilitation (PSpF+S); pure synchronous facilitation (SyncF); pure PSpS; postspike suppression on an underlying synchrony suppression (PSpS+S); pure synchronous suppression (SyncS); and no postspike or synchrony effect (Flament et al. 1992; McKiernan et al. 1998).

All identified postspike and synchrony effects also were assigned a qualitative ranking of weak, moderate, or strong based on visual assessment of the magnitude of the facilitation or suppression effect relative to baseline activity. All effects that were classified as PSpF or PSpS were quantified further in terms of their latency, duration, and magnitude. First, we subtracted any nonstationary, ramping baselines (e.g., see Fig. 6 in Lemon et al. 1986). To do this, a ramp function was calculated using a linear least-squares fit to a selected data range. The ramp function then was subtracted while retaining the record’s baseline mean. EMG values from a range of bins in the pretrigger period then were averaged to derive a baseline mean and SD. The baseline typically was determined by averaging the first 10 ms of each record (~20 to ~10 ms pretrigger). Onset and offset latencies of PSEs then were identified as the points where the envelope of the SpTA crossed a level equivalent to 2 SD above or below the mean of the baseline EMG.

The peak of each effect was defined as the highest point in the PSpF or the lowest point in the PSpS. The magnitude of PSpF was quantified in terms of its peak percent increase (PPI) as follows: PPI = 100 * (Maximum bin value – baseline mean)/baseline mean. A similar measure [peak percent decrease (PPD)] was calculated for PSpS.

Quantification of cell-muscle covariation

Cell-muscle covariation was quantified by computing analogue cross-correlation functions between cortical cells and recorded muscles using the algorithm described by Miller et al. (1992, 1993). Cross-correlations were compiled off-line using the Neural Averager software package (Larry Shupe, University of Washington, Seattle) from data saved on a 28-channel TEAC instrumentation tape recorder. We collected 90 s of continuous data (~20–30 complete trials of the reach and prehension task) for each cross-correlation. Single-unit spikes were discriminated and the resulting pulses were sent to a frequency meter, the output of which was proportional to the inverse of interspike interval. The output of the frequency meter was sent to a 2-pole Butterworth low-pass filter (12 dB/octave attenuation >20 Hz) creating an analogue signal that was a smoothed representation of the cell’s firing frequency over time (Cheney et al. 1998). EMG signals from all recorded muscles were amplified, full-wave rectified, and also low-pass filtered in the same way as unit firing rate. Amplifier gains were adjusted to normalize EMG signal amplitude across all channels. Both the CM cell firing rate and the EMG signals were sampled at 200 Hz before calculating the cross-correlation. The analysis window for each correlation was 2 s. EMG records were shifted in 5-ms increments relative to the CM cell activity record to yield a cross-correlation with an analysis window of ±1 s.
Analysis of cross-correlations

We assigned each cross-correlation to one of eight qualitative groups based on visual inspection of the shape of the cross-correlation plot. The categories are illustrated in Fig. 1 and included: single peak; double peak; single trough; biphasic (initial trough followed by a peak); biphasic (initial peak followed by a trough); phasic/tonic (with identifiable peak); ramp (either rising or falling); and complex. The last category consisted of nonzero correlations with patterns that did not fit clearly in any of the other eight categories.

Three quantitative measures then were calculated for each cross-correlation (Fig. 2). The peak value of each correlation ($p_{\text{max}}$) was defined as the single point with the largest absolute difference from zero (positive or negative) that was straddled on each side by at least one point of lower absolute value. As with the Pearson’s correlation coefficient, $p_{\text{max}}$ could potentially range from $-1.0$ to $+1.0$. The peak lag was defined as the time from $p_{\text{max}}$ to the center of the analysis window (time 0). Positive values indicate that peaks in EMG activity followed peaks in CM cell activity; negative values indicate that EMG peaks preceded peaks in CM cell activity. We also calculated (when possible) the width (duration) of the correlation envelope at a magnitude halfway between the maximum and minimum values in the record.

At what magnitude can a cross-correlation peak be considered statistically significant? As pointed out by Miller et al. (1993), statistical analysis of cross-correlation effects is complicated by a number of factors. To circumvent this problem, they performed a Monte Carlo simulation on their cross-correlation data and concluded that a reasonable level of significance for analogue cross-correlations of this type is $p_{\text{max}} \geq 0.15$. Because our data were obtained using the same cross-correlation method (Houk et al. 1987; Miller et al. 1993), we have adopted $p_{\text{max}} \geq 0.15$ as the level of significance and $p_{\text{max}} \geq 0.25$ as the criterion for identifying the clearest cross-correlations worthy of further detailed study. With these criteria in mind, we constructed the following five-point ordinal scale for characterizing the significance of cross-correlations: strong, $p_{\text{max}} \geq +0.25$; moderate peak, $0.25 > p_{\text{max}} \geq +0.15$; not significant, $0.15 > p_{\text{max}} > -0.15$; moderate trough, $-0.25 < p_{\text{max}} \leq -0.15$; strong trough, $p_{\text{max}} \leq -0.25$.

RESULTS

We tested 174 cells for the presence of PSEs. One hundred twelve cells showed PSEs in at least one of the tested muscles (facilitation or suppression). We chose 23 of those 112 cells for analysis and calculated cross-correlations for 499 cell-muscle pairs. The 23 cells were selected based on the fact that each contained at least two PSpFs that were rated as moderate or strong, although many had additional weak facilitation effects and one or more suppression effects. We targeted cells with two or more moderate or strong facilitation effects based on the fact that Miller et al. (1992) found a tendency for rubromotoneuronal (RM) neurons with strong synaptic linkages (identified by the presence of PSpF in the SpTAs) to also show relatively strong covariation (i.e., cross-correlation peaks).

A small number of cross-correlations (34) had peaks that occurred at the positive or negative limit of the analysis period. These correlations [including 19 shoulder (SHL), 19 elbow (ELB), and 1 wrist (WRS)] were judged to be of little functional interest and were excluded from further analysis. The clear majority (281, 60%) of the remaining 465 cross-correlations had a single peak as their primary qualitative feature and were classified in category 1 (Fig. 1). Fifty two (11%) of the correlations were classified as complex because they could not easily be placed into any of the other seven categories. The remaining 124 correlations (28%) were distributed across the other six categories with the double-peak category being most common after the single-peak category.

One hundred twenty two (26%) of the 465 cross-correlations were associated with either a postspike or synchrony effect in SpTAs. Eighty six of these effects were facilitation (57 PSpF, 19 PSpF+S, 10 SyncF), whereas 36 were suppression effects (11 PSpS, 10 PSpS+S, 15 SyncS). Three hundred forty-three cross-correlations were not associated with any type of facilitation or suppression effect in the SpTA (pure or synchronous).

Figure 3A shows the peak lag times of all 465 cross-correlations plotted against their respective peak magnitudes ($p_{\text{max}}$). The overwhelming majority of $p_{\text{max}}$ values were positive (438 of 465, 94%). This was especially evident in cross-correlations associated with facilitation (Fig. 3B) but was also true for cross-correlations associated with suppression (Fig. 3C) and those not associated with any postspike or synchrony effect (Fig. 3D). There was a weak but statistically significant tendency for cross-correlations with higher $p_{\text{max}}$ to have lower peak lag times ($r = -0.28$ and $P < 0.001$ when plotting the absolute value each $p_{\text{max}}$ against the absolute value of its corresponding lag time). This is especially evident in Fig. 3B where we have plotted only the correlations for the 86 cell-muscle pairs with facilitation in the SpTA (PSpF, PSpF+S, and/or SyncF). Interestingly, the 10 cross-correlations that were associated with SyncF in the SpTA had the smallest aggregate lag times. Lag times for this subgroup ranged from $-95$ to $+55$ ms, and $p_{\text{max}}$ ranged from 0.196 to 0.662. The widest range of lag times occurred for those correlations with the lowest peak magnitudes.

FIG. 1. Eight qualitative categories of cross-correlations based on visual inspection of the “shape” of the cross-correlation envelope. Numbers (right) give the percent of cross-correlations found for that category. Long-term cross-correlations were used as a measure of corticomotoneuronal (CM) cell-muscle covariation during the reach and prehension task.
Cross-correlations that were associated with suppression in the SpTA had generally higher positive and negative lag times and lower peak magnitudes (Fig. 3, C vs. B). The clear majority of cross-correlations with negative $r_{\text{max}}$ were in the group that was not associated with any kind of postspike or synchrony effect (Fig. 3D).

**Magnitude and latency of cross-correlations associated with PSpF**

Before quantitatively examining the relationship between $r_{\text{max}}$ and PPI, we compared the magnitude and timing of the group of cross-correlations associated with pure PSpF ($n = 57$) with those that were not associated with any postspike or synchrony effect ($n = 343$). Figure 4A contains histograms comparing the range of $r_{\text{max}}$ for these two groups. The mean $r_{\text{max}}$ for the group of correlations associated with PSpF ($0.36 \pm 0.15$) was significantly greater than the mean $r_{\text{max}}$ for those cross-correlations not associated with a postspike or synchrony effect ($0.29 \pm 0.16$, $P < 0.001$). The overall distribution of $r_{\text{max}}$ values was somewhat broader for the group not associated with postspike or synchrony effects, and the histogram for that group also shows a small secondary peak for negative $r_{\text{max}}$ values not found in the histogram of correlations associated with PSpF.

The distribution of lag times was also different between the PSpF and no-effects groups (Fig. 4B). Although the distributions appear very similar, closer examination reveals that the cross-correlations associated with PSpF had lag times that were closer to zero (shorter) and significantly different from the lag times for correlations not associated with a postspike or synchrony effect ($1.6 \pm 329.2$ vs. $119.8 \pm 328.3$ ms, $P = 0.013$). This also is reflected in the fact that the median lag time for the group associated with PSpF was $-5$ ms, whereas the median lag time for the group without PSEs was $+75$ ms. The shorter lag times of the facilitation group (Fig. 3B) compared with the no-effect group (Fig. 3D) can be attributed in part to the fact that the facilitation group had a larger fraction of distal muscles (77.9%) and distal muscles tend to have shorter lag times than proximal muscles. For example, in the facilitation group, the median lag for distal muscles was $-10$ ms compared with $125$ ms for proximal muscles. The corresponding lags for the group not associated with any facilitation or suppression effect were $30$ ms for distal muscles and $110$ ms for proximal muscles.

**Does the magnitude of PSpF correlate with $r_{\text{max}}$?**

Figure 5A plots the magnitude of pure PSpF, measured as PPI, against $r_{\text{max}}$ for all cell-muscle pairs that showed PSpF and had >1,000 sweeps in the SpTA ($n = 53$). Spike-triggered averages containing PSpF+$S$ and SyncF as well as those containing <1,000 sweeps were excluded from this analysis because we could not confidently calculate PPI (McKiernan et al. 1998). PPI and $r_{\text{max}}$ for this group of cell-muscle pairs were not correlated ($r = 0.08$, $P = 0.6$). However, when the analysis was limited to the 30 cell-muscle pairs in which the magnitude of PSpF had been rated as moderate or strong, a weak, positive
A correlation emerged between PPI and $\rho_{\text{max}}$ ($r = 0.33$, $P = 0.08$, Fig. 5B) although the correlation failed to reach statistical significance.

Although there were several cell-muscle pairs that had both a high PPI and a high $\rho_{\text{max}}$, there were also many instances where the PPI was large but $\rho_{\text{max}}$ small and vice versa. Figure 6 shows examples of four different cell-muscle pairs to illustrate this point. In Fig. 6A, both PPI and $\rho_{\text{max}}$ are high, indicating that the cell had both a strong synaptic linkage with motoneurons of this muscle and also strongly covarying activity. Although PPI is almost as large for the PSpF in Fig. 6B, $\rho_{\text{max}}$ is very weak. However, the cross-correlation peak lag time is near time 0, suggesting that there were points during the task when both the cell and muscle activity covaried synchronously. The low $\rho_{\text{max}}$ suggests an overall weak covariation. This could arise from a consistent but weak covariation at one or more points throughout the movement cycle. Alternatively, a strong correlation at one point during the movement cycle could be degraded by the absence of covariation or even an inverse relationship between cell firing and muscle EMG at another point during the movement cycle. In Fig. 6C, PSpF magnitude was weak despite a strong $\rho_{\text{max}}$, indicating strong
covariation between the cell and this muscle during the task but only a weak synaptic linkage. Finally, Fig. 6D shows the cross-correlation with the single highest \( r_{\text{max}} \) we found (0.79). However, in this case the muscle showed no identifiable PSpF and therefore demonstrated a very strong covariation between cell firing and muscle EMG in the absence of any detectable synaptic linkage.

We also calculated the correlation coefficients between PPI and the peak lag of the corresponding cross-correlation peaks as well as between PPI and the durations of the cross-correlation envelope at 50% peak magnitude. Neither of these relationships was statistically significant.

Because the relationship between PPI and \( r_{\text{max}} \) was either weak (when considering moderate and strong pure PSpF) or nonexistent (all pure PSpF), we wondered if a clearer pattern would emerge for PSpFs of different magnitudes within a single CM cell’s muscle field. Table 2 lists the PSpF magnitudes for target muscles belonging to several different CM cells. Listed for each muscle is \( r_{\text{max}} \) and its rank order within the cell’s muscle field based on \( r_{\text{max}} \). For some cells (e.g., 21n7, 125k1, and 148n5), the rank orders for PPI and \( r_{\text{max}} \) were identical. However, for several additional cells there was little or no agreement between the respective rank orders of PPI and \( r_{\text{max}} \) within a muscle field.

**Postspike suppression and inverse cell-muscle covariation**

For 17 cross-correlations of CM cell and muscle activity, the predominant or only qualitative feature was a trough near time 0 (Fig. 1, Category 3). For 13 of these correlations, \( r_{\text{max}} \) was

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**Figure 4.** A: histograms of \( r_{\text{max}} \) for cross-correlations associated with pure PSpF and all cross-correlations that were not associated with facilitation or suppression of any type. B: histograms of lag times for cross-correlations associated with pure PSpF and cross-correlations that were not associated with facilitation or suppression of any type.
negative and occurred at the bottom of the trough. However, in
the other four cases, $r_{\text{max}}$ was positive because the trough was
superimposed on an otherwise broad positive correlation. The
existence of a centrally located trough demonstrates the pres-
ence of an inverse pattern of cell/muscle covariation during the
task. Despite the positive values for $r_{\text{max}}$ in some of these
cases, the correlation trough appeared to be the most significant
feature, especially in those cases where the low point of the
trough had a much smaller positive or negative lag than $r_{\text{max}}$.

An additional 36 of 465 cross-correlations had a different
type of trough. These troughs came from categories 2, 4, and 5.
In each case, the cross-correlation contained a positive peak on
one or both sides of a dip in the cross-correlation envelope
yielding a positive $r_{\text{max}}$, which was used in other analyses in
this paper (e.g., Fig. 3). However, on the basis of the premise
that the trough might be the most significant feature in the
cross-correlation, we identified the lowest point in the cross-
correlation occurring within a 500-ms window ($\pm 250$ ms)
around time 0 and measured its lag and magnitude (Fig.
7). Starting with that point, we sequentially evaluated preceding
points in the cross-correlation until we found the point where
the value of the cross-correlation envelope no longer continued
to rise. We labeled this point the “pretrough peak.” Subtracting
the low point from the pretrough peak yielded a “fall magni-

![Graph A](image1.png)

**Graph A:** Scatter plot of peak percent increase (PPI) vs. $r_{\text{max}}$ for 53 cell-muscle pairs. There was no significant correlation between
these 2 measures ($r = 0.08$, $P = 0.6$).

![Graph B](image2.png)

**Graph B:** Scatter plot of PPI vs. $r_{\text{max}}$ for 26 cell-muscle pairs
where the PSpF had been rated as moderate or
strong. Although the correlation between PPI and
$\rho_{\text{max}}$ is much stronger in this case, it did not quite
achieve statistical significance ($r = 0.33$, $P = 0.08$). $\triangle$ and $\Delta$ in A and B illustrate plotting of
$\rho_{\text{max}}$ from cell 21N7 (EDC) as described in Fig.
3 legend. Results of statistical analysis of the
relation between PPI vs. $\rho_{\text{max}}$ were nearly iden-
tical in both A and B no matter which plotting of
21N7 was included. However, the statistics re-
ported here were calculated using the value rep-
resented by $\triangle$. 

---

**Fig. 5.** A: scatter plot of peak percent in-
crease (PPI) vs. $\rho_{\text{max}}$ for 53 cell-muscle pairs.
There was no significant correlation between
these 2 measures ($r = 0.08$, $P = 0.6$). B: scatter
plot of PPI vs. $\rho_{\text{max}}$ for 26 cell-muscle pairs
where the PSpF had been rated as moderate or
strong. Although the correlation between PPI and
$\rho_{\text{max}}$ is much stronger in this case, it did not quite
achieve statistical significance ($r = 0.33$, $P = 0.08$). $\triangle$ and $\Delta$ in A and B illustrate plotting of
$\rho_{\text{max}}$ from cell 21N7 (EDC) as described in Fig.
3 legend. Results of statistical analysis of the
relation between PPI vs. $\rho_{\text{max}}$ were nearly iden-
tical in both A and B no matter which plotting of
21N7 was included. However, the statistics re-
ported here were calculated using the value rep-
resented by $\triangle$. 
Spike-Triggered Average

A

PPI = 13.91

65n6 - FDP

n = 3366

B

PPI = 11.06

65n6 - ECU

n = 4565

C

PPI = 3.84

110n1 - FDI

n = 10901

D

PPI = na

148n5 - BIL

n = 8143

Cross-Correlation

FIG. 6. A: SpTA and corresponding cross-correlation for cell-muscle pair with high PPI and high $r_{max}$ [65N6, flexor digitorum profundus (FDP)]. B: SpTA and cross-correlation for cell-muscle pair with high PPI and low $r_{max}$ [65N6, extensor carpi ulnaris (ECU)]. C: SpTA and cross-correlation for cell-muscle pair with low PPI and high $r_{max}$ [110N1, first dorsal interosseus (FDI)]. D: SpTA and cross-correlation for a cell-muscle pair with no PSpF despite a very large $r_{max}$ [148N5, long head of the biceps (BIL)].

Similarly, subtracting the lag of the low point from that of the pretrough peak yielded a “fall time.” Any cross-correlation with a fall magnitude $\geq 0.25$, the low point of which had a smaller lag time than $r_{max}$, was categorized as a “strong trough.” Likewise, any cross-correlation with a fall magnitude $\geq 0.15$ but $< 0.25$, the low point of which had a smaller lag time than $r_{max}$, was categorized as a “moderate trough.”

Based on these criteria, 24 of the 36 cross-correlations were categorized as containing significant troughs despite the presence of a positive $r_{max}$. The lag of the low point in the trough was between $-50$ and $+50$ ms for 17 of the 24 recategorized correlations. The average fall magnitude for these 24 troughs was 0.29, and the average fall time was 229 ms.

A number of the cross-correlations containing troughs were associated with postspike and/or synchrony suppression. However, as with facilitation effects, there was no clear quantitative relationship between the magnitude of suppression and the magnitude of the troughs. Figure 8 shows examples of troughs that were associated with suppression effects in SpTAs. In Fig. 8A, $r_{max}$ for PDE is weakly to moderately positive throughout except for a visible trough the low point of which has a lag of $+2$ ms. The small lag time for the bottom of the trough in this correlation demonstrates that muscle activity fell at the same time cell activity was rising. This inverse relation between cell and muscle activity is functionally consistent with the presence of clear PSpS in the SpTA. In Fig. 8B, the cross-correlation between cell firing and FCR muscle activity rose early in the correlation to a value significantly greater than zero. Beginning
at a lag of $-155$ ms, the correlation magnitude dropped to near zero, forming a clear trough. Unlike the correlation in $A$, the magnitude of this correlation never dropped below zero, although it was close. Fall magnitude was 0.16 and the trough reached its low point at a lag of $+30$ ms. This inverse covariance between cell and muscle activity is again consistent with the presence of a strong PSpS+S in the SpTA.

The cross-correlation in Fig. 8C is similar in shape to those in $A$ and $B$. The correlation rose to its largest positive value with a lag of $-120$ ms, at which point it began falling to a low of 0.05 at a lag of $+5$ ms. The corresponding SpTA showed synchronous suppression. Although the onset of suppression appears sharp in the SpTA, it begins too early to be attributed solely to a synaptic output linkage between the cell and muscle. Moreover no discontinuity could be identified between the onset and the negative peak of the effect that might indicate the presence of a true PSpS together with the synchrony suppression (McKiernan et al. 1998).

Not every suppression effect in the SpTA was associated with a trough in the cross-correlation. Figure 8D illustrates a case where a clear PSpS appeared in the SpTA despite a strong positive correlation between cell and muscle. Similarly not every trough in the cross-correlation was associated with a suppression effect in the SpTA. Three (12%) of the cross-correlations with significant troughs were associated with PSpF. In each of these cases, $r_{\text{max}}$ was positive and had a negative lag, but a clear trough was present and reached a low point closer to time 0 than $r_{\text{max}}$.

The majority of facilitation effects in SpTAs (89.5%) was associated with significant peaks in the cross-correlation (Table 3, Fig. 9). However, 55.6% of the cross-correlations associated with suppression effects and 84.3% of the cross-correlations not associated with any facilitation or suppression effects also had significant correlation peaks. Therefore the presence of a significant peak in the cross-correlation is clearly not a good predictor of a facilitation effect in the SpTA. It also should be emphasized that this relatively high incidence of significant cross-correlation peaks occurred in nontarget muscles of iden-

<table>
<thead>
<tr>
<th>Cell</th>
<th>Muscle</th>
<th>PPI</th>
<th>$r_{\text{max}}^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>21N7</td>
<td>EDC</td>
<td>5.42</td>
<td>0.18 (1)</td>
</tr>
<tr>
<td></td>
<td>ECR</td>
<td>4.51</td>
<td>0.15 (2)</td>
</tr>
<tr>
<td>125K1</td>
<td>EDC</td>
<td>7.74</td>
<td>0.31 (1)</td>
</tr>
<tr>
<td></td>
<td>ED23</td>
<td>5.29</td>
<td>0.28 (2)</td>
</tr>
<tr>
<td></td>
<td>ADE</td>
<td>2.99</td>
<td>0.28 (3)</td>
</tr>
<tr>
<td>148N5</td>
<td>APB</td>
<td>9.49</td>
<td>0.64 (1)</td>
</tr>
<tr>
<td></td>
<td>ED23</td>
<td>5.51</td>
<td>0.43 (2)</td>
</tr>
<tr>
<td>60N4</td>
<td>ED45</td>
<td>16.88</td>
<td>0.37 (1)</td>
</tr>
<tr>
<td></td>
<td>APB</td>
<td>8.18</td>
<td>0.22 (4)</td>
</tr>
<tr>
<td></td>
<td>ED23</td>
<td>7.25</td>
<td>0.30 (3)</td>
</tr>
<tr>
<td></td>
<td>FDP</td>
<td>4.53</td>
<td>0.32 (2)</td>
</tr>
<tr>
<td>65N6</td>
<td>APB</td>
<td>17.36</td>
<td>0.41 (3)</td>
</tr>
<tr>
<td></td>
<td>FDP</td>
<td>13.91</td>
<td>0.61 (1)</td>
</tr>
<tr>
<td></td>
<td>ECU</td>
<td>11.06</td>
<td>0.12 (5)</td>
</tr>
<tr>
<td></td>
<td>FCU</td>
<td>10.07</td>
<td>0.34 (4)</td>
</tr>
<tr>
<td></td>
<td>ED23</td>
<td>6.01</td>
<td>0.44 (2)</td>
</tr>
<tr>
<td>83K2</td>
<td>APB</td>
<td>9.50</td>
<td>0.29 (2)</td>
</tr>
<tr>
<td></td>
<td>FDP</td>
<td>7.34</td>
<td>0.48 (1)</td>
</tr>
<tr>
<td>110N1</td>
<td>FDI</td>
<td>8.25</td>
<td>0.48 (3)</td>
</tr>
<tr>
<td></td>
<td>ADE</td>
<td>5.25</td>
<td>0.55 (1)</td>
</tr>
<tr>
<td></td>
<td>BIL</td>
<td>4.75</td>
<td>0.54 (2)</td>
</tr>
<tr>
<td></td>
<td>FDI</td>
<td>13.39</td>
<td>0.48 (2)</td>
</tr>
<tr>
<td></td>
<td>ECU</td>
<td>6.71</td>
<td>0.50 (1)</td>
</tr>
<tr>
<td></td>
<td>ED45</td>
<td>6.13</td>
<td>0.45 (3)</td>
</tr>
</tbody>
</table>

Muscles are listed in order of magnitude of peak percent increase (PPI). CM, corticomotoneuron; PSpF, postspike facilitation. * Numbers in parentheses indicate rank order among cross-correlation peak magnitudes for each cell.

FIG. 7. Measurement of cross-correlation troughs. Variables measured included the value and lag of the lowest point in the trough as well as the pretrough peak. These measures were used to calculate fall magnitude and fall duration. We considered the trough to be the most functionally significant feature of a cross-correlation if the fall magnitude was $>-0.15$ and the low point of the trough was closer to the center of the analysis window than $r_{\text{max}}$. 

The cross-correlation between cell and muscle activity is again consistent with the presence of a strong PSpS+S in the SpTA.
tified CM cells. The incidence of significant correlations might be much lower for a random sample of cells in forelimb motor cortex.

There was a greater percentage of strong correlation peaks in cell-muscle pairs with PSpF+S than in cell-muscle pairs with either PSpF or SyncF alone. Almost 95% (18/19) of the SpTAs with PSpF+S were associated with a strong peak in the cross-correlation compared with 75.4% (43/57) of the SpTAs containing only PSpF and 80% (8/10) of the SpTAs with only SyncF.

A large number of muscles with suppression effects (38.9%) were associated with cross-correlations troughs (Table 3, Fig. 9). In comparison, only 3.5% of muscles with facilitation effects were associated with cross-correlation troughs. We visually identified troughs in several additional cross-correlations associated with suppression effects, but these troughs were too small to meet our significance criteria. However, about half of the suppression effects (55.6%) were associated with cross-correlation peaks in which there was no evidence of a trough. Therefore the presence of postsynaptic or synchrony suppression appears to be a relatively poor predictor of the presence of a trough in the cross-correlation. However, if a trough appears in the cross-correlation, there is almost a five-fold greater probability of suppression in the SpTA than facilitation (Table 3). As was true for cross-correlations associated with facilitation effects, a greater percentage of strong troughs was associated with PSpS+S than pure PSpS or SyncS. Overall, it is noteworthy that for all CM cells tested, at least one

![SpTA and cross-correlation](image-url)
muscle of the target muscle field exhibited a functionally consistent relationship between the postspike effect and cell-muscle covariation (PSpF associated with a significant correlation peak or PSpS associated with a significant correlation trough).

SpTAs containing no postspike or synchrony effects were much more likely to be associated with cross-correlations containing significant peaks than correlations without significant features. Nearly two-thirds of the SpTAs in this group were associated with cross-correlations containing a strong peak (64.5%, 221/343).

Analysis of cross-correlations for muscles of different joints

In a previous study, we showed that postspike effects from CM cells are more common and generally stronger in distal forelimb muscles compared with proximal muscles during performance of the reach andprehension task (McKiernan et al. 1998). As a final analysis in this study, we examined our data to determine if the characteristics of CM cell-muscle cross-correlations also might show differences for muscles at different joints. First, we compared the strength of cross-correlations across each of the five joints studied. Figure 10, A and B, were constructed from all correlations lacking postspike or synchrony effects (facilitation or suppression) in the SpTAs. In Fig. 10A, the number of cross-correlations with strong peaks ($\rho_{\text{max}} \geq 0.25$) is expressed as a percentage of all cross-correlations calculated for each of the forelimb joints studied. There was a higher percentage of strong cross-correlation peaks in muscles of the wrist and digits than in muscles at other joints. For example, 88% of the correlations for forearm digit muscles contained strong peaks, whereas only 77% of the correlations for the proximal forelimb muscles contained strong peaks.

Parentheses enclose percentages. SpTA, spike-triggered averaging; PSpF+S, PSpF on an underlying synchrony facilitation; SyncF, synchronous facilitation; PSpS, postspike suppression; PSpS+S, PSpS on an underlying synchrony suppression; SyncS, synchronous suppression. * Strong peak = $\rho_{\text{max}} \geq 0.25$; moderate peak = $\rho_{\text{max}} = +0.15$ and $\rho_{\text{max}} < +0.25$. † Moderate trough = ($\rho_{\text{max}} \leq -0.15$ and $\rho_{\text{max}} > -0.25$) or (fall magnitude $\geq 0.15$ and latency of low point closer to 0 ms than $\rho_{\text{max}}$); strong trough = ($\rho_{\text{max}} \leq -0.25$) or (fall magnitude $\geq 0.25$ and latency of low point closer to 0 ms than $\rho_{\text{max}}$).

### Table 3. Summary of the number and percentage of cross-correlation peaks and troughs sorted according to the type of effect observed in the SpTA

<table>
<thead>
<tr>
<th>Effect Type</th>
<th>Associated with Moderate or Strong Peaks*</th>
<th>Not Associated with Moderate or Strong Peak or Trough</th>
<th>Associated with Moderate or Strong Troughs†</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSpF</td>
<td>57</td>
<td>5 (8.8)</td>
<td>3 (5.3)</td>
</tr>
<tr>
<td>PSpF+S</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SyncF</td>
<td>10</td>
<td>1 (10)</td>
<td>0</td>
</tr>
<tr>
<td>All facilitation</td>
<td>86</td>
<td>6 (7.0)</td>
<td>3 (3.5)</td>
</tr>
<tr>
<td>PSpS</td>
<td>11</td>
<td>0</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>PSpS+S</td>
<td>10</td>
<td>0</td>
<td>5 (50)</td>
</tr>
<tr>
<td>SyncS</td>
<td>36</td>
<td>2 (5.6)</td>
<td>14 (38.9)</td>
</tr>
<tr>
<td>All suppression</td>
<td>343</td>
<td>289 (84.3)</td>
<td>23 (6.7)</td>
</tr>
</tbody>
</table>

Parentheses enclose percentages. SpTA, spike-triggered averaging; PSpF+S, PSpF on an underlying synchrony facilitation; SyncF, synchronous facilitation; PSpS, postspike suppression; PSpS+S, PSpS on an underlying synchrony suppression; SyncS, synchronous suppression. * Strong peak = $\rho_{\text{max}} \geq 0.25$; moderate peak = $\rho_{\text{max}} = +0.15$ and $\rho_{\text{max}} < +0.25$. † Moderate trough = ($\rho_{\text{max}} \leq -0.15$ and $\rho_{\text{max}} > -0.25$) or (fall magnitude $\geq 0.15$ and latency of low point closer to 0 ms than $\rho_{\text{max}}$); strong trough = ($\rho_{\text{max}} \leq -0.25$) or (fall magnitude $\geq 0.25$ and latency of low point closer to 0 ms than $\rho_{\text{max}}$).
had a peak with $r_{\text{max}} > 0.25$, whereas only 53% of the correlations for muscles of the elbow were equally strong. However, Fig. 10B shows that there was no difference across joints in average $r_{\text{max}}$ for these cross-correlations.

The results were somewhat different for cross-correlations associated with facilitation effects (PSpF, PSpF$+S$, and SyncF). Overall, the percentage of cell-muscle pairs with strong correlation peaks in this group was greater than for cell-muscle pairs that did not show a facilitation or suppression effect. This was particularly true of proximal muscles. Most noteworthy is the fact that the percentage of proximal muscles showing strong correlation peaks was equally as great as the percentage of distal muscles when the analysis was limited to muscles associated with facilitation effects in spike-triggered averages (Fig. 10C). The same was true for the average magnitude of correlation peaks (Fig. 10D).

The timing of cross-correlation peaks also showed differences for muscles at different joints. Lag times for cross-correlations of shoulder muscles ranged widely around zero (both positive and negative) compared with those of wrist and digit muscles, which were more tightly clustered around time $0$. The mean cross-correlation lag times for shoulder, elbow, wrist, digit, and intrinsic hand muscles were 97.7, 210.7, 6.3, -28.2, and 55.6 ms, respectively (all cell-muscle pairs).

**DISCUSSION**

The results of this study demonstrate that cortical cells possessing a synaptic linkage with motoneurons tend to show stronger covariation with their target muscles than with non-target muscles. The results also demonstrate a significant relationship between the sign of the postspike effect (facilitation or suppression) and the presence of a peak or trough in the cross-correlation. Of all the target muscles with facilitation effects in spike-triggered averages (PSpF, PSpF$+S$, or SyncF) in the spike-triggered averages, 89.5% were associated with significant cross-correlation peaks, indicating positively covarying muscle and CM cell activity. Seven percent of facilitation effects was not associated with a significant effect in the cross-correlation, whereas only 3.4% of effects was associated with correlation troughs. In contrast, of all the muscles with suppression effects in spike-triggered averages, 38.9% was associated with significant troughs in the cross-correlation indicating an inverse relation between CM cell and muscle activity consistent with the presence of suppression. Fifty-five percent of suppression effects was associated with correlation peaks, whereas 5.6% was not associated with a significant effect in the cross-correlation. Finally, the results also suggest that the magnitude of a PSpF (excluding weak effects) is related, albeit weakly, to the magnitude of the corresponding cell-muscle functional covariation.

One limitation of this approach is the use of PSpF and PSpS as a measure of the strength of the underlying synaptic linkage. A recent computer simulation of the CM system by Baker and Lemon (1998) is highly relevant to this issue. Examination of simulated postspike effects in their model revealed that the magnitude of PSpF can be influenced heavily by the number of CM cells with which the tested cell is synchronized. Synchronization with 10 other CM cells had little effect on magnitude but synchronization with 30 CM cells doubled PSpF magnitude. Of course, little is known about how many CM cells actually may show synchronized discharge during real movements. Baker and Lemon also emphasize that the levels of synchrony they used were probably at the upper limit of what is likely to exist in the CM system. Nevertheless this problem
should be noted as a factor that could degrade the relationship between PSpF magnitude and cell-muscle covariation. Although many CM cell-target muscle pairs covaried during the reach and prehension task in a way consistent with the sign and strength of the CM cell’s synaptic effects on target motoneurons, many exceptions were found. It also should be noted that nearly 50% (231 of 465) of the CM-muscle cross-correlations had a \( r_{\text{max}} \geq \pm 0.25 \) but were not associated with any postspike or synchrony effect in the corresponding SPTA. The existence of many strong correlations between cell and muscle activity in cases lacking any demonstrable synaptic linkages simply means that the presence of a strong cross-correlation is of little or no predictive value for the presence of postspike effects in spike-triggered averages. The presence of such correlations in the absence of synaptic linkages could be related, in part, to the fact that the task involves periods in which different muscles consistently are coactivated as a natural requirement of task execution but without the presence of underlying common synaptic input. For example, a wrist movement task requiring alternation between flexion and extension position zones will produce broad and uniform coactivation of all extensors in one direction and all flexors in the other direction. The highly uniform and stereotyped pattern of EMG activity in this task makes it unsuitable for investigating relationships between effects in spike-triggered averages and cell-muscle covariation. In contrast, the reach and prehension task produced highly fractionated patterns of EMG activity suitable for more rigorous correlational analysis of cell-muscle covariation. However, stereotyped coactivation of some muscles did occur in this task and would have contributed to cases of strong cell-muscle covariation in the absence of demonstrable synaptic connections. Nevertheless it is important to emphasize that the majority of muscles with facilitation effects in spike-triggered averages (89.5%) also had significant cell-muscle cross-correlation peaks. Even more striking is the fact that when we limited the analysis to only the correlations with strong peaks \( (r_{\text{max}} \geq \pm 0.25) \), there was a greater difference between the correlations associated with facilitation effects and the correlations that were not associated with any PSE. Sixty-nine of the 86 correlations associated with facilitation effects (80.2%) contained strong peaks, whereas only 220 of the 343 correlations not associated with any facilitation or suppression effect (64.1%) contained strong peaks. Perhaps our most important observation is that none of the correlations associated with pure PSpF had a \( r_{\text{max}} \geq \pm 0.25 \) but were not associated with any postspike or synchrony effect in the corresponding SPTA. The characteristics of the cross-correlations in this study make an interesting comparison with those reported by Miller et al. (1992, 1993) for red nucleus neurons. The median \( r_{\text{max}} \) was greater in our data, but the most frequent lag times were very similar for our data on CM cells and Miller et al.’s data on red nucleus cells. The most common \( r_{\text{max}} \) fell between 0.30 and 0.35 for cross-correlations associated with PSpF as well as those that were not associated with a postspike or synchrony effect. In Miller et al.’s study (1992), the most common peak magnitude fell between 0.15 and 0.20 for both groups. The larger \( r_{\text{max}} \) values for CM cells in this study may be related to stronger synaptic coupling between CM cells and target muscles (Cheney et al. 1988) and/or the fact that cortical input to motoneuron pools in the primate are likely to be a much more dominant than red nucleus input (Mewes and Cheney 1994). Our results may have been influenced by the fact that we only included CM cells that produced two or more moderate or strong PSpFs in target muscles, although this seems unlikely given the lack of a relation between the magnitude of PSpF (all effects) and the magnitude of \( r_{\text{max}} \). Even if they did not produce PSpF in all target muscles, the cells in this study may have been part of larger functional clusters of cortical cells the summed output of which would tend to facilitate synergist muscles during various phases of the task. This, in turn, would increase the probability of a significant correlation. On the other hand, Miller et al. (1993) did not limit their analysis to RM cells. The fact that they included cross-correlations from at least some cells with no demonstrated synaptic linkage to target muscles may be a further explanation for the difference in cross-correlation peak magnitudes between our study and theirs.

For cross-correlations with \( r_{\text{max}} \geq \pm 0.25 \), Miller et al. (1993) reported that the most frequent lag times occurred between 0 and +25 ms (14% of the sample). When we examined our data with similar 25-ms bins, the most frequent lag times also fell between 0 and +25 ms both for the cross-correlations associated with PSpF and for those not associated with any postspike or synchrony effects (11.3 and 7.8%, respectively). However, we had a much broader range of lag times than Miller et al., even for those cross-correlations with \( r_{\text{max}} \geq 0.25 \).

Functional implications of cross-correlations and spike-triggered averages

Miller et al. (1992) point out that it is particularly difficult to characterize the relationship between activity of premotor cells and target muscles during complex free-form movements because of the phasic nature of both cell and muscle activity.
They maintain that the long-term cross-correlation method is well suited to studying the relationship between randomly varying signals and argue that the identification of “functional linkages” by the cross-correlation method has a number of advantages over other methods.

We would agree with this assessment. However, it is also important to emphasize a point made by Fetz and Finocchio (1975) that temporal correlations are neither necessary nor sufficient evidence to establish a synaptic connection between a premotor cell and motoneurons of a target muscle. Nevertheless, as they point out, there is an intuitive inclination to expect the activity of connected elements to be correlated. The vast majority of the cell-muscle pairs with PSpF in our study, in fact, did have strong cross-correlation peaks. However, there was only a weak relationship between the magnitude of PSpF and the magnitude of cell-target muscle covariation measured as \( p_{\text{max}} \). As pointed out in the preceding text, this most likely reflects the fact that an individual CM cell represents only a very small fraction of the total synaptic input to a particular motoneuron or motoneuron pool. The contribution of any given CM cell to target muscle activation may be modified or completely overshadowed by inputs from other premotor neurons. Of course, as noted earlier, the limitations of PSpF as a measure of the strength of the underlying synaptic linkage also may have contributed. Because of the nature of the reach and prehension task, neither CM cells nor target muscles were tonically active throughout the movement cycle. Rather both cells and muscles tended to burst one or more times during the task, usually in relation to specific phases of reaching and retrieving the food reward. In almost none of the cell-muscle pairs studied was every burst of cell activity consistently accompanied by a similar burst of EMG activity in all the cells target muscles.

Figure 11 contains a conceptual model of the combinations of synaptic (spike-triggered averages) and functional (task covariation) linkages we observed in this study. This model provides a conceptual construct for explaining the various relations between postspike effects and task-related CM cell-muscle covariation that we observed in this study. Four CM cells (A–D) with different muscle fields are represented in Fig. 11. Based on previous findings (Cheney and Fetz 1985), individual CM cells probably are organized as functional groups or cell clusters in which each cell is linked by sharing a common (or similar) muscle field. Therefore although we have represented only single cells this figure, it should be recognized that each cell can be viewed as a cluster of cells with similar muscle fields. Spike-triggered averages compiled from each CM cell show different patterns of facilitation based on their unique connections with spinal motoneuron pools.

S1 and S2 are cortical interneurons modeled after those suggested by Huntley and Jones (1991). These neurons send extensive, horizontally oriented, intrinsic axon collaterals to many different forelimb movement representations and “may be recruited during complex movements to coordinate the activity of motor cortical zones whose predominant output is to forelimb muscle groups acting synchronously.” Once again, S1 and S2 can best be viewed as functional clusters of neurons, although we have only represented one neuron of each type. The activity of cells S1 and S2 is out of phase (Fig. 11D). However, both S1 and S2 will tend to synchronize the discharge of their respective target CM cells.

Consider the case in which movement execution involves powerful input to CM cells A and B from S1. INT 1 and DIG 1 show strong PSpF from CM cell A and strong task-related covariation with A (like cell-muscle pair 65N6-FDP in Fig. 7). The strong PSpF in these muscles and WRS 1 reflects the correspondingly strong synaptic connections from CM cell A to INT 1, DIG 1 and WRS 1 motoneurons. On the other hand, task-related covariation will be determined by the actions of cell clusters S1 and S2. S1 is the dominant input to CM cells A and B during this movement task. Accordingly, S1 will activate CM cells A and B, which in turn, will coactivate INT 1, DIG 1 and WRS 1. Because INT 1 and DIG 1 do not receive input from S2, input from S1 will drive the task-related functional activity of CM cell A and muscles. However, unlike INT 1 and DIG 1, WRS 1 shows little or no covariation with CM cell A despite the presence of strong PSpF. This occurs because input from S2 is out of phase with respect to the activity of S1. Because both S1 and S2 have roughly equivalent synaptic actions on WRS 1 motoneurons (represented by number of synaptic contacts in Fig. 11A), excitation from increasing activity in S1, on average, is opposed by disfacilitation from decreasing activity in S2. As a result, the activity of WRS 1 does not covary with CM cell A. In fact, it is conceivable that the activity of WRS 1 might be related inversely to the activity of CM cell A depending on the relative strengths of synaptic inputs from cell A compared with S2-related CM cells and the degree and phase of modulation of inputs from S1 and S2.

ELB 1 represents the reverse condition in which there is an absence of PSpF but relatively strong covariation (like cell-muscle pair 148N5-BIL in Fig. 6). The lack of synaptic connections between CM cell A and ELB 1 motoneurons results in an absence of PSpF. However, S1 provides common input not only to CM cell A but also to other CM cells, some of which do make synaptic connections with ELB 1 motoneurons. Because ELB 1 does not receive input from S2-related CM cells, S1 is the dominant input for this task. Consequently, ELB 1 will covary with CM cell A and other muscles for which S1 is the dominant or only input.

In conclusion, this example emphasizes the fact that effects in spike-triggered averages will not necessarily correlate with the degree of covariation between the cell and muscles involved in the task. The strength and presence of postspike effects will reflect underlying synaptic connections, whereas the presence of task-related functional covariation, revealed by long-term cross-correlation methods, will reflect the summation of input signals to a particular CM cell relative to the summation of input signals to motoneuron pools of muscles to which the cell activity is being compared. Because a particular CM cell represents only one of potentially many parallel CM and non-CM inputs to a motoneuron pool, the activity of a CM cell and its target muscles can easily be divergent. In view of this, perhaps one of more remarkable findings of this study is the extent to which CM cells and their target muscles do closely covary.

Conclusions

The primary question addressed by this study concerns the extent to which the presence and strength of PSpF and PSpS from CM cells correlates with the magnitude of covariation in activity of CM cells and their target muscles revealed by...
FIG. 11.  A: diagram of a theoretical model to illustrate how the different combinations of synaptic and functional linkages may be produced.  B: expected synchrony components in the spike-triggered averages are not shown.  D: cell A is the cell that all cross-correlations in C were constructed.  See text for details.
computing long-term cross-correlations. We found that the magnitude of cross-correlations is greater for muscles with facilitation effects in spike-triggered averages than for muscles lacking effects in spike-triggered averages. Our results also demonstrate a significant relationship between the sign of the postspike effect (facilitation or suppression) and the presence of a peak or trough in the cross-correlation. Finally, the magnitude of PSpF (moderate and strong effects only) was correlated weakly with the magnitude of the cell-muscle cross-correlation peak. Nevertheless, although many CM cell-target muscle pairs covary during the reach and prehension task in a way consistent with the sign and strength of the CM cell’s synaptic effects on target motoneurons, many exceptions exist. Muscles lacking demonstrable evidence of a significant effect in spike-triggered averages also could show strong covariation with CM cells; however, these cases cannot be viewed as providing a test of the extent to which synaptic effects of a CM cell are consistent with cell-target muscle covariation. The results are compatible with a model in which control of particular motoneuron pools reflects not only the summation of signals from many CM cells, but also signals from other premotor neurons. Any one neuron will make only a small contribution to the overall activity of the motoneuron pool. In view of this, it is not surprising that relationships between postspike effects and CM cell-target muscle covariation are relatively weak with many apparent incongruities.

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