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Effects of synchrony between primate corticomotoneuronal cells on post-spike facilitation of muscles and motor units

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Cross-correlating the activity of neighboring motor cortex neurons recorded with independent electrodes in behaving monkeys has revealed synchronization peaks, largely due to common synaptic input. Corticomotoneuronal (CM) cells produced post-spike facilitation (PSF) of rectified forearm electromyograms (EMG); 15 cells synchronized with CM cells showed no PSF. Five pairs of CM cells with overlapping muscle fields exhibited similar synchrony peaks. The contribution of this synchrony to facilitation of common target muscles was assessed by two new methods: selective spike-triggered averaging and convolution. They showed that the PSF is composed predominantly of effects mediated by output of the triggering cell, but may include a broad, shallow component mediated by synchrony with other CM cells.

The postsynaptic effects of a neuron on its targets may be documented by the technique of spike-triggered averaging [6, 8]; however, a recurring question concerns the degree to which postspike effects may be mediated by other cells synchronized with the trigger cell [3, 4, 7]. For example, spike-triggered averages (STAs) of rectified electromyographic (EMG) activity have been used to document the effects of precentral cortex cells on activity of forelimb motor units in behaving primates [5, 12]. The post-spike facilitation (PSF) of rectified EMG activity at appropriate latencies has been interpreted as evidence that the triggering precentral cell produced the increased firing probability of motor units. Assuming it is mediated by a monosynaptic connection, the PSF identifies the cell as a corticomotoneuronal (CM) cell and the facilitated muscles as its target muscles. Alternatively, it has been argued that the PSF may not be produced by the recorded cell, but rather be mediated by its synchronous firing with one or more CM cells. Since this disynaptic linkage involves a second-order correlation, such effects of synchronization have been judged to be negligible compared

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with directly mediated effects [5]. To investigate these mechanisms quantitatively we documented the degree of synchrony between neighboring cortical neurons, including CM cells, in a behaving monkey and used two methods to calculate the effect of this synchrony on motor unit facilitation.

A *Macaca mulatta* monkey generated alternating, isometric torques about the wrist while pairs of precentral neurons were recorded with independently controlled microelectrodes [5, 16]. One electrode was advanced perpendicular to the dural surface, while the second was driven obliquely in a sagittal plane. Pyramidal tract neurons (PTNs) were identified by antidromic activation from the medullary pyramids. When a PTN was isolated on either electrode, STAs of rectified forearm EMG activity were compiled, and other cells were sought with the second electrode. Cortical unit data and EMGs were recorded on FM tape for off-line analysis of correlations between the two spike trains and, in some cases, single motor units derived from the multiunit EMG [10, 11, 14].

Thirty-five percent of 217 cell pairs exhibited correlogram peaks which straddled the origin, consistent with common synaptic input [11]. For 15 non-CM cells sharing common input with CM cells, the correlogram peaks had a mean width of 28.0 ± 13.3 ms (S.D.) and a mean normalized peak area (counts in correlogram peak divided by mean of target and reference spikes) of 0.078 ± 0.070. None of these 15 non-CM cells showed significant postspike effects in EMGs, confirming that the degree of synchrony between precentral cells is insufficient to indirectly mediate clear PSF from non-CM cells. We also recorded and cross-correlated twelve pairs of CM cells. Correlograms for 7 pairs had no central peak and the muscle fields of these cells did not overlap (two pairs facilitated antagonist muscles). Five pairs had central correlogram peaks with a mean width of 21 ± 9.8 ms and mean area of 0.061 ± 0.033. All five pairs had at least one target muscle in common. We used two methods – selective STA and convolution – to assess the relative contribution of synchrony to the PSF in common target muscles.

Fig. 1 illustrates the post-spike effects produced by a pair of CM cells recorded simultaneously with electrodes separated by 335 μm. Cell B facilitated 4 independent muscles while cell A facilitated two of these; the STA for a common target muscle, flexor digitorum sublimis (FDS), is shown in Fig. 1c,i. The cross-correlogram between these two cells, shown in Fig. 2a, has a central peak straddling the origin. The correlogram peak has a width of 14.8 ms and half-width of 2 ms, and area of 0.056.

To determine how much of the post-spike effect was mediated by synchrony between the cells, we compiled STAs only from spikes selected to eliminate the cross-correlogram peak. This was accomplished by rejecting any trigger spikes associated with spikes of the neighboring cell that would contribute to a central peak above baseline in the continuously updated correlogram. The traces in Fig. 1a,g show the cross-correlograms with the non-triggering unit, with and without spike selection (solid and dotted records, respectively). With the above-chance synchronous spikes removed, STAs of both cells still show PSF in the same muscles (shown for FDS in Fig. 1b,h); moreover, the shapes of the PSF were virtually identical (c.f. Fig. 1b,h

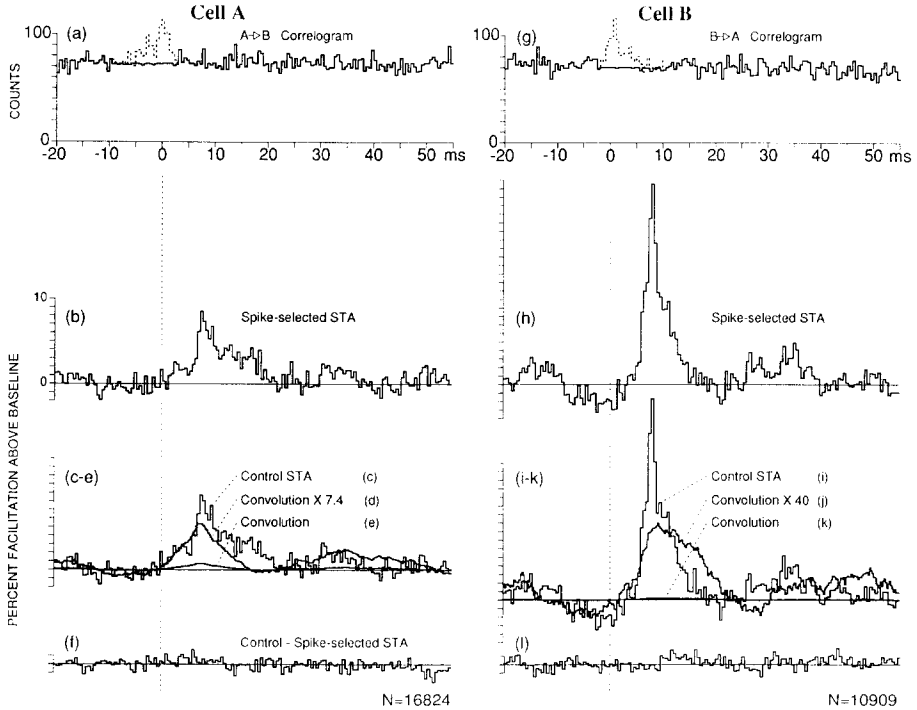

Fig. 1. Spike-selected STA and the expected contribution of synchrony to the STA by convolution analysis. a–b, g–h: spike-selected STAs of flexor digitorum sublimis (FDS) triggered from cell A (b) and cell B (h) and associated correlograms between cells A and B (a, g). The computer rejected any triggers for the correlogram and STA which would result in an above-baseline count to be added to the correlogram over the range of the correlogram peak. The baseline was defined as all bins outside of the peak range and was recalculated for each sweep. This produced STAs with the effects of synchronous spikes removed, as confirmed by the flat correlogram of the target cell (the dotted lines outline the peak that would have occurred without spike selection). c, i: STAs computed for spikes without selection (control) appear identical in time course. f, l: differences between control and spike-selected STAs. e: convolution of A→B correlogram with spike-selected STA_B, representing the component of STA_A expected from the synchronized output of cell B. Vertical scale of c and e are equal. d: convolution expanded 7.4 times (least-squares best fit), contrasting the early, broad component and sharp, late PSF. i, k: same analysis as in c, e with cell B as trigger. j: convolution expanded 40 times. Time zero indicates the time of cortical unit spike. STAs are scaled relative to baseline (first 20 ms of the trace); ordinate marks of b–f, h–l indicate 1% of baseline. N equals the number of sweeps. Traces were compiled with a 250 μs bin width, then adjacent pairs of bins combined for clarity.

with Fig. 1c,i). The differences between control and selected STAs are essentially flat (Fig. 1f,l). However, the magnitude of PSF (percent increase in mean of post-spike feature divided by the mean of the baseline) differed for the two conditions: the PSF produced in FDS declined from 12.76 to 12.71% (6.0–11.25 ms range) for cell B, and from 6.3 to 5.6% (6.0–10.0 ms range) for cell A. This reduction suggests that 0.4–11% of the observed PSF for this muscle is mediated by synchronous firing.

The contributions of synchrony between cells A and B to the STA from A (STA_A)

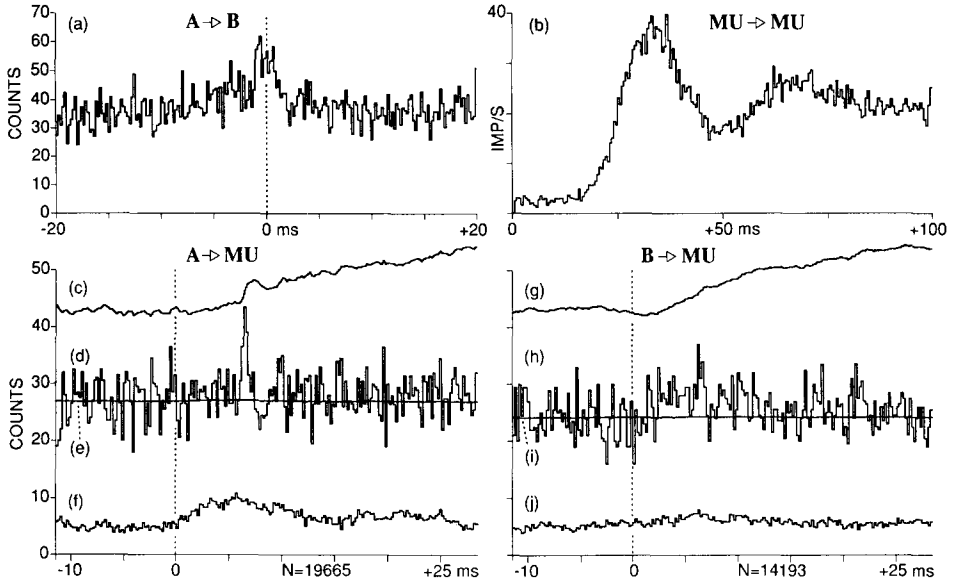

Fig. 2. Demonstration of direct convergence of two CM cells on the same motor unit and the contribution of synchrony to the single motor unit correlogram. a: cross-correlogram between CM cells A and B. b: autocorrelogram of the motor unit recorded in muscle FDS. c, g: cumulative sums of CM-to-motor-unit correlograms shown in traces d and h. Cell A and cell B are used as trigger for the traces c–f and g–j, respectively. Ordinate axis applies to traces d, e, h and i only. e: convolution of the A→B correlogram with the spike-selected B→MU correlogram. i: convolution of B→A correlogram with spike-selected A→MU correlogram. f, j: traces in e and i with vertical scale expanded 20 times. Correlograms are scaled in counts. N equals the number of sweeps. Bin width of trace b is 1 ms. Traces a and c–j were constructed using 100 μs bins, then adjacent pairs of bins combined for clarity.

can also be estimated by convolving the correlogram peak between cells A and B with the output from B, represented as STA_B. STA_A compiled in the control condition (i.e. no spike selection) and the convolution of the correlogram with STA_B (compiled with spike-selection) for FDS are drawn to the same scale in Fig. 1c and e, respectively. The mean of the post-spike feature of the convolution is only 10% of the PSF in STA_A. Drawing the convolution at a higher gain (Fig. 1d) shows that its shape differs from the control STA. STA_A appears to be comprised of two components: a broad shallow component from 0.0 to 20.0 ms and a sharp peak from 6.0 to 10.0 ms. The convolved average closely matches the broad component but fails to match the sharper peak. A similar result is obtained for cell B (Fig. 1i–k). Since the correlogram peak spans several bins, the contribution of correlated spikes to the STA will be a broadened version of the correlated cell's STA. The magnitude of the convolved average is approximately one seventh and one fortieth of the broad component of STA_A and STA_B, respectively. STAs with early onsets and no superimposed sharp components have been observed previously (called 'complex' averages in ref. 5), but these were not interpreted as causal effects of the triggering cell.

The effects of CM cells on motor units can be more accurately quantified by cross-

correlating activity of cells and single motor units, since correlograms are not confounded by potential non-linear summation of rectified EMG averages [10, 13]. Toward this end, a single motor unit was isolated from the FDS recording; the autocorrelogram of its spike train is shown in Fig. 2b. Cross-correlograms between the two CM cells and this motor unit (Fig. 2d,h) both show an increased motor unit firing probability centered 6.2 ms after the trigger. The k value of the correlogram peak (value of peak bin divided by baseline mean) is 1.7 and 1.5 for cells A and B, respectively, which agrees well with a 1.7 average value found for more distal muscles [10]. The cumulative-sums of each correlogram (Fig. 2c,g) exhibit inflections at 5.9 ms, but also show a gradual increase beginning near the trigger, consistent with CM cell synchronization. The widths of both correlogram peaks are less than the duration of the correlogram peak for the CM cells, so the brief features of 1 ms duration cannot be mediated by synchrony between the two cortical units.

The magnitude of the expected contribution of synchrony can be approximated by convolving the spike-selected CM-MU correlogram with the CM-CM correlogram. Fig. 2e,i shows this convolution at the same scale as the control correlogram; the convolution is essentially flat. Expansion by 20 times (Fig. 2f,j) reveals a broad peak. Its amplitude suggests that synchrony accounts for 2.9% and 1.5% of the motor unit facilitation from cell A and cell B, respectively. Since spike selection cannot eliminate synchrony from other, unobserved CM cells, these results are probably overestimates, and establish an upper bound to the contribution of synchrony from one cell.

Although the above analysis indicates that the contribution of *one* CM cell to the STA of a synchronized CM cell is negligible, the effect of synchrony among the entire colony of CM cells would be proportionately greater. The number of CM cells converging to any particular motoneuron can be estimated from intracellular studies: maximal corticospinal volleys produced CM-EPSPs on the order of 1–2 mV in rhesus motoneurons [6a, 15], and 2 mV in baboon forelimb motoneurons [2]. Assuming the mean amplitude of a unitary CM-EPSP to be 100 μV [1], one could surmise that about 10–20 CM cells converge on a forelimb motoneuron. If all pairs of a colony of 10–20 CM cells are synchronized with a probability of 0.061, each spike from a CM cell would be accompanied by an average of 0.6–1.2 spikes from some other members of the colony. Hamm et al. [7] reported that one correlated Ia spike occurring within 7.5 ms of each triggering spike increased the STA estimates of Ia-EPSPs in motoneurons by only 16%. For CM cells, correlated spikes are dispersed over approximately 21 ms. This would produce a correspondingly broad component of the PSF, with an early onset and a late offset, which would sum with the direct effect mediated by the triggering cell. Despite the presence of synchrony, the sharply rising, later component of PSF may still be interpreted as evidence for direct connection from the triggering cell to motor units.

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