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Corticomotoneuronal connections of precentral cells detected by post-spike averages of EMG activity in behaving monkeys

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The observation that activity of certain precentral motor cortex cells covaries consistently with contralateral muscles under different behavioral conditions may suggest a functional relationship, but cannot establish the existence of an anatomic connection[3,4]. Even those precentral cells with monosynaptic connections to motoneurons would be expected to generate unitary EPSPs too small to fire the motoneuron reliably[2,8,9]. However, the effects of such unitary EPSPs on the firing probability of an active motoneuron should, in principle, be statistically detectable by cross-correlation techniques. A convenient approximation to a true cross-correlation is the post-spike average, which is triggered from action potentials of the presynaptic neuron and preferentially summates those postsynaptic events correlated with that cell. By summing motoneuron membrane potentials following action potentials in Ia afferent fibers, Mendell and Henneman[7] first demonstrated that each Ia afferent fiber produces monosynaptic EPSPs in virtually all homonymous motoneurons. Similarly, post-spike averages have revealed monosynaptic connections to motoneurons from secondary afferents[6] and from inhibitory spinal interneurons[5], as well as intracortical connections between motor cortex cells[1]. While these studies summed intracellular membrane potentials, recorded in anesthetized animals, we have investigated the possibility that spike-triggered averages could detect the effects of monosynaptic corticomotoneuronal connections on the firing probability of motor units in alert, behaving monkeys.

Two rhesus monkeys (*Macaca mulatta*) were trained to flex and extend the wrist alternately against a programmed load; wrist position was tonically maintained for 1–2 sec to provide long periods of coactivation of precentral cells and agonist muscles. One monkey moved the wrist between two stops, the other between two electronically detected hold zones. EMG activity of forelimb muscles was recorded with either surface or implanted electrodes, or both. For 201 motor cortex cells whose activity covaried with wrist flexion or extension, post-spike averages were compiled with a Lab 8E computer. The full-wave rectified EMG activity was summed over a 30-msec analysis interval from 5 msec before to 25 msec after the cortical spike. Time bins were usually 250 μsec and at least 2000 events were averaged for each cell. A second type of average, the response average, was also compiled to document the covariation of cell and muscle

activity. The response average was triggered from movement onset and summed cell and muscle activity and wrist position in the 3-sec interval between 0.5 sec before and 2.5 sec after movement onset. Bin width was usually 10 msec and response averages included 50–100 responses.

Of the 201 precentral cells that covaried with the movement, 147 were not associated with any detectable spike-related change in the average level of EMG activity. For 54 cells the post-spike average exhibited a clear transient increase in mean EMG activity, which rose above pre-spike levels at latencies between 4.3 and 11.5 msec after the cortical spike, reached peak amplitude in 2–5 msec, and returned to pre-spike levels thereafter. Figs. 1–3 illustrate precentral cells that exhibited such a transient post-spike facilitation (PSF). Cell P4-6 covaried repeatedly with wrist extension (Fig. 1A); the response average indicates that this cell became active 30 msec prior to extensor EMG activity and maintained a high firing rate throughout the extension (Fig. 1B). The post-spike average shows a transient facilitation in the extensor EMG beginning 6.0 msec after the cortical spike, rising to a peak in 4.7 msec, and decaying back to baseline in 11 msec (Fig. 1C). Similarly, cell P69-1 covaried repeatedly with wrist flexion (Fig. 2A). The response average indicates an initial peak in unit firing in addition to sustained activity during the tonic hold (Fig. 2B). The post-spike average revealed a sharp PSF in the flexor muscles, with an estimated onset at 7.5 msec, rise time of 2.5 msec, and decay back to baseline in 4.5 msec. For both of these cells the EMG activity was recorded with surface electrodes.

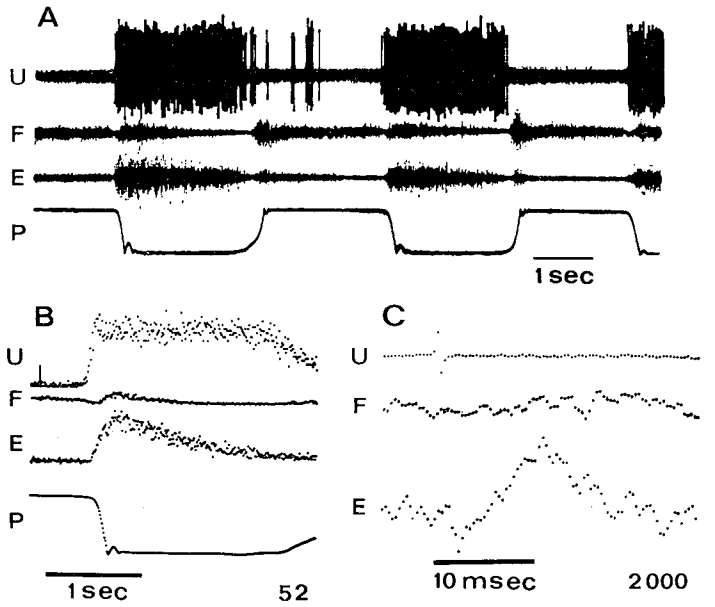

Fig. 1. A: activity of precentral unit P4–6 (U), surface-recorded EMG of wrist flexor (F) and extensor (E) muscles, and wrist position (P). With the hand held between plates hinged at the wrist, the monkey moved between stops at 20° flexion (upper limit) and 10° extension. B: response average triggered from 52 extension movements. Vertical bar = 20/sec. C: post-spike average triggered from 2000 action potentials of the cortical cell. Bin width = 333 μsec. In this and subsequent figures, EMG activity was full-wave rectified before averaging.

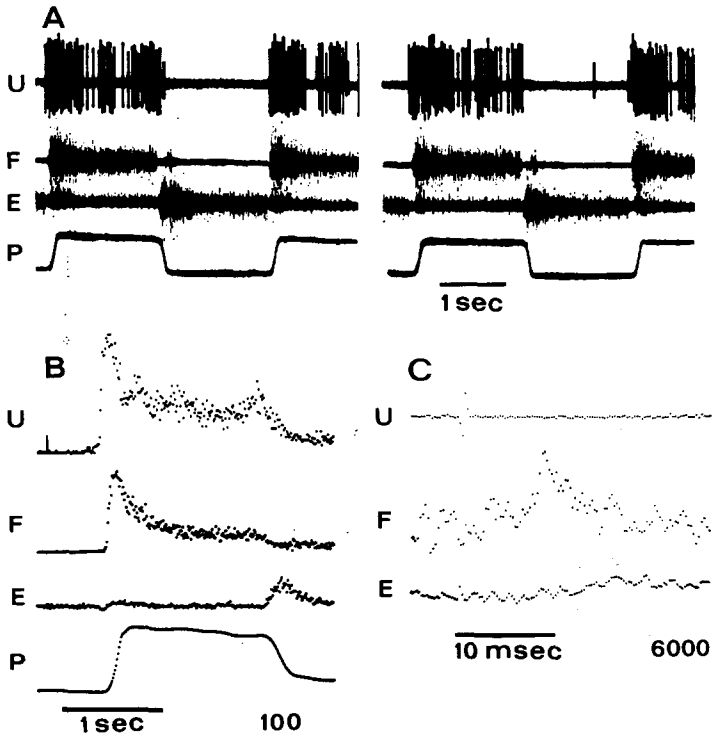

Fig. 2. A: activity of unit P69-1 and surface-recorded EMG. B: response average compiled for 100 flexion movements. Vertical bar = 10/sec. C: post-spike average triggered from 6000 action potentials. Bin width = 250 μsec. Abbreviations as in Fig. 1.

To determine whether motor units of more than one muscle could exhibit such post-spike facilitation we implanted pairs of EMG electrodes into specific synergistic wrist muscles.The precentral cell in Fig. 3 covaried with flexion and was followed by a PSF in each of the 3 implanted wrist flexors: flexor carpi radialis, palmaris longus and flexor carpi ulnaris; the simultaneously recorded surface EMG also exhibited a PSF. This post-spike average was compiled from 4165 action potentials which occurred during the tonic hold period — i.e., the averager was triggered only from spikes occurring 200 msec after the end of phasic flexion, to eliminate any preferential contribution from the onset of unit and muscle activity. (When spikes during phasic flexion were included, the post-spike averages looked quite similar except for larger peaks in PL and SURF.) To test whether the different EMG electrodes had recorded some motor units in common we triggered the averager from each EMG record and summed the rectified activity of all recorded muscles. Such averages showed a peak in the average of the EMG which provided triggers, due to summation of the triggering motor unit potentials, but no correlated peaks appeared in the averaged EMG activity of adjacent muscles; this indicates that the electrodes implanted in different muscles had recorded independent motor units. A correlated peak did appear in the surface EMG, indicating that the surface electrodes had picked up some units in common with implanted muscles,

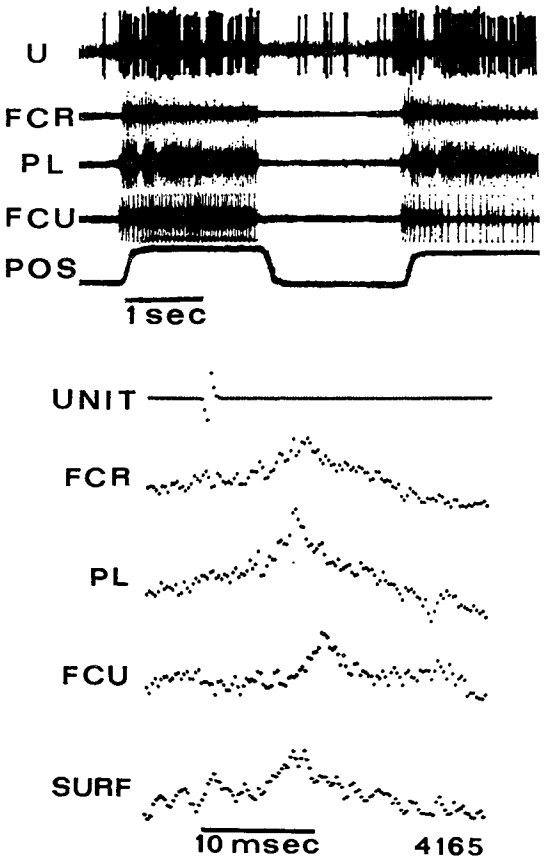

Fig. 3. Top: activity of unit P75-1 (U), flexor carpi radialis (FCR), palmaris longus (PL), flexor carpi ulnaris (FCU), and position (POS). EMG activity of each muscle was recorded with implanted bipolar electrodes. Bottom: post-spike average of unit and rectified EMG activity of the 3 implanted muscles and surface EMG (SURF). This average was triggered from 4165 spikes occurring during the tonic hold periods.

particularly PL. We conclude that this cell produced a PSF in at least 3 different wrist flexor muscles.

To test the descending projections of these cells a bipolar stimulating electrode was implanted in the pyramidal tract (PT) of the first monkey. Sixty-five cells were tested for antidromic responses to PT stimulation by frequency following and/or collision test; the proportion of cells exhibiting PSF was higher for PT cells (4/35) than for non-PT cells (1/30). Finding a low proportion of wrist-related cells exhibiting PSF (23/119), we suspected that the stimulating electrode might have severed the axons of some PT cells. In the second monkey, with no PT electrode, post-spike averages were compiled for 82 cells covarying with wrist movements; of these, 31 showed clear PSF in one or more muscles. The larger proportion of PSF cells may also be due to the fact that in the second monkey we preferentially sampled precentral cells which covaried strongly with wrist movement and also sampled more muscles with each cell.

The latency and time course of these post-spike facilitations are entirely consistent with the expected effect of monosynaptic corticomotoneuronal connections. If the PSF is mediated by such a connection, its latency would represent the sum of conduction times along corticospinal and motoneuron axons, plus two synaptic delays. The latencies of monosynaptic EPSPs evoked in cervical motoneurons by cortical stimulation have been reported to be between 2.0 and 4.5 msec[2,8]; the conduction time from motoneurons to wrist muscles would be expected to range from 2 to 5 msec. The observed latencies of the PSF ranged from 4.3 to 11.5 msec. The rise time and decay of PSF are also consistent with the known time course of cortically evoked monosynaptic EPSPs[2,8,9]. Of the post-spike averages that showed any transient modulation of EMG activity, virtually all showed a facilitation. The simplest explanation of these results is that the enhanced post-spike probability of motor unit firing was mediated by monosynaptic excitatory connections between the cortical cell and motoneurons of the recorded muscles. The shape and magnitude of the observed PSF would depend on several variables, including the size of the unitary corticomotoneuronal EPSPs, the number of recorded motor units affected, the tonic firing rate of these units, and the shape of the recorded motor unit potentials. Although unitary EPSPs are likely to be 100 μV or less[7,9], their effects on the firing probability of active motoneurons would appear to be statistically detectable in spike-triggered averages of 2000 events.

Although post-spike averages of the firing probability of the postsynaptic cell require greater ensemble averages to detect the effect of a connection than averages of postsynaptic potentials, they have the advantage of being observable in behaving animals, for cells whose covariation with behavioral responses can also be documented. In this study, the cells exhibiting a PSF covaried consistently with either flexion or extension, rarely with both. The activity of most cells began 20–60 msec prior to onset of EMG activity in muscles showing PSF. Many cells were driven by passive wrist movements in a direction opposite the active movement which involved the cell, i.e., passive movements that stretched the muscles exhibiting PSF.

Assuming the PSF is mediated by monosynaptic connections, the post-spike averaging technique could be used to establish the extent of divergent terminal connections of single PT cells to motoneurons of different muscles. With this aim we compiled post-spike averages for 111 cells with at least 5 covarying wrist muscles. Of these cells, 41 were followed by PSF in one or more muscles; 10 were followed by PSF in more than half of the recorded muscles. Thus, the set of muscles whose motoneurons are contacted by a corticomotoneuronal cell — called the cell's 'muscle field'[3] — may include at least several synergists of one joint. The activity of such motor cortex cells would facilitate a group of muscles involved in a given movement. Other precentral cells (14 of 41) were followed by PSF in only one of the 5 recorded muscles, suggesting a more restricted muscle field. The activity of these cells would preferentially affect specific muscles. Many of the negative results were obtained with post-spike averages of 2000 events; it is possible that larger ensemble averages could have revealed some PSF in additional muscles.

In summary, the post-spike averaging technique appears to be capable of establishing the existence and extent of terminal connections of individual corticomoto-

neuronal cells in behaving monkeys. The covariation of such corticomotoneuronal cells with behavioral responses can also be independently documented.

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