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J. Physiol., Paris, 1978, *74,* 239-245
Symposium Pyramidal micro-connexions
and motor control.
Marseille, July 1977.

Muscle fields of primate corticomotoneuronal cells*

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SUMMARY :

1° The effects of single precentral cortex cells on the activity of identified forelimb muscles were documented by spike-triggered averages (STA) of rectified EMG activity. Some averages revealed a transient post-spike facilitation (PSF), with a latency and time course consistent with the expected effects of monosynaptic corticomotoneuronal (CM) connections.

2° For 216 cells STA's were compiled for five or six independent forelimb flexor or extensor muscles. The action potentials of 103 cells were followed by PSF in either one muscle (n = 32) or two (n = 34) or more (n = 37).

3° The independence of the EMG recordings was confirmed by averages triggered from the EMG of specific muscles ; these revealed negligible pickup of the same motor units by leads in adjacent muscles.

4° The synchronization of cortical cells was tested by cross-correlating neighboring pairs of strongly covarying cells. No evidence of spike synchronization of covarying cortical cells was found in the cross-correlograms or in averages of rectified spike activity. Thus, the distribution of PSF in multiple muscles could not be due to redundant motor unit recording or to synchronization of cortical cells.

5° Defining the cell's « muscle field » as the set of muscles which exhibit PSF, these results suggest that over two-thirds of cells showing PSF have muscle fields including more than one synergistic muscle. If the PSF is mediated by monosynaptic CM connections, the terminals of most CM cells appear to be distributed to motoneurons of several muscles.

Key-words : Motor cortex. Corticomotoneuron. Spike-triggered average. Cross-correlation. Monkeys.

* Reçu le 5 septembre 1977 et accepté en première ecture.

** This research was supported by grants RR00166, NS5082, NS12542 and NS0966 from the National Institute of Health, U.S. Public Health Service.

INTRODUCTION

The functional organization of connections between precentral corticomotoneuronal (CM) cells and motoneurons of specific forelimb muscles is fundamental to understanding the cortical control of limb movement. We know considerably more about the convergence of colonies of CM cells to individual motoneurons than about the divergence of single CM cells to different motoneurons. By electrically stimulating cortex and recording EPSP's evoked in primate motoneurons, PHILLIPS and colleagues (CLOUGH *et al.,* 1968 ; LANDGREN *et al.,* 1962 ; PHILLIPS and PORTER, 1964) and JANKOWSKA *et al.* (1975) have demonstrated that motoneurons receive monosynaptic input from colonies of CM cells distributed over wide and overlapping cortical areas. The extent to which such CM cells may send divergent terminal connections to motoneurons of multiple muscles remains largely unresolved by standard stimulation and ablation techniques, which affect many cells. Recent electrophysiological evidence that single PT cells in the cat may be antidromically activated from several spinal segments (SHINODA *et al.,* 1976) confirms the possibility that such PT cells may influence diverse groups of segmental spinal cells. We have recently used spike-triggered averages (STA's) of rectified EMG activity to detect the effects of single cortical cells on the firing probability of covarying motor units (FETZ *et al.,* 1976 ; FETZ and FINOCCHIO, 1975). Such STA's sometimes reveal a clear, transient post-spike facilitation (PSF) of activity in one or more covarying forelimb muscles. The latency and time course of such PSF are consistent with values expected to result from monosynaptic CM connections. We report here further evidence concerning the extent to which EMG averages triggered from single precentral cells reveal PSF in different forelimb muscles, and evidence concerning the possible mediation of such PSF's by monosynaptic CM connections of the recorded cells.

TECHNIQUES

Four rhesus monkeys (*Macaca mulatta*) where trained to alternately flex and extend the wrist against programmed spring-like loads. These movements included a static hold period of 1-2 sec in electronically detected hold zones, to provide long periods of tonic coactivation of cell and muscles. The monkey's right hand was held with fingers extended between padded plates, to optimize separate activation of flexor and extensor muscles. Activity of up to 12 identified forelimb muscles (6 flexors and 6 extensors of wrist and fingers) was monitored with implanted pairs of EMG wires. In early experiments wires were surgically implanted into specific muscles and led subcutaneously to a terminal on the skull. More recently we have injected pairs of wires transcutaneously with hypodermic needles into muscles identified (1) by their relative anatomical location, and (2) by characteristic responses to electrical stimuli ; these wires were held in place with adhesive tape and led to a terminal plug taped to the upper arm. Such electrodes were well tolerated and provided stable recording for over four weeks.

Activity of precentral cortex cells was monitored during active wrist movements and STA's compiled for those cells which covaried with flexion and/or extension. If STA's of 2,000 sweeps or less showed evidence of PSF in any muscle, we recorded cell and muscle activity as well as position and torque on a 16-channel FM tape recorder. STA's could also be computed off-line ; these usually resembled the averages compiled on line. For a given cell, STA's compiled from separate samples were usually similar in shape and muscle distribution. Most STA's were computed for the 30 msec period from 5 msec preceding to 25 msec following the spike ; bin width was typically 250 μsec. Analysis periods longer than 30 msec (*e.g.,* — 40 msec to + 60 msec) rarely revealed any additional features in the STA comparable in size to the PSF ; since longer averages took considerably more time to compile, we chose the 30 msec period for detecting PSF.

RESULTS

1) *Post-spike facilitation revealed by spike-triggered averages.*

The cell in Fig. 1 illustrates many of the results and the controls employed. This unit was recorded simultaneously with three implanted wrist flexor muscles [flexor carpi radialis (FCR), palmaris longus (PL) and flexor carpi ulnaris (FCU)] and with flexor muscle activity recorded through surface electrodes (SURF). Response averages aligned on phasic flexion movements revealed the average muscle and unit activity during successive flexion movements (Fig. 1B). Activity of this unit exhibited a pronounced phasic peak at flexion onset, followed by a gradually increasing ramp of activity during the hold period. Such a « burst-ramp » pattern was typical of 8 % of CM cells ; more often, CM cells fired tonically during the hold period at rates at or slightly below the dynamic component (cf. FETZ *et al.,* 1976, Figs. 1 and 2). For this cell the STA of rectified EMG activity computed for 6,000 spikes (Fig. 1D) reveals a PSF in each muscle.

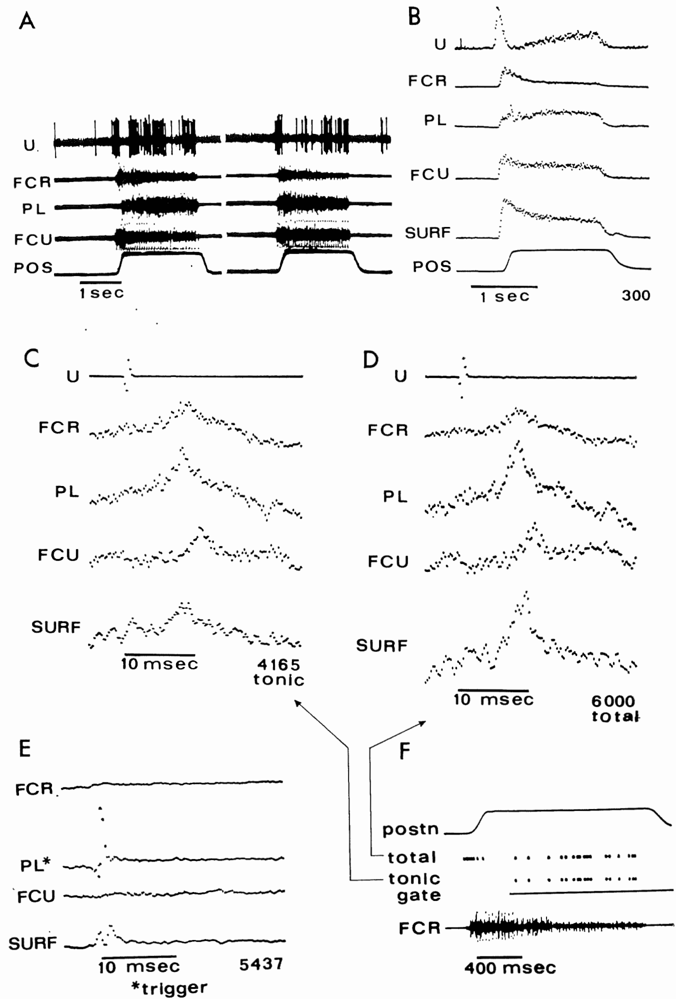

FIG. 1. — *Precentral cell with PSF in three wrist flexor muscles.*

A : Examples of two flexion responses, showing EMG of flexor carpi radialis (FCR), palmaris longus (PL) and flexor carpi ulnaris (FCU), as well as position (POS).

B : Response average of unit activity (U), and full-wave rectified EMG activity of implanted flexor muscles and surface EMG (SURF) (300 responses). Analysis period, 3 sec ; bin width, 20 msec.

C : Spike-triggered average of rectified EMG activity for 4 165 spikes occurring during tonic holds. Analysis period, 30 msec ; bin width, 250 μsec.

D: STA for 6 000 spikes from both phasic movements and tonic holds.

E : Average of rectified EMG activity triggered from motor units in PL ; same parameters as STA's ; N = 5437.

F : Single trial illustrating gating signal which began 200 msec after the end of phasic movements, used to gate pulses for STA in C.

Onset latency was slightly longer for FCU (8.8 msec) than for FCR (6.0 msec) or PL (6.8 msec), perhaps due to different motoneuron conduction times.

To determine whether appearance of the PSF depended on the phasic activity occurring during movement onset, we compiled separate STA's using only action potentials occurring 200 msec or more after the phasic peak. The single trial in Fig. 1F illustrates the gating of pulses from spikes occurring during the tonic hold ; such pulses were used to compile the STA in Fig. 1C. The resemblance between the STA's in Fig. 1C and 1D suggests that the PSF involved motor units active during the tonic hold, and was not an artifactual consequence of averaging non-stationary activity at movement onset. The fact that the peak for PL and SURF is higher in Fig. 1D than in Fig. 1C may be due to contributions of additional motor units active only during the phasic movement or to a greater efficacy of high frequency cell activity in producing PSF.

A trivial way in which PSF could appear in recordings from « several muscles » might be redundant recording of the same motor units through different EMG electrode pairs. This possibility was routinely rested in all experiments by computing EMG-triggered averages. As shown in Fig. 1E, when the EMG averages were triggered from motor units in PL the surface-recorded activity reveals a peak simultaneous with the triggering peak in PL, suggesting pickup of some units in common. However, there was no sign of a comparable peak in the average of either of the two adjacent muscles, FCR or FCU. Similarly, averages triggered from motor units in FCR and FCU showed no evidence of correlated peaks in the average activity of the other two implanted muscles. This confirms that the implanted leads had indeed recorded independent motor units. We conclude that the spikes of this unit were followed by facilitation of activity in motor units of three separate wrist flexor muscles.

Figure 2 illustrates a second precentral cortex cell, which covaried with wrist extension and was recorded with six extensor muscles of wrist and fingers. STA's of 4,600 events revealed a strong PSF in extensor digitorum communis and extensor digitorum 4 and 5, which began at 4.8 and 4.0 msec respectively and peaked at 9.5 and 8.5 msec after the cortical spike. In addition, the STA's of extensor carpi radialis longus and extensor digitorum 2 and 3 exhibit evidence of a weaker PSF. The STA's of the remaining two muscles showed no convincing evidence of any facilitation.

2) *Cross-correlation of covarying precentral cells.*

It has been suggested that the appearance of PSF in multiple muscles may be mediated by synchronized activity in multiple CM cells, each of which produces PSF in a subset of the affected muscles. We have sought evidence for synchrony of spikes in pairs of adjacent precentral cells whose activity covaried strongly. Such covarying motor cortex cells were usually driven by similar passive movements and fired during the same active movements. In the course of previous experiments, twelve such pairs of strongly covarying cells were recorded from the same microelectrode and separated on the basis of action potential wave form. They were thus within a few hundred microns of each other and likely to share common

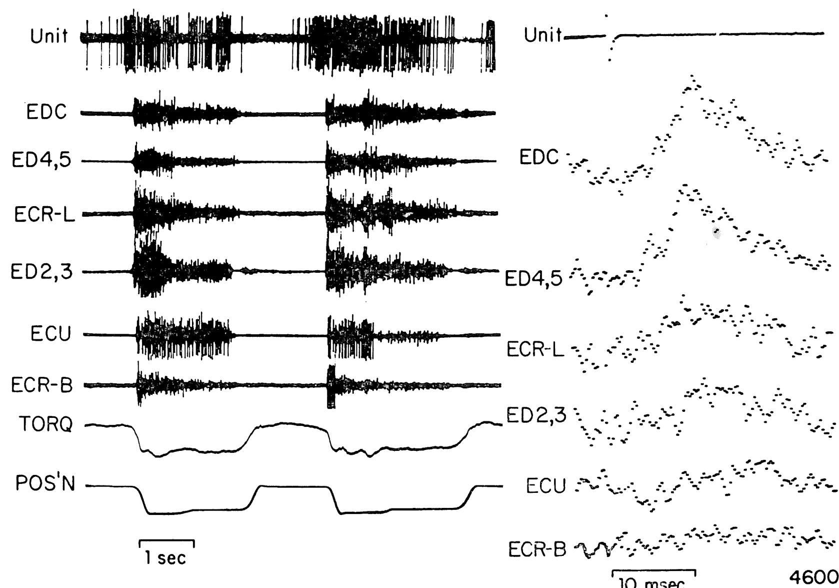

FIG. 2. — *Responses (left) and spike-triggered averages (right) for precentral unit covarying with wrist extension.*

Muscle activity was recorted with pairs of wires implanted into extensor digitorum communis (EDC), extensors digitorum 4 and 5, and 2 and 3 (ED4, 5 and ED2, 3), extensor carpi radialis longus and brevis (ECR-L and ECR-B), and extensor carpi ulnaris (ECU). Bin width for STA, 250 μsec ; N = 4 600.

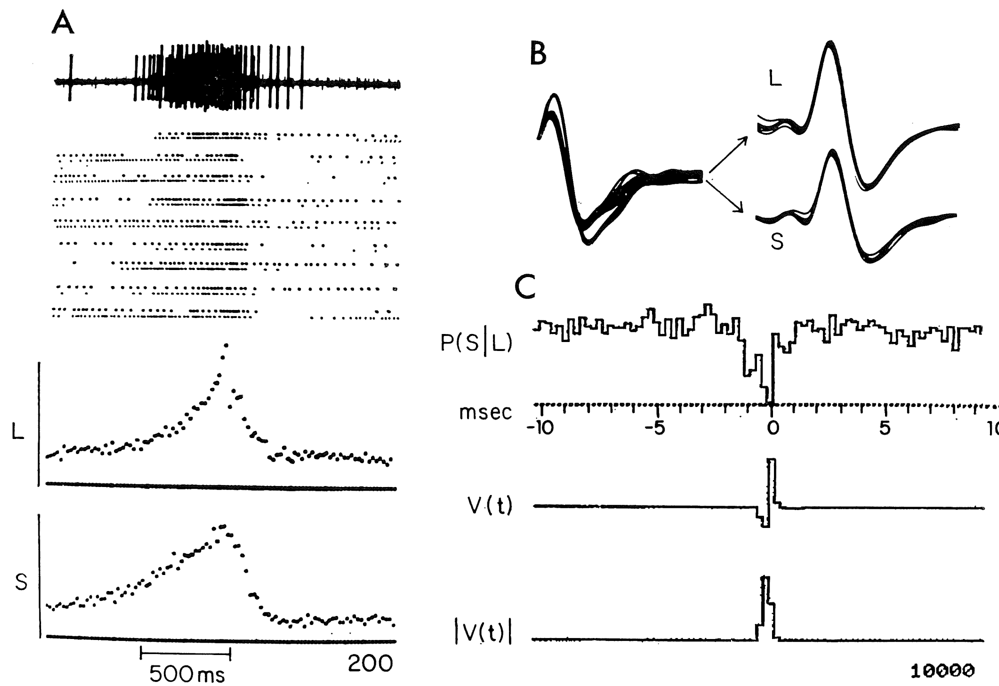

FIG. 3. — *Cross-correlation of strongly covarying precentral cells.*

A : Examples of large (L) and small (S) units during successive operant bursts of the large unit. Double dot rasters show spikes of large (upper) and small (lower) units ; response average of 200 bursts at bottom. The covariation of these cells in successive minutes of an operant conditioning session is shown in Fig. 6A of FETZ and BAKER (1973).

B : Superimposed action potentials of large and small cell, illustrating spike separation.

C : Cross-correlogram shows probability, P(S|L), of small unit firing given a spike in large unit ; except for a dip near zero caused by refractoriness of spike separator, P(S|L) is flat for ± 10 msec, (N = 10,000 events.) V(t) is average of recorded unit spikes, and | V(t) | gives average of rectified unit activity.

connections. Examples of two such pairs are illustrated in Fig. 3 and 4. The two cells in Fig. 3 were recorded while burst activity of the large unit was operantly conditioned (FETZ and BAKER, 1973). Both units responded to passive ankle flexion and covaried consistently during successive minutes of the operant conditioning session (FETZ and BAKER, 1973, Fig. 6A). Operant bursts of the large cell were consistently associated with bursts of the small cell (Fig. 3A). Cross-correlating these units by triggering an averager from the large unit and summing simultaneous activity of the small unit revealed no evidence of a peak indicative of spike synchronization (Fig. 3C). The brief reduction in the correlogram within 1 msec of zero is an artifactual consequence of the refractoriness of the spike separator. Evidence of spike synchronization during this brief interval was sought by summing both unrectified and rectified microelectrode potentials. The average unrectified potential —V (t) — represents the action potential of the large unit, which, triggered the averager. The average rectified voltage — |V(t)| — would also include the action potentials of the small unit ; if these consistently occurred synchronously with the large unit they would contribute to the rectified peak near zero. The fact that the two averages have equal width, even at much higher gains, suggests the absence of appreciable small unit synchronization. Evidence of synchrony was further sought by directly inspecting superimposed oscilloscope sweeps triggered from both action potentials ; these revealed negligible superposition of spikes (Fig. 3B).

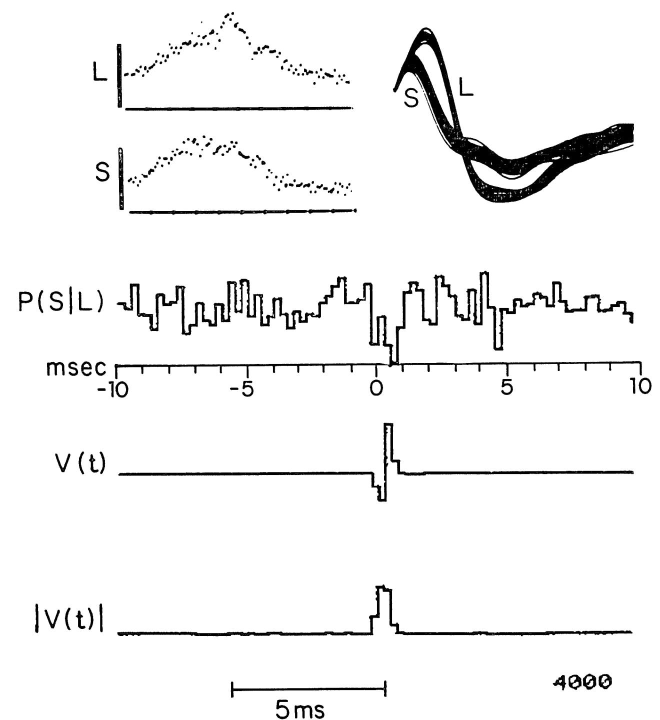

Fig. 4. — *Cross-correlation of adjacent precentral cells which covaried consistently during isometric muscle contractions.*

Top left shows response averages during isometric ECR contraction ; the covariation under other conditions is illustrated in Fig. 9 of FETZ and FINOCCHIO (1975). Cross-correlogram P(S | L), as in Fig. 4.

A second pair of cells that covaried consistently, recorded during operant conditioning of isometric muscle activity (FETZ and FINOCCHIO, 1975, Fig. 9), is shown in Fig. 4. Both units responded to passive elbow extension. The cross-correlogram of these cells reveals no evidence of a peak. Twelve pairs of strongly covarying precentral cells previously recorded were similarly cross-correlated ; none could be shown to exhibit any evidence of spike synchronization.

3) *Muscle fields of corticomotoneuronal cells.*

The relative number of muscles exhibiting PSF is plotted in Fig. 5 for 216 precentral cells recorded and averaged with five or six different synergistic forelimb muscles. This histogram represents a sample of precentral units biased in favor of cells with response properties characteristic of CM cells — namely, they covaried stongly with flexion or extension movements. Earlier experiments had included more cells that covaried weakly or variably with wrist movement, or exhibited only phasic responses or fired with both flexion and extension ; such cells generally showed no evidence of PSF. Other factors potentially contributing to a lower proportion of PSF cells in early experiments were the presence of a PT electrode and sampling of fewer muscles. Of the cells in Figure 5, recorded with five to six implanted synergists, for which STA's of at least 2,000 events were compiled, less than half (48 %) were followed by PSF in any muscle. The threshold for acceptability as a PSF is illustrated by the weak PSF for extensors digitorum 2 and 3 in Fig. 2. Of the cells that exhibited a PSF, two-thirds were followed by PSF in two muscles or more ; the mean number of muscles affected was 2.3.

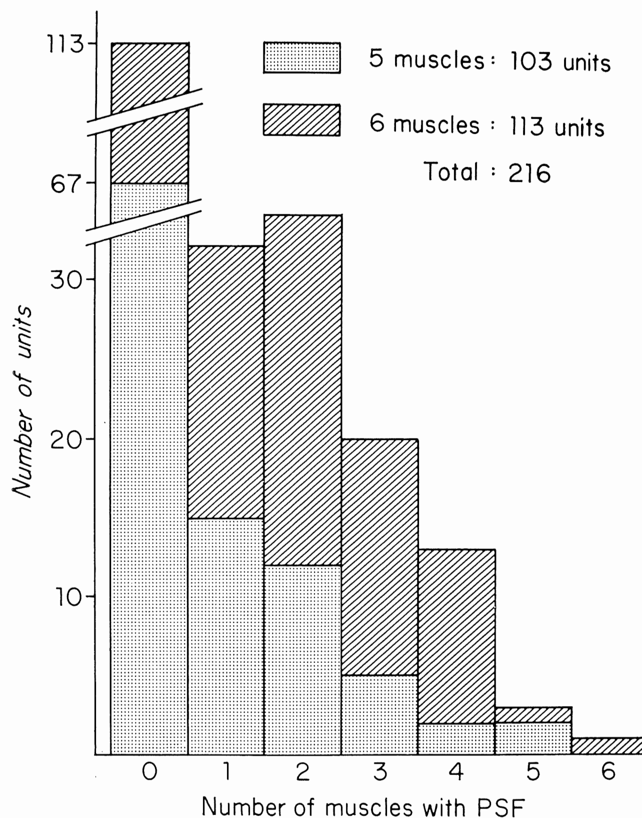

FIG. 5. — *Number of precentral cortex cells showing PSF in indicated number of synergistic wrist muscles.*

For these cells STA's of at least 2,000 events were compiled for five or six covarying wrist muscles. Both strong and weak PSF's are included.

DISCUSSION

1) *Is the post-spike facilitation produced by a monosynaptic corticomotoneuronal connection ?*

The feature in STA's of rectified EMG activity that we have called post-spike facilitation (PSF) is a transient increase in average EMG activity, *i.e.,* a rise to a peak followed by decay back to prespike baseline. Such a pattern would not be produced simply by summing nonstationary EMG activity, *e.g.,* at movement onset. Preferential sampling when muscle activity is increasing would produce a steadily rising level of average EMG activity throughout the analysis interval ; this was occasionally observed, but never counted as a PSF. In most cases the PSF's also had a clear onset time after the cortical spike ; of 233 cases, the average onset latency was 7.1 ± 3.3 msec (mean ± standard deviation). If such PSF's are mediated by monosynaptic CM connections, their onset latency would represent the sum of the conduction times from cortical cell to motoneurons [2.0-4.5 msec (CLOUGH *et al.,* 1968 ; LANDGREN *et al.,* 1962 ; PHILLIPS and PORTER, 1964)] and from motoneuron to muscle fiber [2.0-5.0 msec (CLOUGH *et al.,* 1968)] plus two synaptic delays. The observed rise (3.2 ± 1.8 msec) and decay times (7.0 ± 3.4 msec) would be functions of the number of motor units affected, the shape of their muscle fiber potentials, the time course of the CM EPSP's and the dispersion of conduction times.

Of all the pathways that might result in PSF, a monosynaptic CM connection would probably produce the strongest PSF. We would expect such a connection to make the largest contribution to the conditional probability, P (M | C), that the motor unit (M) fires given a spike in the cortical cell (C). The relative contribution of disynaptically mediated effects would be expected to be smaller, since the intercalated neuron would itself be statistically affected and would in turn have a comparably small effect on the motoneuron. Thus, if cortical cell C contacts interneuron A, which in turn contacts motoneuron M, the conditional probability that the motoneuron fires given

a spike in C is the product of two conditional probabilities :

$$P(M|C) = P(M|A) \cdot P(A|C)$$

In other words, the probability of observing a disynaptically mediated effect is of the order of the square of the probability of detecting a monosynaptically mediated effect. The same relationship would pertain if A were a corticomotoneuronal cell with a collateral connection to recorded cell C, which may be non-CM. Such second-order correlations would make relatively smaller contributions. Still smaller effects would be produced by third order correlations, such as a common input to CM cell A and recorded cell C, or trisynaptic mediation.

The strong PSF's which appeared in averages of 2,000 events or less, and which had sharply rising slopes, were very likely mediated by monosynaptic CM connections. The effects detected in averages of 2,000 events were virtually always facilitation. Some PSF's had shallow onset and smaller amplitude (*e.g.,* ED2,3 in Fig. 3) ; we believe that such weak PSF's may have been produced by weak monosynaptic connections ; however, it is conceivable that they may also have been mediated by strong second-order correlations. Averaging another order of magnitude more events (ca. 20,000) sometimes revealed more subtle effects in additional muscles — usually post-spike facilitation ; occasionally post-spike suppression of EMG activity was also observed, particularly in rare records that included activity of antagonistic muscles. Such very weak effects may have been mediated polysynaptically.

2) *Factors affecting the estimation of muscle field.*

The extent to which PSF appears in different muscles would define the extent of the cell's physiological muscle field. The observed distribution of PSF in different muscles could overestimate the actual muscle field of individual cells (1) if different EMG leads had recorded motor units in common or (2) if spikes of CM cells were highly synchronized. The possibility of redundancy in EMG records was directly tested by cross-correlating muscle activity. In all cases included here, EMG-triggered averages revealed no evidence that adjacent leads had recorded the same units. A few examples of common pick-up were detected and such redundant records were eliminated from the data base. Furthermore, the EMG leads were confirmed to be in different muscles by direct observation during implantation and by characteristic wrist and finger movements evoked by electrical stimulation. Thus, the distribution of PSF — and hence the muscle field — is not artifactually exaggerated by redundant EMG recording.

The possibility that CM cells with restricted muscle fields fired so synchronously that triggering from one could reveal effects mediated by the other would require a very high degree of spike synchrony ; a sufficiently large proportion of spikes would have to occur within a few milliseconds of each other to account for the brief PSF observed. This would require exceptionally potent synaptic connections, which have never been detected in intracellular recording of cortical cells (ASANUMA and ROSEN, 1973) but remain conceivable. No evidence for sharp peaks in the cross-correlogram of strongly covarying adjacent cortical cells could be obtained in this study. Such cell pairs would be most likely to exhibit synchronization, since they would be most likely to share common inputs or be synaptically interconnected (ASANUMA and ROSEN, 1973). Thus, spike synchronization does not appear to be sufficiently strong to produce artificially broad distribution of PSF in different muscles.

On the other hand, the observed distribution of PSF could easily underestimate the true muscle field if a significant proportion of correlated motor units in these and additional muscles were not recorded. The overall proportion of spike-triggered EMG averages that exhibited PSF was 20 % (233 cases in 1,193 cell-muscle pairs). Since the EMG leads sampled only a small fraction (perhaps 10 %) of the total number of motor units in the muscles, they may well have missed detecting PSF in unrecorded units. Thus, some of the « non-PSF » muscles might have exhibited PSF with more complete sampling of motor units. To this should be added the possibility that unrecorded muscles might have had correlated units. Thus, the muscle field of these CM cells is probably larger than the number of PSF muscles observed in these limited EMG samples. An interesting, unresolved issue is whether some CM cells might produce PSF in muscles of other joints.

3) *Functional significance of muscle field.*

The physiological muscle field was operationally defined as the set of muscles exhibiting PSF. If the PSF is mediated by monosynaptic CM connections, as seems probable for at least the strong PSF's, the physiological muscle field would also be the anatomical muscle field, *i.e.,* the muscles with motoneurons contacted by terminals of the CM cell. It may well be that some anatomical connections are too weak to be detected by STA's of firing probability. At this point the degree to which the distribution of PSF gives a realistic estimate of the anatomical muscle field remains debatable.

These results bear on the long-standing issue of whether movements or muscles are « represented » in

precentral motor cortex. Insofar as this question can be answered for individual CM cells, these results suggest that those cells with restricted muscle fields — consisting of single muscles — would directly affect only one muscle and no others. Although activity of such a cell may covary with additional synergistic muscles during many movements, its activity would have no direct facilitatory effect on those muscles. Of the CM cells recorded with five or six muscles, 31 % exhibited PSF in only one muscle. However, about two-thirds of the CM cells had muscle fields including more than one synergistic muscle. Activity of such CM cells would clearly affect a group of muscles involved in a movement. It seems likely that more extensive sampling of muscle activity may reveal a greater proportion of CM cells with multiple muscle fields.

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