PROPERTIES OF SINGLE CELLS IN VERTEBRATE MOTOR SYSTEMS REVEALED BY SPIKE-TRIGGERED AVERAGING

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Reporter: *Richard Martin*

Participants: *Elwood Henneman, Lorne Mendell, Richard B. Stein, Douglas G. Stuart*

Additional Participants: *Paul Cheney, Hans-R. Lüscher, John Munson, William Roberts, George Sypert, Anthony Taylor, and Victor Wilson*

Elwood Henneman *(Harvard Medical School, Boston, Massachusetts)*, introduced the subject of spike-triggered averaging (STA) by referring to his original study with Lorne Mendell (Mendell and Henneman, 1971), which employed the technique to investigate individual EPSPs produced by impulses in single Ia afferent fibers in homonymous gastrocnemius motoneurons. Since most individual EPSPs were too small to distinguish from synaptic and electrical noise, it proved useful to average several hundred EPSPs with a computer, triggered from the action potentials of the Ia afferent fiber. The amplitudes and time courses of these averaged EPSPs suggested that most Ia synapses were located at specific sites on the motoneurons. This study also revealed that each single Ia afferent distributed terminals to virtually all homonymous motoneurons.

Dr. Henneman reviewed the diverse studies in which spike-triggered averaging is now being applied, referring to the work of the speakers in the symposium. He closed with a word of caution to those who would use the technique to be certain that the trigger events used to compile averages are not synchronized with any other unwanted signal, such as 60 cycle noise.

Richard B. Stein *(University of Alberta, Edmonton, Alberta, Canada)* discussed the use of STA to investigate the electrical and contractile properties of motor units in human muscles, in particular, the first dorsal interosseous muscle of the hand. Dr. Stein reviewed several possible methodological problems which require attention in such studies. STA of force triggered on the action potentials of single motor units revealed the average increase in force correlated with that single unit. Under certain conditions, such averages could

be interpreted as the mean twitch tension developed by the single motor unit supplying the triggering spike. To be confident of this interpretation, Dr. Stein stated that one must eliminate several potentially confounding situations. First, the twitch-tension average should not be contaminated by later contributions from the same single motor unit due to action potentials following the triggering event within the analysis interval. This possibility can be tested by compiling a histogram of interspike intervals between the motor unit's action potentials. Dr. Stein indicated that the time-course of the averaged tension was a valid record of the twitch tension if it was short compared to the briefest interspike intervals. A second potential problem was the possibility of recording triggering spikes from more than one single motor unit. This could be recognized by differences in action potential waveform, and presence of abnormally brief intervals. A third potential problem is synchronization of action potentials from different motor units. To test for synchronization, it is useful to compile STA of surface EMG recordings with and without full-wave rectifications. If no motor unit synchronization occurs, then averaging the unrectified surface EMG reveals the contribution of the single triggering motor unit to the surface record. The use of AC recording prevents any net contribution from other units. On the other hand, when the full-wave rectified surface records are averaged, other units can make a new contribution to the averages. This contribution was found to be about 0.8 times the root mean square voltage of the surface EMG (Milner-Brown *et al.*, 1973). If the discharge of several motor units is synchronized, there will be a larger increase in the rectified signal than can be accounted for by these contributions from nonsynchronized units. Twitch-tension averages would tend to be erroneously large in averages triggered from units which were synchronized with other units, so these were eliminated from the data base.

The twitch tensions of motor units determined by STA showed a systematic relation to threshold force at which they became active. Dr. Stein observed that individual motor units were recruited in order of size of twitch tensions in agreement with Dr. Henneman's "Size Principle". In addition, a correlation was also noted between a motor unit's speed of contraction and the amount of force it developed. Motor units with fast twitch times typically produced large forces of contraction, while slow twitch units produced relatively smaller forces.

However, in patients who severed their ulnar nerves, Dr. Stein and his colleagues did not observe an orderly pattern of recruitment after muscles had been reinnervated. To systematically examine this phenomenon in a preparation more amenable to experimental manipulation, Dr. Stein's group studied the cat's gastrocnemius muscle. A cuff containing chronic nerve electrodes was implanted on the

sciatic nerve, and both EMG activity and contractile forces were monitored. Single motor units were stimulated via a needle electrode, care being taken to isolate the effective stimulation to a single unit. All-or-none thresholds to stimuli and a clear unitary twitch development were taken to indicate a single unit activation. The sciatic nerve electrode recorded compound spike activity if the needle electrode placement was too near nerve twigs to give pure motor unit activations. With this preparation, the averaged single motor unit spike and twitch tension could be correlated to assess changes in motor unit-tension relationships induced by reinnervation.

In summarizing, Dr. Stein stressed that STA methodology should be supplemented with other techniques, such as EMG recordings and single motor unit-stimulation, to confirm that the correlations shown by STA are due to real phenomena and not to synchronized artifactual events.

Lorne Mendell *(Duke University, Durham, North Carolina)* discussed the use of STA to estimate connectivity of Ia afferent fibers to motoneurons. The connections between presynaptic and postsynaptic elements typically display widespread divergence of presynaptic afferent input to many postsynaptic neurons as well as extensive convergence of many afferents onto single motoneurons. To analyze this situation at the Ia motoneuron synapse mediating cat hindlimb monosynaptic reflexes (typically involving about 60 Ia fibers and 300 motoneurons), Dr. Mendell and his co-workers have used the STA technique to examine averaged EPSPs produced by single Ia afferents in single motoneurons. Analysis of averaged EPSP amplitudes and projection frequencies (*i.e.*, the number of motoneurons which receive input from a single Ia afferent) is possible, as is determination of the characteristics of EPSP time course such as the rise time (time to peak) and the half-width (duration of half amplitude).

This utilizes Rall's analysis (Rall, 1967) indicating that an excitatory synapse on the dendrites of a neuron produces an EPSP with slower rise time and half-width than did the same synaptic current on the soma, as recorded by an electrode in the soma. Thus, it may be possible to use the time course data of averaged Ia EPSPs as an index of the locations of Ia terminals on motoneurons.

The cat hindlimb is denervated except for the muscle under study, leaving the dorsal roots in continuity with the spinal cord. Action potentials in single Ia fibers are elicited by muscle stretch or electrical stimulation of dorsal rootlets. These Ia spikes serve as triggering events for a computer average. The averaged intracellular records from a motoneuron show an EPSP if the Ia fiber under study synapses on that motoneuron. Synaptic potentials produced by other Ia afferents are averaged out of the record, as they do not occur

synchronously with the triggering Ia spikes. Since the EPSPs are of variable amplitude (Kuno, 1964; Mendell and Weiner, 1976) and latency (Collatos *et al.*, in press; Kuno and Llinás, 1970) in single sweeps, averaging over a large number of events is necessary to define the EPSP amplitude and time course. The resolution of the averaged EPSPs improves with increased numbers of sweeps.

EPSP shapes seem not to be determined by characteristics of either the afferent fiber (Scott and Mendell, 1976) or the motoneuron (Mendell and Henneman, 1971), but rather by the particular combination of these elements. These findings tend to strengthen the idea that EPSP time course reflects the location of the Ia synapse on the motoneuron. Occasionally, a compound averaged EPSP is seen which is interpreted to indicate a dendritic as well as a somatic locus of synaptic termination on a motoneuron from a single Ia afferent.

Averaged EPSP amplitudes in homonymous motoneurons typically were on the order of 100 μV, those of lateral gastrocnemius and soleus being slightly larger than medial gastrocnemius and semitendinosus. Rise time and half-widths were similar except in soleus motoneurons in which they were longer, due perhaps to the longer time constants of these motoneurons.

Dr. Mendell pointed out that in determinations of projection frequencies, imperfect resolution of the STA technique could cause the loss of small amplitude events and thus lead to underestimates of afferent connectivity. However, he noted that while the EPSPs of the lateral gastrocnemius motoneurons had the largest amplitudes of these pools, the projection frequency of this muscle was the lowest. These data indicated that resolution of the technique was probably not a major problem causing underestimates of connectivity.

In considering the relationships of various factors to EPSP amplitude, it was found that rise time is not as strongly correlated with amplitude as Rall's model would indicate. Apparently, other factors tend to balance out the attenuation of the local potentials in the dendrites, so that these relatively remote EPSPs appear larger than one might predict. This is discussed by Redman (1976), Mendell and Weiner (1976), and Iansek and Redman (1973). There is a tendency for EPSPs to be larger in smaller motoneurons than in larger motoneurons (Burke, 1968; Mendell and Henneman, 1971). There is, as well, a tendency for larger afferents to produce larger EPSPs than do smaller afferents (Mendell and Henneman, 1971). A more striking tendency is for EPSPs to be larger in homonymous motoneurons than in heteronymous motoneurons (Nelson and Mendell, 1978; Scott and Mendell, 1976).

Recently, Dr. Steven Nelson in Dr. Mendell's laboratory

observed that within hours after spinal cord transection at T13 or L5, amplitudes of individual EPSPs were larger by at least a factor of two compared to before transection. This observed increase in EPSP amplitude is not instantaneous, but rather begins to develop about three hours after transection.

Dr. Mendell discussed various factors influencing projection frequency. The first was the size of the afferent fiber. Small Ia afferents typically had lower projection frequencies than did larger fibers. The size of the motoneuron receiving input did not appear to play an important role. Projections to homonymous motoneurons were found to be more frequent than to heteronymous motoneurons (Nelson and Mendell, 1978; Scott and Mendell, 1976). Some afferents appear to project to more motoneurons than do others, and by this criterion, Dr. Mendell's group has classed Ia afferents as either Type X (projecting to more than 65% of the motoneurons sampled) or Type Y (projecting to less than 65%). This classification was implemented purely for convenience, and there is a continuum of fibers' projection frequencies. This is discussed by Scott and Mendell (1976). Spinal cord transection alters this, and, in fact, eliminates the Type Y class of fibers.

Besides this loss of Type Y fibers, spinal cord transection results in increased Ia fiber connectivity from an average of 78% to 80% to very near 100%. The time course of this phenomenon has not yet been determined with confidence.

Following chronic cord transection at T13, Ia fibers from medial gastrocnemius muscle produce EPSPs of normal amplitude, but projection frequency has increased to 97% or 98% from about 80% prior to cord section. Thus, an increase in projection frequency appears to be independent of any increase in EPSP amplitude.

Dr. Mendell summarized by saying that although there was a large degree of variability in EPSP properties from one motoneuron to the next, the results described above were quite consistent across experiments. This is especially true if one compares the averages of EPSPs of a single Ia afferent seen in many motoneurons in a given experiment. The STA technique has enabled the group to examine the specificity of connections and to quantify these connections in the Ia system in order to analyze the effects of experimental manipulations such as crossing peripheral nerves (Mendell and Scott, 1975), axotomy (Mendell *et al.*, 1976), or spinal cord transection (Nelson and Mendell, in press; Nelson *et al.*, in press).

Douglas G. Stuart *(University of Arizona, Tucson)* prefaced his presentation by suggesting that the STA techniques used by his group could have broad applicability for investigators studying

systems quite diverse from the segmental motor control system. They now obtain in-continuity single unit extracellular recordings from the soma of spindle and tendon organ afferents in the L7 and S1 dorsal root ganglia of anesthetized preparations and unanesthetized decerebrate cats undergoing considerable spontaneous activity. The in-continuity recording is achieved quite rapidly by manually inserting short "floating" microelectrodes into the ganglion. This permits several hours of unusually stable recording from a single afferent (Coensgen *et al.*, in press). This approach is obviously appropriate for a variety of other preparations and to other primary group I to IV sensory neurons at various incoming levels of the neuraxis.

Also in preface were comments on the "ground rules" for STA including: (1) a securely triggered and unequivocally identified reference spike train; (2) proof that this train is indeed in-continuity; and (3) proof that the train is not phase-locked to any other neuronal activity that could influence the averaged event.

In-continuity proof is a routine procedure for primary sensory neurons, if the reference spike train is used to trigger an averaging computer whose signal is the volume-recorded activity at the dorsal root entry zone (recorded with either a subdural- or extradural-positioned electrode). If the reference spikes are in-continuity, 2 K sweep averages invariably reveal a short-latency deflection indicative of the spike's arrival at the cord's surface (Figure 1, top traces in *A* and *B*).

The test for neuronal synchrony is a more complex issue. Initially, it was thought sufficient to record on analog tape a short epoch of the afferent's spike train together with a recording of multi-unit activity from the parent muscle nerve. This recording was then replayed in reverse and the afferent's spike train at the dorsal root level was again used to trigger the averaging computer using the multi-unit muscle nerve recording as the input signal. A similar technique has been used by Kirkwood and Sears (1974; 1975) for determining conduction velocity of afferent fibers recorded in peripheral nerve branches. The lower traces of parts *A* and *B* of Figure 1 reveal typical muscle nerve averages (128 sweeps) for two Ia afferents recorded simultaneously at the S1 (*A*) and L7 (*B*) dorsal roots. As a control for another study (Binder *et al.*, 1977) 18 Ia, four spindle group II and two tendon organ Ib afferents were isolated in thirteen cats. In each case, the muscle nerve averages revealed only a single action potential waveform. A variation of this muscle nerve averaging technique has also been used in motor unit studies (Binder *et al.*, 1978). By stimulating a ventral root filament and using the stimulus as a trigger for averaging a muscle nerve record of the parent muscle, one can determine whether functional isolation of a single motor unit axon has been achieved. In some cases, a double-peak waveform has

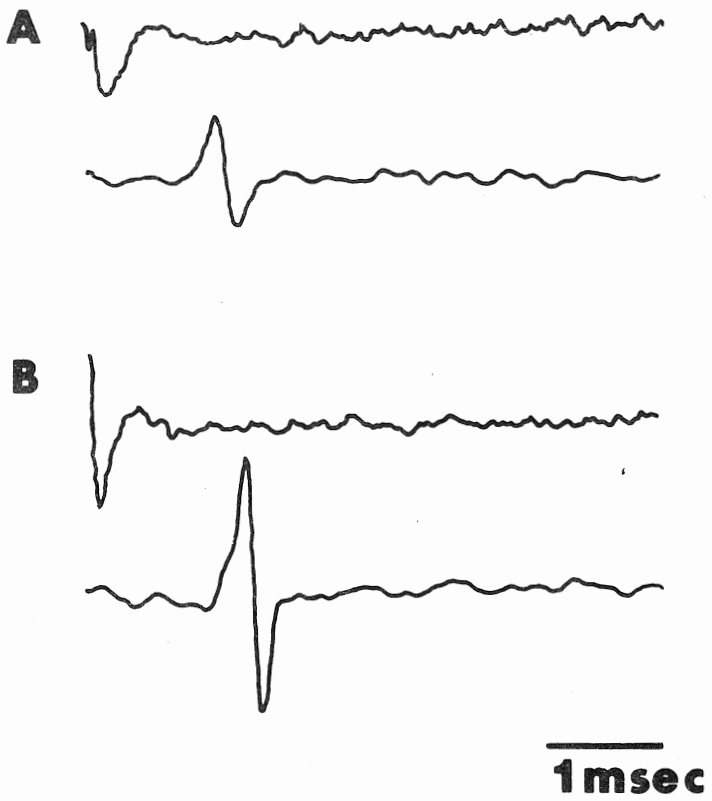

FIGURE 1

Examples of dual "in-continuity" afferent recording. Top traces in A *and* B *represent single afferent average waveforms (2 K sweeps) recorded at the dorsal root entry zone. Bottom traces represent same afferent waveform (128 sweeps) as recorded peripherally from the medial gastrocnemius muscle nerve. Multi-unit muscle nerve activity is recorded simultaneously with dorsal root isolated afferent spikes. The analog tape is then played backwards and the averaging computer is triggered by the isolated afferent spike train at the dorsal root level. The emerging waveform represents the same afferent action potential at the periphery. Both traces in* A *and* B *allow conduction velocity measurements--muscle nerve to dorsal root, dorsal root to cord entry. (From the unpublished work of M.D. Binder, J.L. Smith, R.M. Reinking and D.G. Stuart; see also their 1977 abstract.)*

been observed in these averages, even when the EMG recording and tension transient appeared to indicate single unit isolation. Subsequent ventral root dissection routinely reveals that, in fact, two motor

units were being activated with one of the pair generating far less EMG activity and tension than the other.

It has subsequently become obvious that while this technique is perfectly acceptable for conduction velocity measurements and for functional isolation of single motor unit axons, it cannot resolve partial synchrony between two neuronal spike trains each of which has minor irregularities in its discharge pattern (Matthews and Stein, 1969). A technique is obviously required that tests for synchronous activity between neuronal signals in a fashion similar to the motor unit synchronization test that was developed in Dr. Stein's laboratory (Milner-Brown *et al.*, 1973).[1]

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[1] *Subsequent to the symposium, Dr. Stuart's group used the multi-unit muscle nerve recording as the input to a signal averager triggered by a spike train from: (1) a dorsal root ganglion cell and (2) a random signal source. With appropriate delay of the muscle nerve input signal, rectified and non-rectified waveforms are obtained. The unitary afferent waveform of the non-rectified average is compared to the rectified average containing the unitary waveform and the rectified "random" average. On the basis of these three recordings, the unitary afferent waveform is extracted from the rectified average in addition to the activity of any other afferents in synchrony with it (Roscoe* et al., *in press). By use of this procedure it has been possible to validate the argument (Watt* et al., *1976) that when cats are spinalized and maintained on α-chloralose, or on a 0.5% halothane, N_2O, and O_2 gaseous mixture (Binder* et al., *1977), the spike trains of Ia and spindle group II afferents innervating a single passive muscle at fixed length are truly asynchronous. This means that the monosynaptic EPSPs recorded in motoneurons by STA* under these experimental conditions *can be confidently ascribed to the action of the reference afferent spike train alone. This is particularly important in the case of the monosynaptic projections of spindle group II afferents onto their homonymous motoneurons (Kirkwood and Sears, 1974; Kirkwood and Sears, 1975; Stauffer* et al., *1976) where one might possibly attribute the average response to a Ia afferent fiber firing in synchrony with the trigger spindle group II spike. It must also be cautioned that the boundary conditions for use of this improved test for neuronal synchronization have not yet been established. This means that, at this stage, the striking absence of synchrony in muscle afferent discharge in the passive muscle at fixed length cannot be assumed for other preparations in which this testing procedure or a similar test for afferent synchronization* (e.g., *Inbar* et al., *in press) has not been rigorously applied.*

The main body of Dr. Stuart's presentation was concerned with use of the STA technique to study the spinal motoneuronal connections of muscle receptor afferents. While delineation of these pathways is fundamental to the understanding of segmental motor control, a great deal is yet unknown about them (for review, see Matthews, 1972). A major difficulty has been finding a suitable means of selectively activating the different afferent species. For example, it is well known but rarely documented that both graded electrical stimulation and its combination with various forms of nerve blockade have many uncertainties and pitfalls.

The potential value of STA in this area was underscored when Kirkwood and Sears (1974; 1975) established the existence of a previously unsuspected monosynaptic connection between spindle group II afferents and their homonymous motoneurons. In confirming this finding, Stauffer and co-workers (1976) took advantage of the "presynaptic spike (Pre-SS)" to contrast the spinal conduction time and synaptic delay of Ia and spindle group II pathways (Figure 2). The

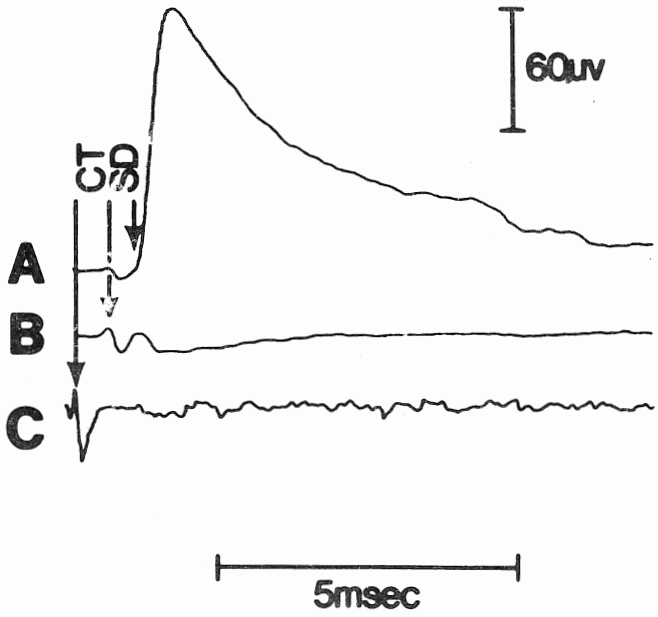

FIGURE 2

A spindle group II-produced EPSP in an MG motoneuron to show timing and value of the Pre-SS. A shows the IC-recorded average of 1,824 sweeps. B shows the corresponding EC control. C is the dorsal root spike average. The intervals CT and SD are taken to indicate the central conduction time and synaptic delay of this particular afferent to this particular motoneuron. (From Stauffer et al., 1976.)

Pre-SS is thought to indicate the arrival of propagated impulses in the presynaptic terminals or closely related branches (Brooks and Eccles, 1947). The Pre-SS of a single neuronal impulse was first recorded by Jankowska and Roberts (1972). They used it to measure the synaptic delay of the Ia inhibitory interneuronal connection with motoneurons supplying antagonists. This procedure (and the use of the Pre-SS to study the central conduction time of primary sensory axons) has recently been validated by Munson and Sypert (1978; see also Sypert and Munson, 1978). Taken together, these various findings suggest that the STA technique can contribute further to our understanding of even monosynaptic muscle afferent connections with motoneurons.

The question was next addressed as to whether it is possible to detect disynaptic segmental connections between muscle afferents and motoneurons by use of the STA technique. Figure 3 from

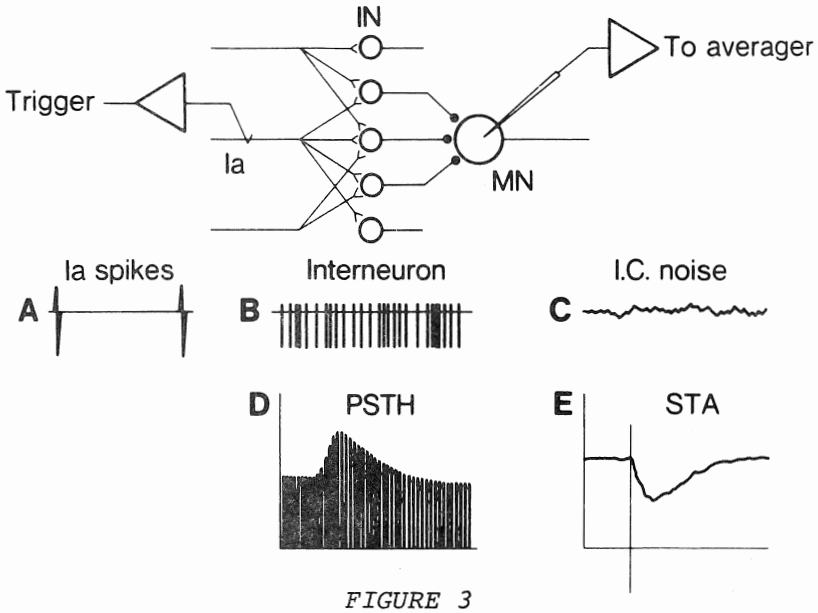

FIGURE 3

Schematic diagram to illustrate the way in which STA may detect activity crossing two synapses. Upper part shows three incoming Ia afferents, one of which is being recorded in-continuity (A). Some unspecified pattern of overlapping excitatory connections is made on interneurons (IN) *which are firing at some mean level* (B) *modulated by the Ia EPSPs. This modulation might show in a post-Ia spike histogram of an interneuron as in* D. *Intracellular noise* (C) *from an antagonist motoneuron* (MN) *contains a demodulated and partially smooth version of frequency-modulated interneuron firing. Spike-triggered averaging of this would then yield an IPSP waveform,* E, *which is a slightly smooth version of* D *with one synaptic and a little conduction delay. (From Watt* et al., *1976.)*

Watt and colleagues (1976) shows how STA may work across two synapses and, as recently pointed out by Dr. D. G. D. Watt (personal communication), further theoretical considerations of relevance to this type of analysis are contained in a recent report on a sun-weather correlation (Hines and Halevy, 1977). To test the arguments presented in Figure 3, Watt and colleagues (1976) first studied the well-known disynaptic inhibitory connection between medial gastrocnemius Ia afferents and extensor digitorum longus. STA effects thought to reflect unitary disynaptic inhibitory connections (Figure 4) were then compared to effects attributable to Ib afferents (Figure 5).

The main features of this work included: (1) the need to average a particularly high (4 to 16 K) number of sweeps to obtain an effect, thereby precluding current passage to check the validity of apparent IPSPs; (2) in view of (1), the need for an as yet undeveloped test that can distinguish when hyperpolarizing (depolarizing) effects are attributable to inhibition (excitation) rather than disfacilitation (disinhibition); (3) the small amplitude of the effects (generally < 10 μV) that were, however, validated by extracellularly-recorded

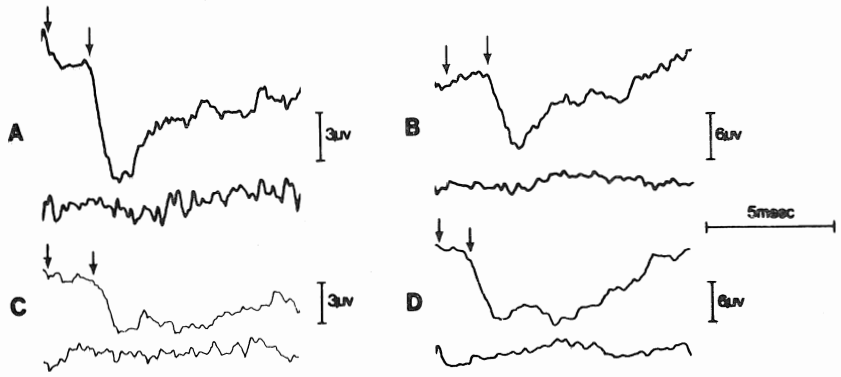

FIGURE 4

Disynaptic effects thought to be Ia-IPSPs measured by spike-triggered averaging. A *and* B: *different TA-EDL cells with same Ia afferent.* C *and* D: *different TA-EDL cells and different Ia afferents. In each case the upper record is IC and the lower EC control is at identical gain. Records derived from 8,192, 2,048, 8,192, and 4,096 sweeps, respectively. Note different voltage scales but common time scale. Arrows indicate estimated dorsal root (DR) entry time and onset of IPSPs. (From Watt* et al., *1976.)*

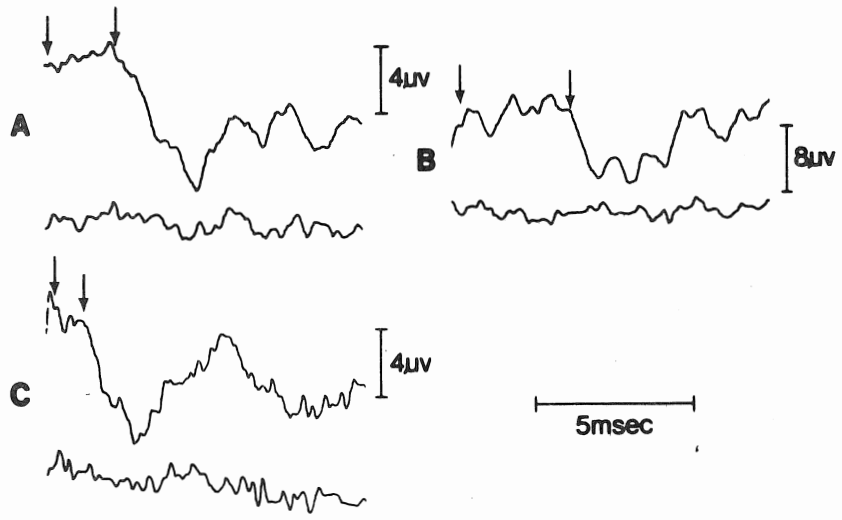

FIGURE 5

Examples of effects thought to be disynaptic Ib-IPSPs found in various MG motoneurons by triggering from an MG Ib afferent. A: *latency 2.1 msec, 16,384 sweeps.* B: *latency 3.4 msec, 4,096 sweeps.* C: *latency 0.9 msec, 4,096 sweeps. Arrows marked DR entry times and onset of IPSPs. (From Watt* et al., *1976.)*

controls based on an identical number of sweeps; (4) the more clear-cut and consistent demonstration of Ia inhibitory connections with motoneurons supplying antagonists than Ib connections with homonymous and other motoneurons, a difference that was attributable only in part to the generally lower firing rates of the Ib afferent spike trains (for further details, see Watt *et al.*, 1976).

Taking all these data on their face value, Dr. Stuart concluded that use of STA to study disynaptic segmental connections was of more theoretical than practical significance. He suggested that a more appropriate procedure would be to modify the STA technique to obviate the problem of stretch-induced afferent discharge. Spindle and tendon organ afferents cell bodies can be impaled in the S1 and L7 dorsal root ganglia and stimulated at 50 to 100 pps. The resultant afferent action potentials can then be used in the standard STA procedure while recording intracellularly from relevant motoneurons. The feasibility of this stimulation technique was first tested by Willis and co-workers (1968). Data generated in this way could help to clear up many of the uncertainties associated with the motoneuronal

connections of the Ib afferent population in particular, and provide more quantitative data on the diverse Ia and spindle II pathways to motoneurons than have hitherto been available.

Eberhard E. Fetz *(University of Washington, Seattle)* discussed the use of STA in behaving monkeys to identify precentral motor cortex cells whose action potentials produce detectable output effects on forelimb muscle activity. Experiments in collaboration with Dr. Paul Cheney employed monkeys trained to alternately flex and extend the wrist against elastic loads. Multi-unit EMG activity was recorded with implanted pairs of electrodes from six flexor and six extensor muscles of the wrist and fingers. Muscles were identified by their anatomical location and their responses to direct stimulation. Action potentials of task-related motor cortex cells were used to trigger averages of full-wave rectified EMG activity. For some cells, averages of two to four thousand sweeps revealed a transient postspike facilitation (PSF) of average EMG activity (Figure 6); the PSF had an onset latency (mean 6.7 msec) and a shape consistent with mediation by monosynaptic corticomotoneuronal (CM) connections. Such PSF was used to identify those cells, called CM cells, whose action potentials are followed by correlated enhancement of motor unit activity (Fetz *et al.*, 1976).

Most CM cells (70%) produced PSF in more than one of the synergistic muscles. EMG recordings were confirmed to be of independent motor units by cross-correlating muscle activity; averages of EMG activity triggered from motor unit spikes in each muscle confirmed that other loads did not redundantly record the same units. The set of independent muscles facilitated by a motor cortex cell has been called its "muscle field" (Fetz and Cheney, 1978). The fact that the muscle fields of most CM cells included two or more forelimb muscles suggests that the activity of these cells affects coactivation of several muscles in a movement. If the PSF is mediated by monosynaptic connections, these results would suggest that CM cells generally distribute divergent terminals to target motoneurons of more than one forelimb muscle. The extent of the muscle fields does not appear to be artifactually exaggerated by redundant motor unit recording or by synchronization of cortical cells (Fetz and Cheney, 1978); in fact, the number of target muscles may have been underestimated by the limited sampling of motor units.

The response properties of CM cells were documented during controlled ramp and hold wrist movements against different loads (Cheney and Fetz, 1978; Fetz and Cheney, in press). Since the PSF represents the average effect of single spikes on motor unit activity, the net effect of a CM cell firing pattern is the PSF convolved with the firing rate. On the basis of their dynamic and static responses, all CM cells could be classified into one of four types:

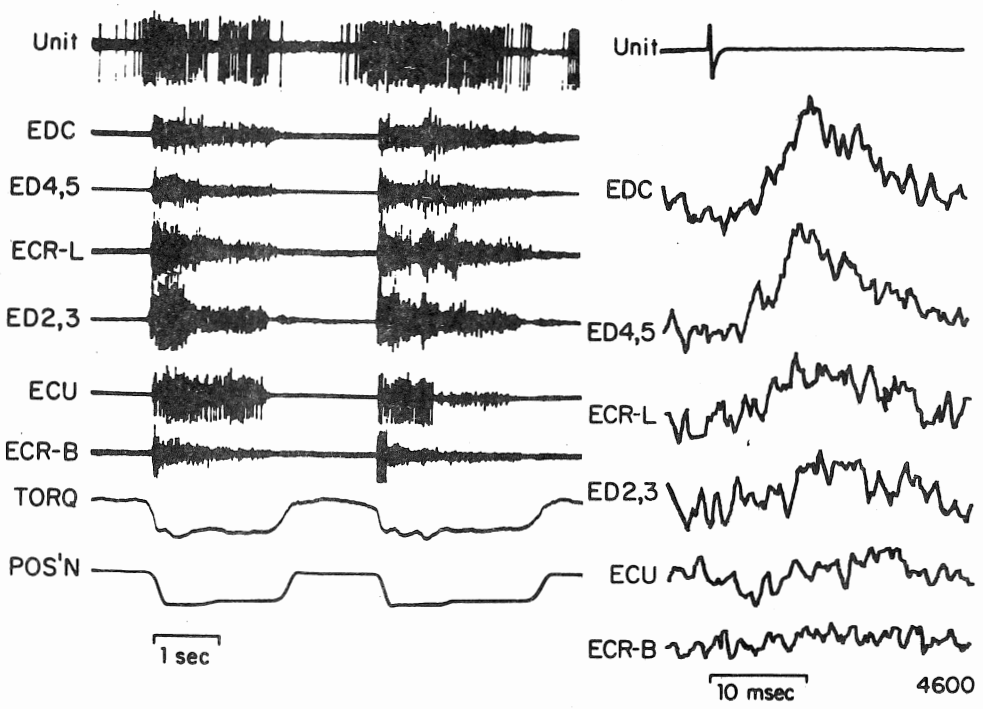

FIGURE 6

Postspike facilitation of forelimb extensor muscles. Left: two successive wrist movements showing, from top, activity of precentral CM cell and extensor muscles of wrist and fingers, torque and position. Right: spike-triggered averages of rectified EMG activity; analysis interval included 5 msec prior to spike and 25 msec following spike. N = 4600. (From Fetz and Cheney, 1978.)

phasic-tonic (57%), tonic (28%), phasic-ramp (80%) and ramp (5%). All CM cells fired during the static hold period, either at steady rates (tonic types) or gradually increasing rates (ramp types). In addition, some cells of each type showed a greater phasic burst of activity at onset of movement, which would preferentially contribute to initiation of muscle activity. When monkeys displaced the wrist against different elastic loads, the tonic firing rate of all CM cells was found to be linearly proportional to static torque over part of the range. The load sensitivity--*i.e.*, the increment in firing rate per increment in active torque--was twice as great for extension-related CM cells than flexion-related cells.

To investigate the output effect produced by microstimulation of these cells, single stimulus pulses were delivered through the microelectrode at sites of CM cells during comparable active movements (Cheney and Fetz, 1977). Stimulus intensities were too weak to evoke consistent EMG responses (2 to 10 μa), and stimulus rates were low enough to preclude temporal summation (< 10 Hz). Nevertheless, stimulus-triggered averages revealed a pattern of poststimulus facilitation which very closely resembled the postspike facilitation produced by the CM cell. While single microstimuli produced the same distribution of average facilitation as the spikes of the CM cell, they evoked stronger effects, suggesting that the microstimuli activated a cluster of CM cells whose terminals were similarly distributed to motoneuron pools. During movements in the opposite direction, when the CM cells would normally be silent, microstimuli often produced poststimulus suppression of average EMG of antagonist muscles, suggesting that the same CM cells may also contribute to reciprocal inhibition of antagonist muscles. In concluding, Dr. Fetz stated that the use of STA in awake, behaving animals is useful not only for identifying those cells which produce correlated output effects, but also in quantifying the magnitude and distribution of that output; such information makes it possible to meaningfully interpret the functional consequences of the cells' response patterns.

In a final general discussion period, the floor was opened to others in the audience who had used STA in their work to summarize their results.

John Munson and George Sypert (*University of Florida, Gainesville)* discussed a collaborative study mapping the terminal of Ia afferent fibers in the spinal cord, and investigating the minimal synaptic delay. Dr. Munson described the use of STA to detect the afferent fiber spike within the spinal cord, and the anatomical mapping of the rostro-caudal distributions, branching patterns, and terminal distributions of single Ia afferents of the medial gastrocnemius muscle of the cat. Conduction velocities and EPSP generating potentials of afferent branches could also be determined. Of interest was the observation that these properties differed widely in different branches of the same Ia fiber. Afferent fiber spike potentials were recorded in the motoneuronal pool over an 8600 μM rostro-caudal extent.

Dr. Sypert briefly described the use of STA to study correlations of Ia presynaptic terminal spikes with motoneuron EPSPs. Of particular concern was the latency between these two events to determine distance between the two recording sites. The mean delay between afferent fiber spike and onset of EPSP was found to be 448 μsec; this irreducible delay suggests chemical mediation of the EPSP. Parameters of the EPSP such as half-width, rise time, amplitude, and slope were also related to electrotonic distance.

Anthony Taylor *(St. Thomas Hospital Medical School, London, England)* described the techniques used by his group to study the distribution of afferents of the cat jaw elevator muscles to motoneurons. Single afferents recorded in mesencephalic nucleus of V produced monosynaptic EPSPs in only 15% of motoneurons. The hypothesis was proposed that much of the jaw reflex is due to multisynaptic pathways involving excitatory interneurons. Dr. Taylor also described the use of extracellular STA to plot the distribution of excitatory synaptic fields due to single afferents in the jaw elevator motoneuron pool. By this simplification it was demonstrated that most primary and secondary spindle afferents projected monosynaptically to the pool, though each did so to only a small proportion of the motoneurons.

Hans-R. Lüscher *(Harvard Medical School, Boston)* described a technique for studying the EPSPs caused by a single Ia or group II impulse in a large number of motoneurons; potentials were recorded from L_7 and S_1 ventral roots with a sucrose suction electrode to minimize current shunting. Averages triggered from single Ia and group II muscle afferents revealed postsynaptic population potentials with a waveform similar to EPSPs, but having a longer time course. The amplitudes of these potentials were positively correlated with the conduction velocities of the afferent fibers; the largest potentials were produced by the fastest conducting Ia afferents, and smallest potentials were produced by group II afferents. This suggests that the net postsynaptic effects of primary sensory afferents are significantly related to their size.

Dr. William Roberts *(Neurological Sciences Institute, Good Samaritan Hospital, Portland, Oregon)* described experiments in collaboration with Dr. Elzbieta Jankowska to study the unitary IPSPs produced in motoneurons by the inhibitory interneurons mediating reciprocal disynaptic inhibition from Ia afferents to antagonistic motoneurons. Single Ia inhibitory interneurons were activated by iontophoretic application of glutamate, and their unitary IPSPs analyzed by averaging potentials recorded intracellularly in target motoneurons. IPSP amplitude and distribution were examined, and chloride reversal tests and time course analysis indicated these inhibitory synaptic contacts are predominantly onto the soma or proximal dendrites of the motoneurons.

Victor Wilson *(Rockefeller University, New York City)* mentioned that his group had used similar techniques to investigate inhibitory synaptic connections of vestibular cells to cervical motoneurons. In this situation, motoneurons with unitary IPSPs from a given cell were extremely difficult to find, and he advised other investigators who employ STA in the CNS to investigate questions which can be answered by a relatively small sample of successful recordings.

Dr. Paul Cheney *(University of Kansas, Kansas City)* showed evidence that spike-triggered and stimulus-triggered averages of forelimb muscle activity in behaving monkeys sometimes revealed purely inhibitory effects from some motor cortex cells. Certain cells which co-varied with extension actually showed only postspike inhibition of flexor muscles, with no evidence of facilitating extensors. Stimulus-triggered averages at the same cortical site confirmed this result. He suggested that some motor cortex cells exert only inhibitory effects on motoneurons and their role in movement is to suppress antagonist muscles. Dr. Cheney also showed evidence of postspike facilitation of EMG activity in averages triggered from action potentials of red nucleus cells. These effects were presumably mediated by rubromotoneuronal connections. The response properties of such rubromotoneuronal cells differed from corticomotoneuronal cells in having less task-related tonic activity during static hold periods.

In summarizing the symposium, Dr. Fetz indicated that spike-triggered averaging has elucidated the physiological and anatomical properties of single cells at various levels of vertebrate motor systems. The major contribution of STA has been to quantify the output effects of specific cells, including the twitch tensions of individual motor units, the unitary EPSPs and IPSPs produced in motoneurons by afferent fibers and by spinal interneurons, and the facilitation of motor unit activity by corticomotoneuronal cells. At the same time, the technique has revealed the extent to which single afferent and descending cells distribute divergent terminal connections to different motoneurons. Such information becomes particularly significant when other physiological properties of these cells can be determined, such as their recruitment threshold, size, and response patterns under defined behavioral conditions. In the future, we can expect that the functional organization of motor systems will be further elucidated by continued applications of spike-triggered averaging.

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