J. Physiol. (1984), **352**, *pp.* 147–161
With 11 *text-figures*
Printed in Great Britain

SIMULTANEOUSLY ACTIVE AND INACTIVE SYNAPSES OF SINGLE Ia FIBRES ON CAT SPINAL MOTONEURONES

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(*Received 16 June 1983*)

SUMMARY

1. A technique is described for recording large numbers of individual or single-fibre excitatory post-synaptic potentials (e.p.s.p.s) from single motoneurones by means of spike-triggered averaging.

2. The cable properties of the motoneurones were calculated from the decay time course of a voltage transient in the motoneurone following a current pulse applied to the soma. From this response a theoretical shape index curve was calculated. Most individual or single-fibre e.p.s.p.s elicited by impulses in different I a fibres had simple decay time courses and shape indices that fitted the theoretical shape index curve of the motoneurone from which they were recorded very well. This suggested that the active terminals of these afferent fibres were located within limited post-synaptic areas.

3. In a few cases the original amplitude, latency and shape of individual e.p.s.p.s changed dramatically when they were re-averaged 40 min later after the membrane potential had decreased, but was still at an acceptable level. E.p.s.p.s with simple decay time courses changed to e.p.s.p.s with composite decay time courses, presumably due to activation of previously silent synapses.

4. The results suggest that impulses conducted in a single afferent fibre from a muscle spindle do not necessarily activate all of the synapses which the fibre forms on a motoneurone, but may repeatedly fail to activate some endings during prolonged periods of spike-triggered averaging, while consistently activating others.

5. Evidence regarding the site of transmission failure and the possible mechanism of its relief is discussed.

INTRODUCTION

The axons of almost all neurones in the mammalian central nervous system (C.N.S.) end in terminal arborizations. The number of synapses formed by the terminal branches of a group I a fibre on a single, homonymous motoneurone may vary from two to forty as shown by recent studies with horseradish peroxidase (HRP) (Burke, Walmsley & Hodgson, 1979; Brown & Fyffe, 1981; R. E. Burke, personal communication). The prevailing notion of conduction in the mammalian C.N.S. is that axons are simple, unfailing transmission lines. According to this view, impulses are faith-

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fully transmitted by the axon into all of the branches of its terminal arborizations and to all of the synapses. A more dynamic role for terminal arborizations is suggested by certain features of their morphology. For some time it has been recognized that conduction failure occurs at branch points of peripheral axons in a variety of species (Parnas, 1972; Smith, 1980). Indirect evidence has been obtained recently (Lüscher, Ruenzel & Henneman, 1983*b*) indicating that conduction failure and its relief in the terminal arborizations of primary afferents from muscle spindles might be responsible for certain states of depression and facilitation observed at Ia–motoneurone junctions. Conduction failure would result in silent or inactive synapses or groups of synapses in a terminal arborization. The inability to demonstrate the presence of inactive synapses directly has made it difficult to obtain evidence regarding this hypothesis.

We have developed a technique for recording many individual or single-fibre excitatory post-synaptic potentials (e.p.s.p.s) evoked in motoneurones by trains of afferent impulses in Ia or spindle group II fibres. This technique, which involves spike-triggered averaging (Mendell & Henneman, 1971) of signals recorded on magnetic tape, has provided the large amount of data necessary to distinguish between the majority of Ia projections which send terminals to just one restricted site on the motoneurone's surface and the few that send terminals to more than one site. This has been possible because release of transmitter from synapses located at different electronic distances from the soma elicits e.p.s.p.s with different latencies and time courses (Rall, 1967). In particular, synapses given off by a single Ia fibre to several discrete sites on the somatodendritic tree would produce e.p.s.p.s with composite decay time courses as Mendell & Henneman (1971) reported, whereas synapses clustered in a single restricted area would produce e.p.s.p.s with simple decay time courses. Using these criteria, the presence of previously silent or inactive synapses can be recognized by the shape changes produced in e.p.s.p.s when they become active.

Some of these results have already been presented in abstract form (Lüscher, Henneman & Mathis, 1982).

METHODS

Experiments were carried out on adult cats anaesthetized with 40 mg/kg of sodium pentobarbitone I.P., plus supplements as required. The lower lumbar and sacral segments of the spinal cord were exposed by laminectomy. The left hind limb was denervated except for the medial gastrocnemius (m.g.) muscle, the nerve of which was placed on bipolar electrodes for either electrical stimulation or recording. An extensive denervation of the tail, buttock and hip was also carried out. The exposed tissues were covered with warmed mineral oil. Core temperature and the oil pool covering the spinal cord were held constant at $37 \pm 1{\cdot}0$ °C. The Achilles tendon with a small bone chip was cut from its insertion. Stretch was applied to the m.g. muscle with a rack-and-pinion device connected by a strong thread to the bony attachment of the Achilles tendon.

Stretch-evoked activity in Ia or spindle group II fibres was recorded in continuity with five pairs of platinum hook electrodes from five different, uncut dorsal root filaments (d.r.f.s). D.r.f.s were dissected under a microscope until each filament contained two to six spindle afferents from the m.g. muscle. Stretch of the m.g. muscle was adjusted to cause continuous discharge in all these units, as monitored on an oscilloscope. To test the continuity of impulse conduction in each afferent unit, a ball electrode recorded activity of each afferent from the dorsal surface of the spinal cord above the entry point of the d.r.f. by spike-triggered averaging. Once functional continuity of all units was assured, the remaining L7 and S1 d.r.f.s were cut except for the five small filaments which

were mounted on the electrodes. Thus, afferent input to spinal segments L7 and S1 was greatly reduced. In addition L6 d.r. was completely severed and folded back to gain free access to the surface of the spinal cord with the micro-electrodes.

Intracellular potentials were recorded from motoneurones with glass micro-electrodes, pulled from 'omega dot' glass filled with 3 M-KCl. The tips of the electrodes were etched with hydrofluoric acid (Muheim, 1977), giving tip resistances of 4–8 MΩ. The electrodes were mounted on a Burleigh Inchworm piezoelectric microdrive, permitting advances in 2 μm steps. Potentials were measured with a WPI electrometer and current could be injected through the recording electrode by means of a balanced bridge circuit. Only cells with resting potentials larger than −55 mV were accepted for study. In order to rank the sizes of the motoneurones impaled, the conduction velocities of the axons were measured and the input resistance (R_i) of each cell was calculated by the 'spike-height method' (Frank & Fuortes, 1956).

The trains of multi-unit signals recorded from the five d.r.f.s, as well as the synaptic noise recorded from the impaled motoneurones and the neurogram recorded from the m.g. nerve, were stored simultaneously on different channels of an FM tape recorder (Honeywell 101) at 30 in./s. During repeated playback the signals from one of the five d.r.f. recordings were led to a specially designed time–voltage window discriminator (Lüscher, Mathis & Schaffner, 1983*a*). This instrument could be adjusted to emit a trigger pulse whenever a particular afferent unit discharged and, thus, to trigger the sweep of the averaging computer (Tracor 1710 Modular Analysis System). The input to the averaging computer was the intracellular recording played back from the tape recorder. By repeated playback of the tape, using the window discriminator to pick out the input of individual afferent units, the e.p.s.p. elicited by each unit was averaged out of the synaptic potentials evoked by the other inputs and the background noise in the taped recording. Using the same units, the conduction time, and thus, the conduction velocity of the afferent impulse could be determined by 'back-averaging' the neurogram recorded from the m.g. nerve.

RESULTS

Fig. 1 is a schematic representation of eight m.g. motoneurones and seventeen m.g. muscle spindle afferents studied in a single experiment. Five dorsal root filaments labelled F1–F5 are shown at the rostrocaudal locations where they entered the spinal cord in the L7 and S1 segments. These filaments each contained one to four units, U1–U4, responsive to stretch of the m.g. muscle. No other d.r.f.s were left intact except for the ones shown. Ninety-four individual e.p.s.p.s were recorded by means of spike-triggered averaging from the eight motoneurones whose rostrocaudal and dorsoventral locations are shown in the Figure. The numbering of the motoneurones corresponds to the order of their impalement. The results to be described in this paper will mainly be restricted to the detailed analysis of the ten connexions made with motoneurone No. 1 in order to illustrate some of the advantages of this new technique and to present a particular group of data with new and interesting implications. Ten out of seventeen afferent units projected functionally to motoneurone No. 1. Of these ten afferents one was a spindle group II fibre (dotted line), the other nine were Ia fibres (continuous lines) as determined by their conduction velocity of more than 72 m/s (Hunt, 1954). For simplicity, connexions with the other motoneurones are not shown. The results obtained from this motoneurone were of special interest because they provided an opportunity to distinguish between simultaneously active and inactive synapses arising from the telodendron of the same afferent fibre. In this particular experiment the spinal cord had been transected at T13, 6 h 45 min before recording from motoneurone No. 1.

The electrotonic length of the combined motoneurone soma and dendritic tree was estimated from the voltage transient following a long (75 ms) current pulse (−4 nA)

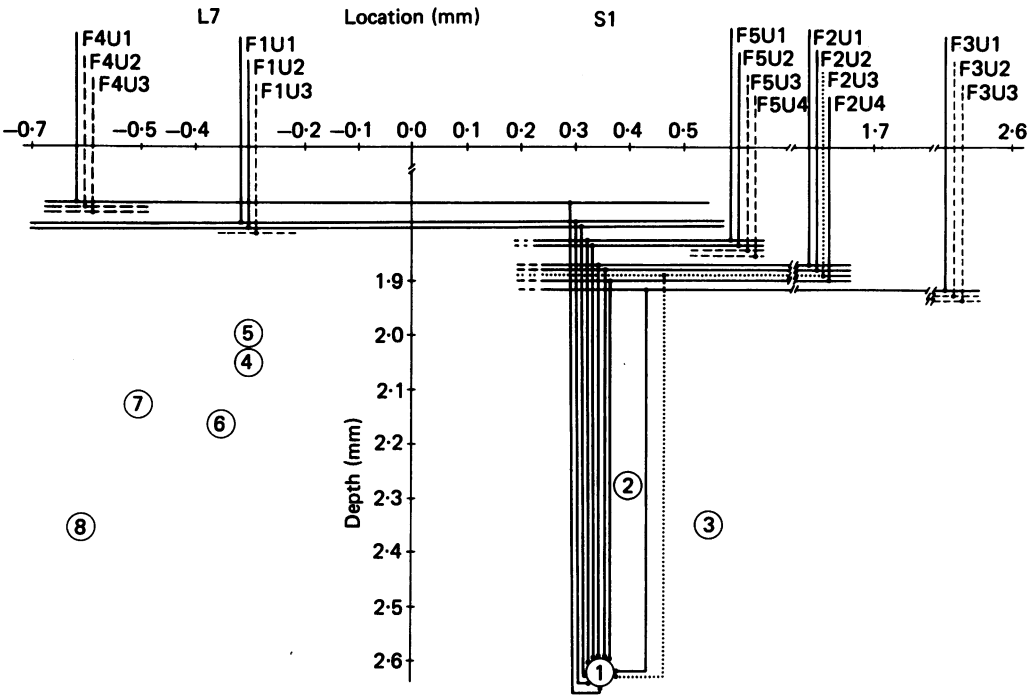

Fig. 1. Diagram of eight m.g. motoneurones and seventeen m.g. muscle spindle afferents studied in a single experiment. Five dorsal root filaments labelled F1–F5 are shown at the rostrocaudal locations where they enter the spinal cord. The locations of the motoneurones are illustrated by circles. Their numbers correspond to the order of their impalement. For simplicity only the functional connexions between ten afferent fibres and motoneurone No. 1 are shown. The dotted line represents a spindle group II fibre whereas continuous lines stand for Ia fibres. The connexions shown were established by spike-triggered averaging.

applied to the soma via the intracellular micro-electrode. This voltage transient can be expressed as the sum of an infinite series of exponential terms. According to Rall's analysis (1969), the exponential with the longest time constant, τ_m, represents the membrane time constant, whereas the exponentials with the shorter time constants have been called 'equalizing' time constants and represent the process of current redistribution within the dendrites. Using the numerical differentiation procedure outlined by Burke & Ten Bruggencate (1971), τ_m was obtained from the averaged transient records ($n = 16$) by fitting a straight line through the tail portion of the graph illustrated in Fig. 2. The first equalizing time constant, τ_1, was estimated by the 'peeling' procedure described by the same authors. The combined soma–dendritic length, L, was calculated according to eqn. (3) in the same paper (Burke & Ten Bruggencate, 1971). The following electrical parameters for motoneurone No. 1 were measured or calculated: $\tau_m = 6{\cdot}4$ ms, $\tau_1 = 0{\cdot}85$ ms, $L = 1{\cdot}25$, $R_i = 1{\cdot}9$ MΩ. A value of 10 for the dendritic-to-somatic conductance ratio, ρ, (Burke & Ten Bruggencate, 1971) was assumed in order to calculate the theoretical shape index curve for individual e.p.s.p.s generated at restricted areas of the somatodendritic tree. In addition, it was assumed that the time course of the synaptic current was equal and

of short duration for all synapses (the form $Te^{-\alpha T}$ ($\alpha = 100$) was used, where T is normalized time (Jack & Redman, 1971)). The theoretical shape index curve obtained from these values is illustrated in Fig. 3. Cross bars indicate the electrotonic distance in intervals of $0{\cdot}25\lambda$. This curve was compiled from the graphs given by Jack & Redman (1971) and Jack, Noble & Tsien (1975), using the parameters given above. In Fig. 3, along with the theoretical shape index curve, are plotted the shape indices of the ten individual e.p.s.p.s recorded simultaneously from motoneurone No. 1. The measured shape indices fit the theoretical curve rather well, suggesting that all of the ten afferent fibres projecting to the motoneurone in this experiment sent all of their

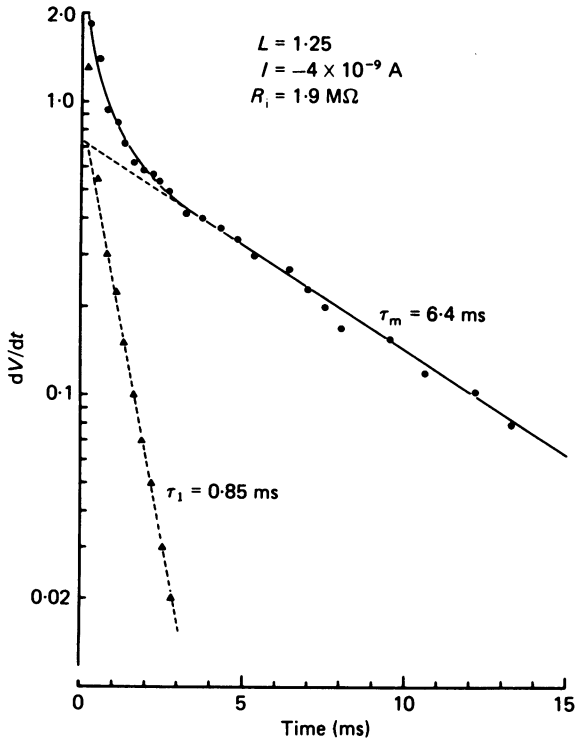

Fig. 2. Semilogarithmic plot of the time derivative (dV/dt) of a voltage transient recorded from motoneurone No. 1 in response to a long current pulse applied to the soma via an intracellular electrode. The result of numerical differentiation is plotted against time (t) in milliseconds. The straight line is the best fit by eye through the tail portion of the curve at times $t > 6$ ms. It represents the membrane time constant of the motoneurone. The difference between the data curve and the extrapolated line for $t < 4$ ms is plotted as triangles (▲) and the straight line (dashed) fitted by eye through these points represents the 'first equalizing time constant'. See text for further explanation.

active terminals to one limited post-synaptic area. In fact, none of the e.p.s.p.s showed a composite time course. In their original study with spike-triggered averaging, Mendell & Henneman (1971) were surprised to find that the majority of individual e.p.s.p.s actually had a single peak and simple time course, suggesting a very restricted input site. This was consistent with the later studies of Brown & Fyffe (1978, 1981) showing that the terminals of single Ia fibres were generally limited to a

restricted post-synaptic site. However, Glenn, Burke, Fleshman & Lev-Tov (1982) have shown that in some cases the synaptic sites of Ia fibres on motoneurones may be considerably separated. Mendell & Henneman (1971) illustrated two examples (p. 177) of individual e.p.s.p.s with compound time courses and suggested that each was elicited by two active groups of synapses from the same fibre.

Intracellular recording was carried out on motoneurone No. 1 for more than an hour. During the first 40 min the membrane potential remained constant at −71 mV. After 40 min the membrane potential drifted slowly to −55 mV and stayed there for many minutes. Another set of e.p.s.p.s was recorded at this level. Two of the most interesting e.p.s.p.s recorded at the two levels of membrane potential are illustrated in Fig. 4*A* and *B*. Four e.p.s.p.s recorded under the same conditions are illustrated in Fig. 9.

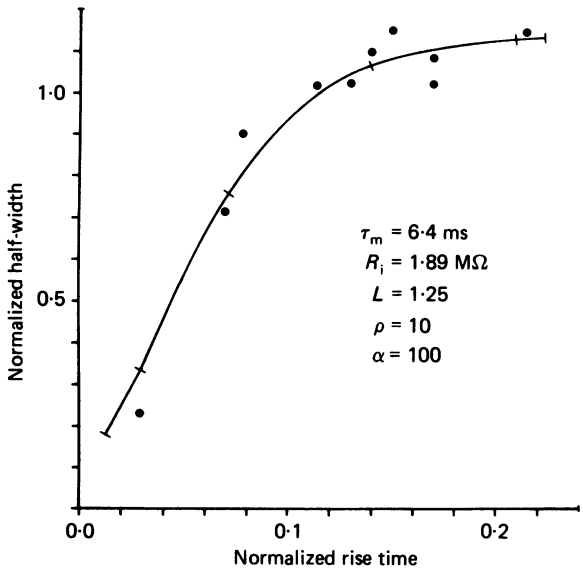

Fig. 3. Shape indices of the ten single fibre e.p.s.p.s recorded from motoneurone No. 1, together with the theoretical shape index curve for motoneurone No. 1. Cross bars indicate the electrotonic distance in intervals of 0·25λ.

The two e.p.s.p.s recorded at −71 mV (arrowheads in Fig. 4*A* and *B*) had slow rates of rise and simple decay time courses. The shape of the two e.p.s.p.s changed dramatically when recorded at a membrane potential of −55 mV. They both exhibited a very fast rise time and a clearly composite decay time course, suggesting that synaptic transmission was no longer restricted to a limited area. As can be seen in Fig. 5 the half-widths of these two e.p.s.p.s are too long for their rise times (□, e.p.s.p. elicited by F1U2; △, e.p.s.p. elicited by F5U2). When the e.p.s.p.s recorded at −71 mV were subtracted from the superimposed e.p.s.p.s recorded at −55 mV (Fig. 4*A* and *B*), the traces below the superimposed e.p.s.p.s were obtained. These potentials, each with a single peak and a simple time course, indicated the e.p.s.p.s recorded at −55 mV were both composed of two subcomponents generated at different sites on the motoneurone surface. The shape indices of these subcomponents of the e.p.s.p.s again fit the theoretical shape index curve well (Fig. 5). These changes

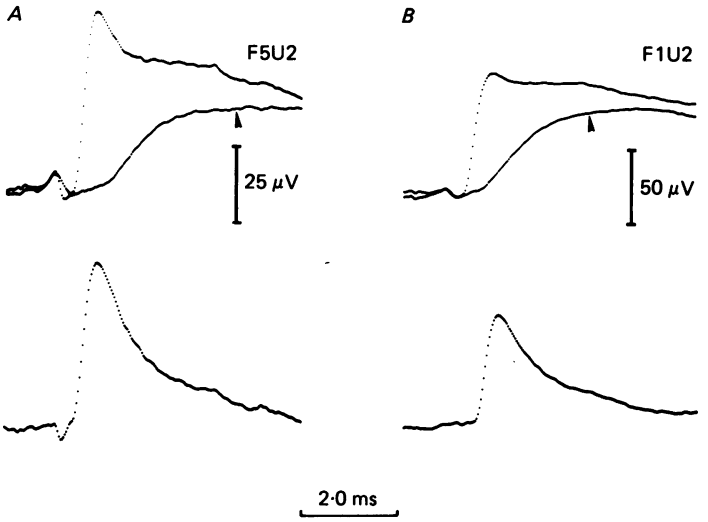

Fig. 4. Two examples (*A* and *B*) of individual e.p.s.p.s that changed their shape and amplitude during the recording period. The two e.p.s.p.s elicited by impulses in Ia fibres F5U2 and F1U2 during the first recording period are identified by arrowheads. Both had slow rates of rise. During the second recording period the e.p.s.p.s elicited by impulses in the same fibres had fast rise times and composite decay time courses, as illustrated in the uppermost traces. Subtraction of the e.p.s.p.s recorded initially from those obtained later resulted in e.p.s.p.s with fast rise times and simple time courses (lowest traces).

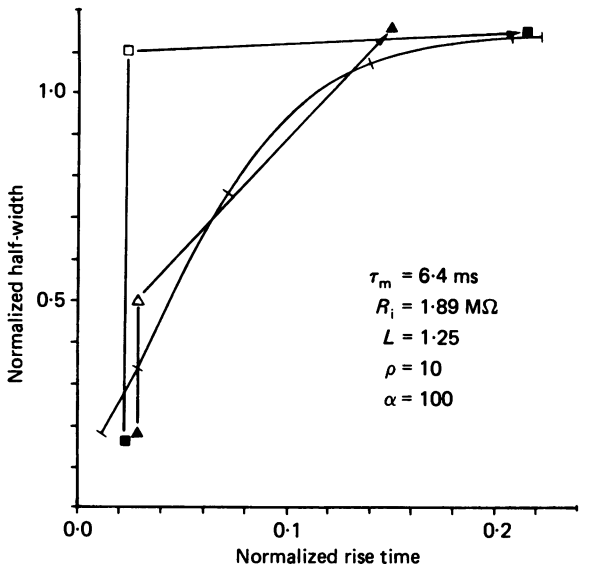

Fig. 5. Theoretical shape index curve for motoneurone No. 1 and the shape indices of the two e.p.s.p.s elicited by impulses in the Ia afferents F1U2 (□) and F5U2 (△) during the second recording period (illustrated in Fig. 4*A* and 4*B*). The half-widths of these two e.p.s.p.s are too long for their rise times. The shape indices of the subcomponents of these two e.p.s.p.s are indicated by arrows to filled symbols (■, ▲), which fit the theoretical shape index curve well.

in the shape indices indicate that one or a cluster of synapses, previously inactive at −71 mV membrane potential were active at −55 mV, and that the newly recruited synapses were located close to the motoneurone soma in both cases.

In order to draw these conclusions, it was important to make sure that the e.p.s.p.s recorded at −71 mV and at −55 mV were elicited by the same afferent unit, that no phase locking occurred between the trigger unit and some other afferent units and that the averager was not accidentally triggered by two different afferent units. In order to exclude the possibility that the averager was triggered accidentally from two different spikes, interpulse interval histograms were compiled for both units during the two recording periods. Fig. 6*A* shows the histograms for unit F5U2 (conduction velocity: 86 m/s) during the first (upper) and second (lower) recording period. Fig. 6B shows the histograms for unit F1U2 (conduction velocity: 93 m/s) during the first (upper) and second (lower) recording period. All histograms show a discrete distribution with no very short intervals, confirming that a single afferent unit was always triggering the averager. The histograms also reveal that the mean firing frequency of units F5U2 and F1U2 slowed down from 25·6 to 23·8 impulses/s and from 23·6 to 17·8 impulses/s respectively during the recording period.

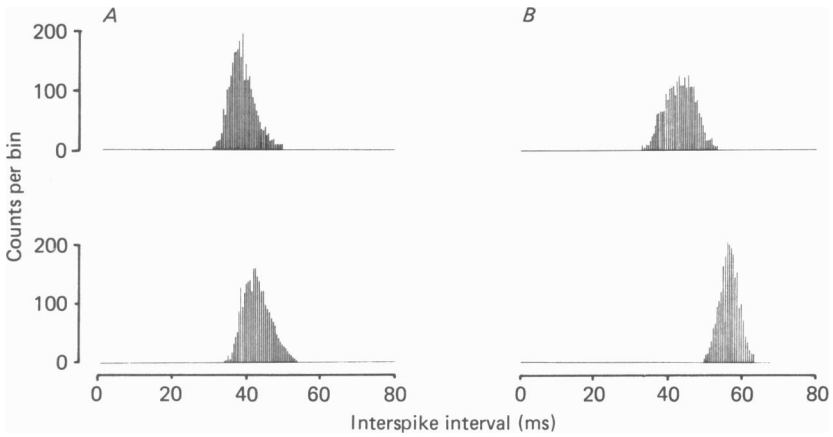

Fig. 6. Interpulse interval histograms for unit F5U2 (*A*) and F1U2 (*B*) compiled during the first (upper illustrations) and second (below) recording periods. All four histograms show unimodal distributions. The shift of the peaks of the histograms to the right during the second recording period indicates that the firing rates of both units decreased.

To exclude the possibility that the observed changes in the e.p.s.p. shape indices might be due to synchronized input from other muscle spindle afferents, the stretch-evoked activity in the d.r.f.s was averaged on a second channel of the averager. The recordings were passed through a two-channel analog delay line in order to visualize the full time course of the d.r. action potential. Fig. 7*A* and *B* show the e.p.s.p.s elicited by the afferent unit F5U2 in cell No. 1 recorded at a membrane potential of −71 and −55 mV respectively. The simultaneously averaged record of the action potential of this unit is shown below. The shape of this action potential is the same during both recording periods, confirming that the same unit was always used to trigger the averager. The recordings from d.r.f. F1 to F4 were then averaged in

sequence always using the same afferent unit (F5U2) to trigger the averager. This procedure should detect synchronization between the unit (F5U2) which was used for spike-triggered averaging to record the e.p.s.p.s and any other afferent input to the spinal segments L7 and S1. Since no other d.r.f.s were entering the spinal cord than the one we were recording from, and no sign of synchronization in the afferent input was revealed in traces F1–F4 of Fig. 7 *A* and *B*, the possibility that the observed changes in the shape of the e.p.s.p.s might be due to synchronization can be excluded. The same test for synchronization and identity of the trigger unit was employed, as described above, for unit F1U2. The results of this test are summarized in Fig. 8 *A* and *B*. Again, no synchronization between the trigger unit and the afferent input can be seen, and the shape of the trigger units is the same during both recording periods. These tests, together with the unimodal distribution of the interpulse interval histograms illustrated in Fig. 6 *A* and *B* confirm that the observed changes in the e.p.s.p. shape must be due to the activation of previously inactive synapses in the terminal arborization of single Ia fibre.

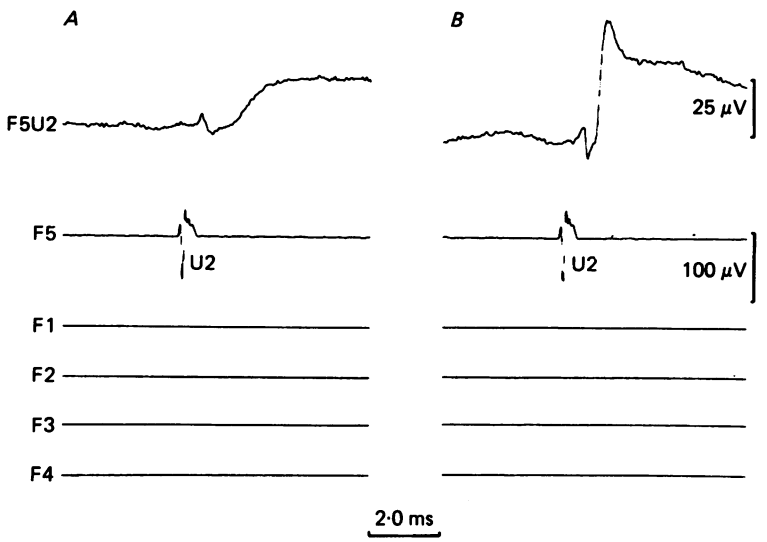

Fig. 7. The single-fibre e.p.s.p. recorded during the first (*A*) and the second (*B*) recording periods together with the averaged impulse of afferent fibre F5U2. The shape of the action potential is the same, whether recorded during the first or the second recording period, thus confirming that the same unit was triggering the averager in both cases. The recordings from dorsal root filaments F1–F4 were averaged in sequence, always using the action potential U2 recorded from filament F5. These averages (F1–F4) are flat, indicating that no strong synchronization between the triggering unit (F5U2) and any other afferent input occurred during the two recording periods.

Fig. 9 *A*, *B* and *C* illustrates three other examples of e.p.s.p.s that changed when recorded at different membrane potentials. The e.p.s.p.s recorded at −71 mV are identified with arrowheads and they are superimposed on the e.p.s.p.s recorded 40 min later and at a membrane potential of −55 mV. The afferent units eliciting the e.p.s.p.s are indicated by their filament and unit number. The change in the shape indices of the e.p.s.p.s illustrated in Fig. 9 *A* suggests that synaptic transmission

switched from a juxtasomatic site to one slightly farther from the soma. In Fig. 9*B*, the e.p.s.p. recorded at −55 mV had a shorter latency than the one recorded at −71 mV and had a slight inflexion (arrow) in the rising phase indicating that at least one additional synapse had become active. The e.p.s.p. shown in Fig. 9*C* had a larger amplitude while the shape index remained the same when recorded at the lower

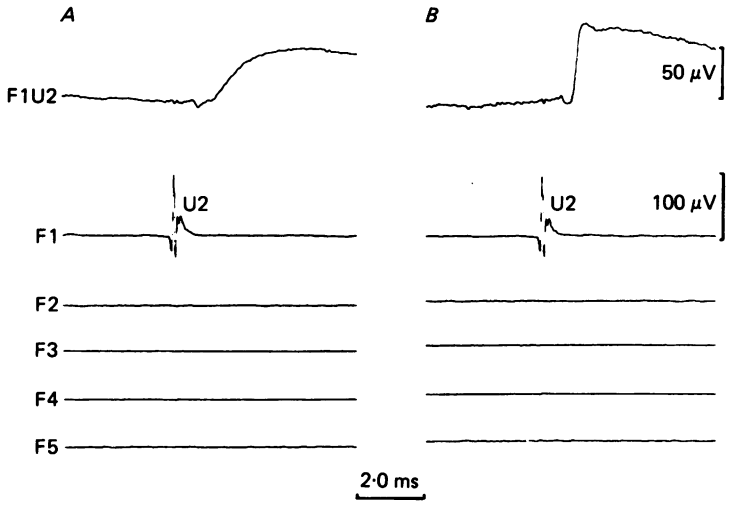

Fig. 8. Same Figure legend as for Fig. 7, but impulses in unit F1U2 were used to trigger the averager.

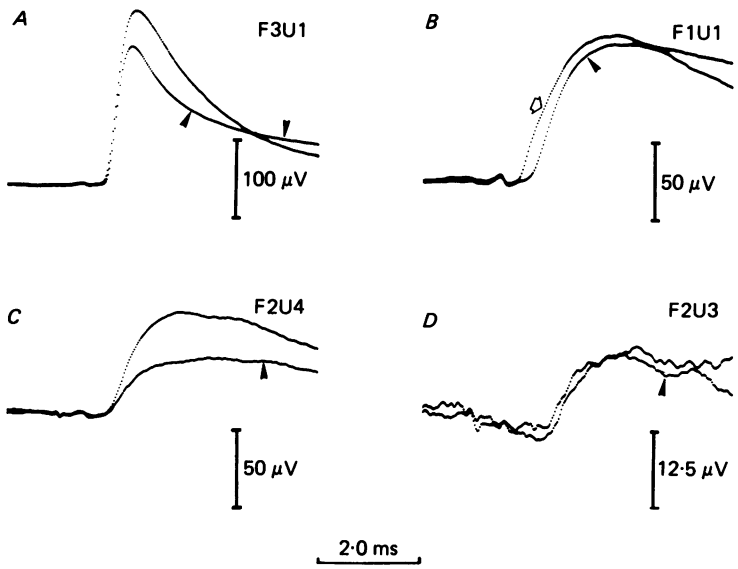

Fig. 9. *A*, *B* and *C*, three additional examples of single-fibre e.p.s.p.s the shape or amplitude of which changed in the second recording period. The e.p.s.p.s recorded first are identified with arrowheads, and are superimposed on the e.p.s.p.s recorded 40 min later. The afferent units eliciting them are indicated by their filament and unit number. *D*, one example of an e.p.s.p. which neither changed in amplitude nor in shape.

membrane potential. Five of the ten e.p.s.p.s were nearly identical in shape, as well as in amplitude, whether recorded at −71 or −55 mV. One of them is shown in Fig. 9*D*.

The shape indices of the individual e.p.s.p.s and their components were used to estimate the site of synaptic transmission on the membrane of the motoneurone. The results are compiled in Fig. 10. The dendrites are lumped into a single cable and schematically illustrated as a straight line. The location of the soma is at the far left.

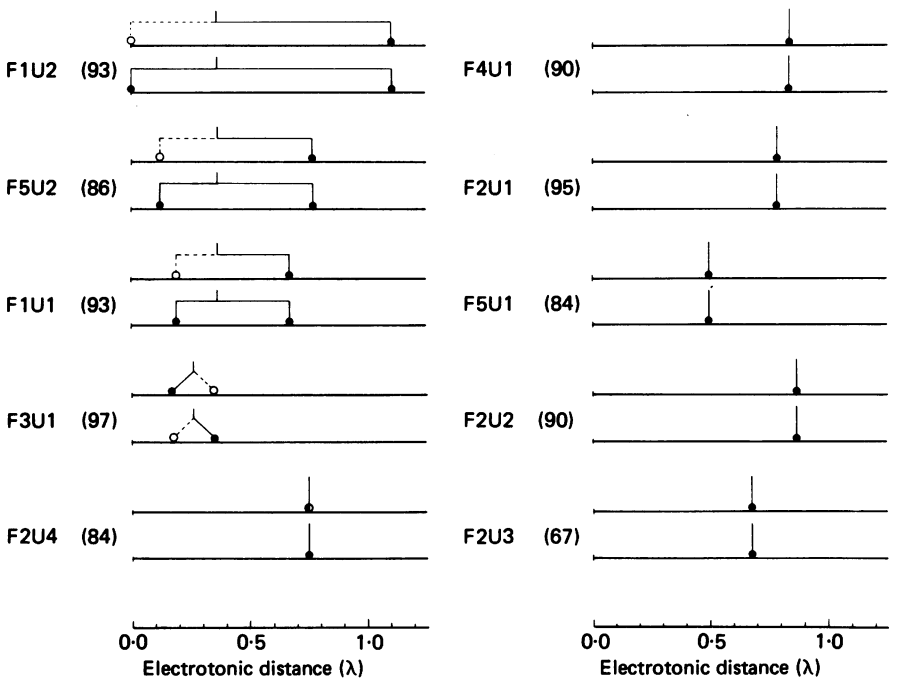

Fig. 10. Location of active (●) and inactive (○) synaptic endings or clusters of endings on a simplified cable representation of the soma and dendritic tree of motoneurone No. 1. The soma is at the far left and is assumed to be the site of electrode penetration. The afferent units are identified by their filament and unit number. The axonal conduction velocities (m/s) are given in parentheses. The upper half of each pair illustrates the state of the synaptic contact systems during the first recording period. The lower half illustrates the state of the same contact systems 40 min later. The right half of the Figure illustrates the location of the synaptic sites which did not change their activities.

The state of the synapses at −71 mV is represented on the upper line of each pair. ●, denote active sites, whereas ○ indicate inactive sites. The number of synapses at these sites is not known, and of course, we have no information on the trajectories of the afferent fibres. In the lower representation of the dendrites the activity or inactivity of the synapses is indicated at the same sites when the membrane potentials had shifted to −55 mV. The numbers in parentheses are the conduction velocities of the afferent fibres. The location of the active synaptic sites of the five connexions, the e.p.s.p.s of which did not change during the recording periods, are shown on the right-hand part of the illustration.

The results presented in the preceding paragraphs have been obtained from a single

motoneurone. They have been chosen for presentation because, in the first place, the electrophysiological characterization of the motoneurone was sufficiently reliable to make the detailed analysis of the e.p.s.p. shapes and, in the second place, some of the observed changes in the e.p.s.p. shapes were unambiguously interpretable. The results, however, are typical, as can be seen in Fig. 11, which illustrates spontaneous

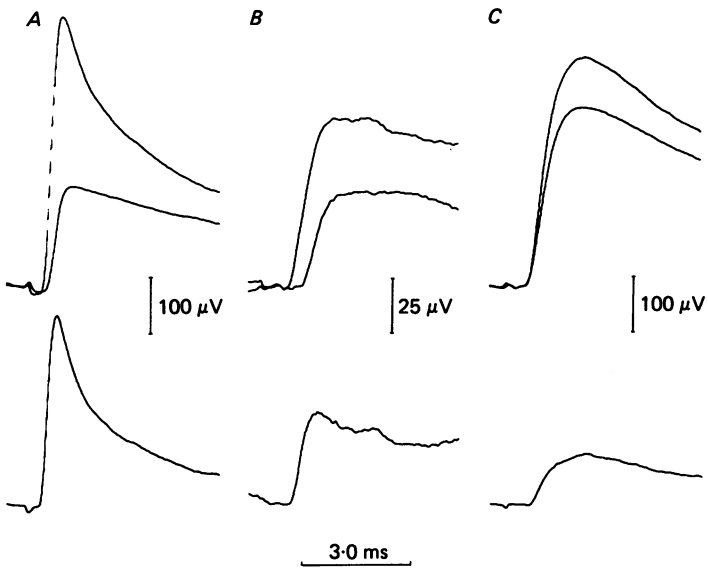

Fig. 11. Three sets of single-fibre e.p.s.p.s recorded from a single motoneurone which changed their shapes and amplitudes. In each case, the smaller e.p.s.p.s have been recorded first at a membrane potential of −68 mV. The larger e.p.s.p.s have been recorded 30 min later at a membrane potential of −62 mV. Below each set the differences between the two e.p.s.p.s are plotted.

changes in the shape and amplitude of three single-fibre e.p.s.p.s, again recorded from a single motoneurone but in a different experiment. The time between the two recording periods was 30 min and the membrane potential drifted slowly from −68 to −62 mV. In all three cases, the e.p.s.p.s recorded first at −68 mV were smaller and they are superimposed on the e.p.s.p.s recorded later at −62 mV. Below each set of e.p.s.p.s the differences between the two e.p.s.p.s are shown. Fig. 11 *A* illustrates an example where the new component had an amplitude of more than 300 μV. The example given in Fig. 11 *B* illustrates a pronounced shortening of the latency which was accompanied by a moderate increase in the amplitude of the e.p.s.p. The third example (Fig. 11 *C*) shows an increase in amplitude of about 100 μV. The shape of this additional component is quite different from the shape of the e.p.s.p. averaged during the first recording period. A total of nine single-fibre e.p.s.p.s was recorded simultaneously from the same motoneurone. Three of them did not change their shape or amplitude. The remaining three e.p.s.p.s showed only minor changes.

DISCUSSION

The observations presented above suggest that afferent impulses conducted in a single afferent fibre from a muscle spindle do not necessarily activate all of the synapses it forms on motoneurones, but may repeatedly fail to activate some endings during prolonged periods of spike-triggered averaging, while consistently activating others. Silent synapses may result (1) from conduction block at axon non-homogeneities in the telodendron or (2) from transmission failure at synapses with a very low probability of releasing transmitter, as proposed by Hirst, Redman & Wong (1981).

If an I a fibre terminates on a very limited post-synaptic surface of a motoneurone, resulting in a clustering of the synaptic endings at about the same electrotonic distance from the soma, it would be difficult to distinguish between alternatives (1) and (2) by analysing the shape and amplitude of the individual e.p.s.p.s. Relief of conduction failure in the terminal arborizations, as well as increase in the probability of transmitter release would both result in increased e.p.s.p. amplitude without affecting the shape of the e.p.s.p., as observed and illustrated in Fig. 9*C*. Furthermore, it is likely that conduction block within a tight cluster of synaptic endings would not necessarily result in inactive synapses, because the electrotonic spread of the action potential might suffice to depolarize all the synaptic endings given off by short terminal branches. Our results, however, indicate that synapses, or groups of synapses, may sometimes be widely separated on the somatodendritic tree, confirming the HRP study of Glenn *et al.* (1982). Conduction failure at axonal branch points would result in silent synapses, or groups of silent synapses, which would become active when conduction block was relieved. This would result in a sudden change of e.p.s.p. shape as illustrated in Figs. 4*A* and *B* and 11. It should be emphasized that whereas the originally inactive synapses were silent for thousands of trials and then became consistently active, the initially active synapses apparently did not change their release characteristics (Fig. 4*A* and *B*) when the e.p.s.p.s were re-averaged. If one assumes that impulses are faithfully conducted through the terminal arborization to all of its synapses, it is difficult to explain (1) how a synaptic ending or group of endings could fail to release transmitter during thousands of trials and (2) how the release mechanism could change in all the synapses within one cluster, but not within the other. This reasoning assumes that the additional components represent the effect of a small cluster of synaptic endings. The magnitudes of the two components illustrated in Fig. 4 are both less than 100 μV suggesting that they may reflect the action of a single synaptic release site (Jack, Redman & Wong, 1981). The amplitude of the new component illustrated in Fig. 11*A* is, however, more than 300 μV suggesting that a small group of probably three synaptic boutons had become active.

Edwards, Redman & Walmsley (1976) attributed the increased amplitude of e.p.s.p.s they observed, at lower membrane potential of the motoneurone during depolarizing current injection, to active dendritic membrane responses. In our results, the following four observations argue against the possibility that the observed changes in the e.p.s.p. shape and amplitude might be due to active dendritic responses: (1) we have observed an increase in e.p.s.p. amplitude without a significant decrease in the motoneuronal membrane potential (Fig. 11); (2) the newly recruited

components sometimes preceded the e.p.s.p. recorded at the higher membrane potential instead of developing out of it (Fig. 4); (3) only a fraction of the total number of single-fibre e.p.s.p.s recorded simultaneously from the same motoneurone changed their shape or amplitude; (4) the e.p.s.p.s with the composite decay time courses in Fig. 4 recorded at the low membrane potential of −55 mV could be subdivided into two components, each of which fits the theoretical shape index curve of motoneurone No. 1 almost perfectly.

In considering how silent synapses might have become active in these experiments, at least three possibilities should be mentioned: (1) the observed spontaneous decrease in the mean discharge frequency of the afferent impulses (Fig. 6*A* and *B*) might decrease the probability of conduction failure at the branch points in the telodendron as it does at branch points in peripheral axons (Parnas, 1972; Smith, 1980); (2) there have been several reports of electrical interactions between motoneurones and primary afferent fibres suggesting a low resistance pathway from the motoneurone to the presynaptic fibre (Decima & Goldberg, 1973; Galindo & Rudomin, 1978; Curtis, Lodge & Headley, 1979). It is conceivable that the small depolarization observed in the motoneurones was somehow carried into the very terminal branches of the telodendron of the afferent fibre. Depolarization just distal to an axon branch point would presumably increase the safety factor for impulse propagation through that branch point; (3) activation of silent synapses might reflect the so far unexplained enhancement of synaptic transmission after acute spinal cord transection as reported by Nelson, Collatos, Niechaj & Mendell (1979).

Previously published findings from this laboratory (Lüscher, Ruenzel & Henneman, 1979; Lüscher *et al.* 1983*b*) and others (Jack & Redman, 1971; Nelson *et al.* 1979; Hirst *et al.* 1981), taken as a whole, strongly suggest that transmission failure occurs somewhere in the projection of Ia fibres to motoneurones. The present findings verify this suggestion in a more direct and specific way than we have been able to demonstrate previously. The implication of these results is that the functional connectivity of Ia fibres with motoneurones is not fixed nor necessarily equivalent to anatomical connectivity. The available evidence from this and other studies suggests, rather, that the functional connectivity and transmission capacity of the Ia projection varies dynamically with the state of the spinal cord and central nervous system.

This research was supported by grants from the Swiss National Science Foundation (No. 3.536.0.79), the Stiftung für Wissenschaftliche Forschung der Universität Zürich and the Jubiläumsspende der Universität Zürich to H.-R. Lüscher. E. Henneman was on leave from the Department of Physiology and Biophysics, Harvard Medical School, Boston, MA, U.S.A.

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