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THE GENERATION OF IMPULSES IN MOTONEURONES

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It has been shown that the intracellularly recorded spike potential of motoneurones is compounded of three separate responses, each normally having an all-or-nothing character: the M spike of the medullated axon; the IS spike of the initial segment of the axon; and the SD spike of the soma-dendritic membrane (Coombs, Curtis & Eccles, 1957). With all three methods of stimulation antidromic, synaptic or direct, the IS spike precedes the SD spike (Araki & Otani, 1955; Fatt, 1957*b*; Fuortes, Frank & Becker, 1957; Coombs *et al.* 1957), but only with antidromic stimulation has the small M spike been observed to precede the IS spike and even to occur in the absence of the IS and SD spikes when antidromic transmission is depressed by various procedures (Brock, Coombs & Eccles, 1953; Coombs, Eccles & Fatt, 1955*a*; Coombs *et al.* 1957). The present paper describes an attempt to determine the factors governing the generation of spike potentials in these three components, and the way in which they are related to the discharge of an impulse down the axon of the motoneurone. The experimental procedures have been described in the previous paper (Coombs *et al.* 1957).

RESULTS

The discharge of impulses along the motor axon

By testing for the refractoriness of the motor axon in the ventral root, Fuortes *et al.* (1957) showed that the discharge of an impulse along the motor axon always occurred when direct or synaptic stimulation evoked an IS spike response of a motoneurone. The SD spike was not concerned. This finding has been confirmed in a number of experiments, but a much more satisfactory experimental procedure has been to record the impulse that is discharged along the ventral root, for in this way the timing of the discharge can be investigated with precision.

In the usual experimental procedure the ventral root was divided into a

number of filaments that were mounted separately on pairs of electrodes that could be used for both stimulating and recording. A third electrode was often placed more proximally on the filament in order to record, relative to an indifferent earth lead, the spike potentials of the propagated impulses and so to minimize errors in assessing the time of initiation of the antidromic impulse. The filament containing the axon of the motoneurone under investigation can be readily identified because an antidromic impulse can be fired into the motoneurone from that strand (Fig. 1*A*, *B*), and the antidromic propagation time can be measured between the first reversal point of the root potential (marked by arrow in Fig. 1*B*) and the onset of the IS spike potential.

When a motoneuronal spike potential was generated by a direct current, as in Fig. 1*D*, *E*, there was always an accompanying spike potential in the ventral root filament, i.e. an impulse was always discharged down the motor axon. When the intensity of the direct current was increased above the threshhold value, the motoneuronal spike potential arose at a progressively shorter latency, as in Fig. 1*E* to *D*, but the discharge of the impulse along the ventral root always occurred at precisely the same time after the onset of the IS spike potential. The measurements were always made from the sharper origin of the IS spike in the differentiated record (cf. Fig. 1*C*).

Similarly, when a motoneuronal spike potential was generated by synaptic stimulation, an impulse was always discharged down the motor axon. It will be seen in Fig. 1*G–J* that the spike potential in the ventral root filament differed from that occurring with direct stimulation in that there was often complication by other impulses discharged in the filament. However, the spike potential belonging to the motoneurone under observation could usually be identified because its form and size were revealed uncomplicated by other potentials in the responses produced by direct stimulation. For example, if Fig. 1*G–J* be compared with Fig. 1*D* and *E*, the spike can be identified even when it occurred amongst other spikes, as in Fig. 1*J*. For any one motoneurone the interval between the onsets of the IS spike and of the spike potential in the ventral root filament has invariably been constant for all strengths of synaptic stimulation, e.g. it was about 0·23 msec in Fig. 1*G–J*, and it has not significantly differed from the latency for direct stimulation, which was also about 0·23 msec for Fig. 1*D* and *E*.

On the other hand, the latency for antidromic propagation up the same length of motor-axon and initiation of the IS spike was about 0·22 msec in Fig. 1*A* and *B*. Usually it has been longer than the times measured as above for orthodromic propagation along the same pathway, and with some motoneurones the discrepancy has been considerable. However the conditions of propagation are very different in the two directions. The measured time for the antidromic propagation is occupied not only in traversing about 15 mm of the medullated axon, but also in initiating the IS spike potential, a process

which normally involves a delay of 0·05–0·1 msec, the M–IS interval (Coombs *et al.* 1957). There were no records at an amplification sufficiently high to enable the M–IS interval to be determined for the motoneurone of Fig. 1, but no significant error would be introduced by assuming a value of 0·07 msec. Thus the antidromic propagation up to the first node of the medullated axon would occupy 0·15 msec in Fig. 1. If the same propagation time is assumed for the orthodromic propagation down the axon, both synaptic and direct

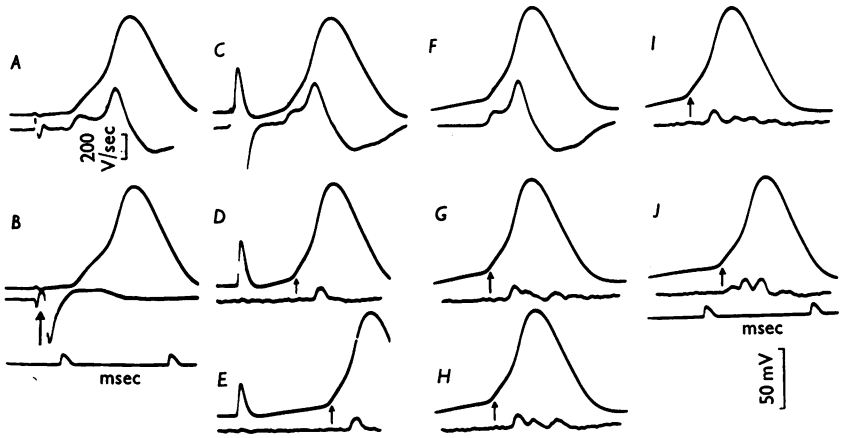

Fig. 1. Upper traces are intracellularly recorded spike potentials evoked in a biceps-semitendinosus motoneurone (resting membrane potential −60 mV) by an antidromic impulse (*A*, *B*), by a depolarizing pulse that began at the artifact and continued throughout the traces (*C–E*) and by monosynaptic activation by an afferent volley from the nerve to biceps-semitendinosus (*F–J*). In *A*, *C* and *F* the lower trace is an electrically differentiated record of the upper trace. In *D*, *E*, *G–J* the lower trace is recorded monophasically from an isolated ventral root filament of L7. In the lower trace of *B* the electrode that records monophasically is used to record the antidromic volley relative to an indifferent earth lead, negativity being downwards. Arrows in *D–J* indicate time of initiation of the impulse in the medullated axon, as calculated from the spike in the ventral root, the measured antidromic conduction time after allowance of 0·07 msec for the M–IS interval (see text). Same voltage scale for all intracellular records, and time scale obtains for all records. A compensatory circuit was employed with the depolarizing pulses of *C–E*.

stimulation would have initiated an impulse in the medullated axon 0·08 msec after the onset of the IS spike potential, as signalled by the arrows in Fig. 1 *D*, *E*, *G–J*. Actually the propagation time in the medullated axon should be a little briefer than with antidromic propagation, because it was occurring along an axon already somewhat depolarized by electrotonic spread of the direct or synaptic depolarization; hence in Fig. 1 *D–J* the initiation of the impulse would be even a little later than the arrows. Fig. 1 thus shows that the IS spike has already developed a potential of about 5 mV at the time of initiation of the impulse in the motor axon. Since the initiation always occurred in the same time relationship to the IS spike, it can be concluded that, with synaptic

and direct stimulation of this motoneurone, the IS spike potential was responsible for depolarizing the medullated axon and so evoking the discharge of the impulse orthodromically propagating along it. Actually it would be adding to the depolarization electrotonically spreading from the soma during the process of synaptic or direct stimulation. As diagrammatically illustrated by Coombs *et al.* (1957, fig. 20*A*) there would be expected to be a very effective electrotonic spread of the SD spike to the central end of the medullated axon, and with the more prolonged potentials occurring with synaptic or direct stimulation this spread would be even more effective.

In some motoneurones the calculated time between the onset of the IS spike and the initiation of the impulse in the first node of the medullated axon was considerably briefer than the 0·08 msec calculated for Fig. 1. However, for 15 motoneurones the value was always positive, the mean being about 0·05 msec, which indicates that a small, but significant, depolarization by the

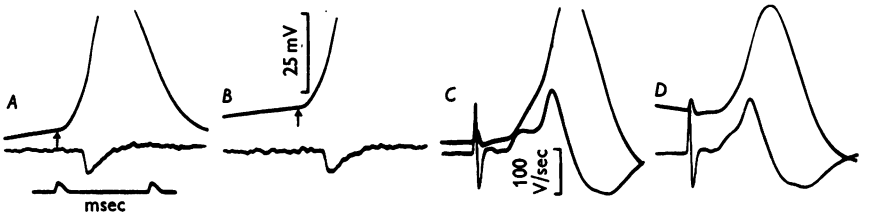

Fig. 2. *A*, *B*, show spike potentials generated in a motoneurone by a depolarizing pulse with the lower trace showing a monophasic record from a filament of L 7 ventral root as in Fig. 1 *D*, *E*. *C*, *D* show antidromic spike potentials set up by a stimulus of three times threshold strength and applied with the cathode at the electrode that was used for the monophasic recording in *A*, *B*. In *D* the motoneurone was conditioned by a depolarizing pulse of 14 nA that was just below threshold for evoking a spike response. On the basis of the antidromic conduction time in *D*, the time of initiation of the discharge is indicated by the arrows in *A* and *B*, and is seen to precede the onset of the IS spike.

IS spike was necessary to generate an impulse at the first node, which already will be depolarized almost as much as is the IS segment by the electrotonic spread of the direct or synaptic depolarization of the soma. Towards the end of three experiments antidromic propagation times became greatly lengthened, even more than double the earlier values, while there was little change in the timing of the discharged impulse relative to the IS spike potential. Presumably the deteriorated motor axon was then conducting asymmetrically, conduction in the orthodromic direction being aided by the preliminary depolarization. In part the lengthening may have been in the M–IS interval. In two other experiments (cf. Fig. 2), even when antidromic conduction time was diminished by a direct or synaptic depolarization that was just subthreshold for initiating an impulse (Fig. 2*D*), the antidromic time was 0·07–0·09 msec longer than the interval between the onset of the IS spike potential and the

arrival of the impulse in the ventral root filament (Fig. 2*A*, *B*). Hence the possibility must be envisaged that at least sometimes the discharged impulse is initiated at the first node and secondarily invades the initial segment.

In Fig. 1*D*, *E*, *G–I* and Fig. 2*A*, *B*, the SD spike potential always began after the initiation of the axonal discharge, which has also been the case in all other motoneurones so investigated. Thus, in the synaptic or direct excitation of a motoneurone, the sequence of events usually is: initiation of the IS spike potential; the IS spike potential initiates the discharge of an impulse down the motor axon; the IS spike potential still later initiates a spike potential in the soma-dendritic membrane. A similar sequence has been observed by Arvanitaki & Chalanozitis (1956) for the ganglion cells of *Aplysia*. Occasionally, as in Fig. 2, the first two stages may be reversed.

In agreement with Fuortes *et al.* (1957) it has been found that the discharge of an impulse along the motor axon was invariably linked with the IS spike. For example in Fig. 3*E*, *G*, a suitably timed hyperpolarizing pulse caused

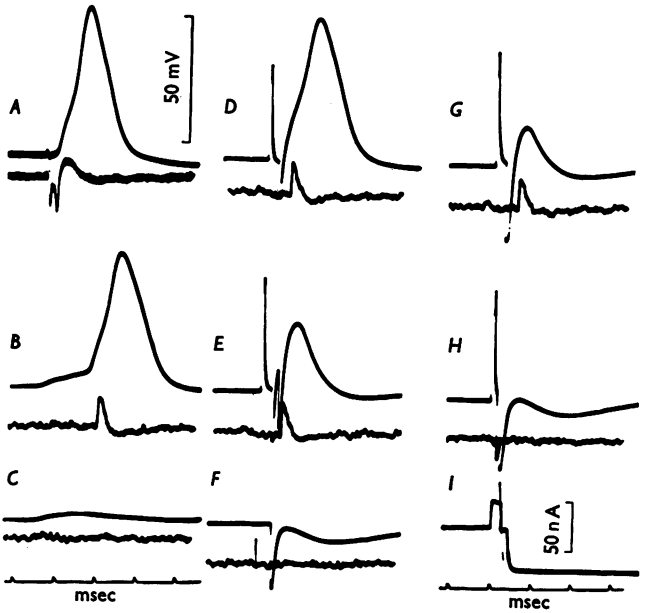

Fig. 3. Conditions of recording resemble those of Fig. 1, but the depolarizing pulse in records *D*, *E*, *G*, *H* was only 0·26 msec in duration, and in *E*, *G*, *H* a prolonged hyperpolarizing pulse was applied at progressively briefer intervals after the onset of the depolarizing pulse, 0·44, 0·30 and 0·16 msec respectively, *F* giving the control record for the hyperpolarizing pulse alone, and *I* the record of the currents when the hyperpolarizing pulse commences 0·41 msec after the onset of the depolarizing pulse. Records *B* and *C* were evoked by monosynaptic excitatory action, that was just above threshold in *B* and below in *C*. Resting membrane potential, −48 mV. Lower traces in *B–H* were recorded from a filament of L7 ventral root as in Fig. 1, while the lower trace of *A* was similar to that of Fig. 1*B*. Same time scales throughout, and same voltage scale for all upper traces.

blockage of IS–SD transmission (cf. Fuortes *et al.* 1957; Coombs *et al.* 1957), but the impulse appeared as usual (cf. Fig. 3*D*) in the ventral root. On the other hand, when the hyperpolarizing pulse caused suppression of the IS spike potential as well, it also suppressed the impulse in the ventral root (Fig. 3*H*). Similarly, with synaptic stimulation of the motoneurone, the impulse only appeared in the ventral root when an IS (and SD) spike was generated (Fig. 3*B*, *C*). These observations are, of course, to be expected on the basis of the earlier finding that the discharge of the impulse precedes the onset of the SD spike.

If with direct and synaptic stimulation an impulse was initiated in the first node of the medullated axon significantly earlier and at a lower threshold than in the initial segment, it would be expected that a suitably timed hyperpolarizing pulse would allow the discharge of an impulse down the motor axon, but suppress the IS spike. Since this has never been observed, it can be concluded that, even if the discharged impulse is sometimes initiated in the first node, the initial segment has virtually the same threshold and latency of response. With its usual intrasomatic location, the micro-electrode is unfavourably placed for discriminating between impulses arising in the first node or in the initial segment under such critical conditions.

The threshold depolarization for initiation of impulses

The IS spike potential. It has already been shown that both direct and synaptic stimulation of motoneurones evoke first an IS spike potential from which arises the SD spike potential (Araki & Otani, 1955; Fuortes *et al.* 1957; Fatt, 1957*b*; Coombs *et al.* 1957). Furthermore, a large part of the surface membrane of the initial segment is in such close electrotonic relationship to the presumed site of the intracellular electrode in the soma that, with such relatively prolonged depolarizations, it would have virtually the same potential as that recorded in the soma (cf. Rall, 1955; Coombs *et al.* 1957, fig. 20*A*). Hence the threshold depolarization for the IS membrane is directly given by the recorded intracellular potential at which the spike arises. The instant at which this occurs is most effectively signalled in the differentiated records (cf. arrows in Fig. 4*B–D*, and Fig. 9*B*). For example, in Fig. 4*B* depolarization to 17 mV by an EPSP is seen to be just critical for evoking an IS–SD spike. Under such critical conditions this spike appeared after a very long latency (1·3 msec in *B*), while in *C* and *D* larger EPSP's were effective at about the same level of depolarization, the spike consequently arising after a shorter latency (0·65 msec in *D*). A comparable series of records is seen in Fig. 11*K–M*, where the EPSP's have been increased by post-activation potentiation. When the depolarization was directly produced by current through one barrel of a double micro-electrode, a spike potential was initiated at approximately the same critical level, which is about 16 mV in Fig. 4*E–H*. The intracellular records of Fig. 4*E–H* give approximately the time course of the membrane

potential change, for the effects of the capacitive coupling and the coupling resistance between the two barrels (Coombs *et al.* 1955*a*) have been virtually eliminated by the operation of a compensatory circuit (Coombs, Curtis & Eccles, 1956). Approximately equivalent thresholds for the origin of the IS spike have also been illustrated in the preceding paper (Coombs *et al.* 1957, Fig. 9*B*, *C*).

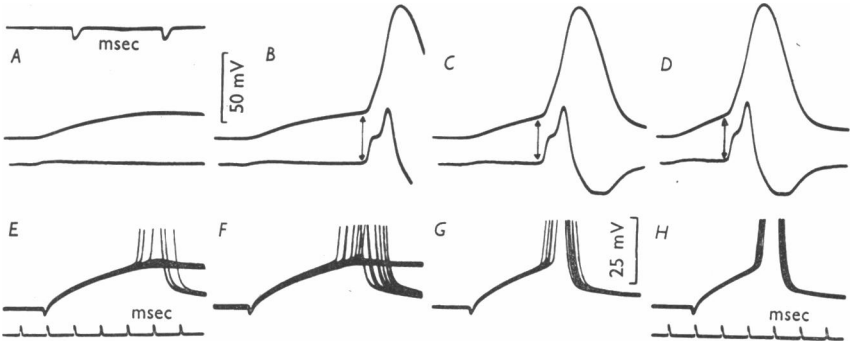

Fig. 4. Intracellularly recorded potentials of a gastrocnemius motoneurone (resting membrane potential −70 mV) evoked by monosynaptic activation that was progressively increased from *A* to *D*, and by a prolonged depolarizing pulse that was applied through the other barrel of a double micro-electrode and progressively increased from *E* to *H*. A compensatory circuit was employed to eliminate capacitive artifacts and also the effect of the coupling resistance. The lower traces in *A*–*D* are the electrically differentiated records, the double-headed arrows indicating the onsets of the IS spikes in *B*–*D*. Separate potential and time scales are shown for the two series. Records *E*–*H* were each formed by the superposition of about twenty faint traces. The spikes are truncated and their steep rising phases have been strengthened to avoid losses in reproduction.

The same critical level of depolarization is observed when different afferent volleys are effective in generating a spike potential of a motoneurone. This is illustrated in Fig. 5 for three different motoneurones: *A*, *B*; *C*, *D*; *E*–*H*. In all three the critical depolarization, as indicated approximately by the arrows, was at the abnormally low value of about 5 mV, which is attributable to the low resting potentials (about −50 mV). Usually the monosynaptic EPSP's from two different muscle nerves were not sufficiently large to evoke spike potentials in motoneurones that had a membrane potential at the normal level of about −70 mV. With the motoneurone of Fig. 4 the critical level for initiating an impulse (17 mV) was one of the highest ever observed (cf. also Figs. 11, 12), the membrane potential being −70 mV and the spike potential recorded at 94 mV. Usually the threshold depolarization was within the range 5–18 mV for motoneurones having resting potentials of −60 to −80 mV and spike potentials above 70 mV (cf. Table 1). A mean value of about 10 mV is also given in Table 1 of Frank & Fuortes (1956) for the threshold synaptic depolarization of the four motoneurones that had spike potentials in excess

of 80 mV. It should be noted that Frank & Fuortes compensated for capacitive losses, whereas the spike potentials in our experiments were probably depressed by as much as 20% (cf. Brock, Coombs & Eccles, 1952).

In all experiments where synaptic and direct stimulation have been effective in generating impulses in a motoneurone, there has, as in Figs. 4 and 5, been no significant difference in the threshold levels of the respective depolarizations. It would appear that the results of Frank & Fuortes (1956) can be similarly interpreted, for they report mean threshold values that presumably do not differ significantly, viz. 7·4 and 8·1 mV for synaptic and direct stimulation respectively, the latter being calculated from the threshold current and

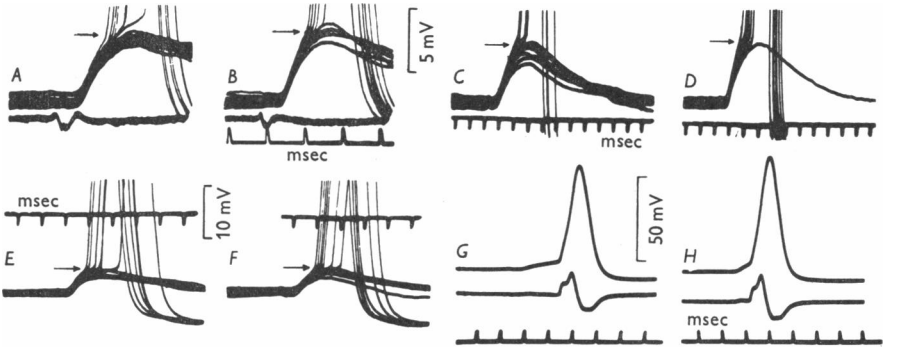

Fig. 5. *A*, *B*; *C*, *D*; *E–H* are intracellularly recorded potentials of three lateral gastrocnemius motoneurones which were evoked by homonymous and heteronymous monosynaptic activation, i.e. by afferent volleys from the lateral gastrocnemius-soleus and the medial gastrocnemius nerves respectively. *A–D* have the same potential scales, and *A*, *B* the same time scales. Different potential and time scales for *E*, *F* and *G*, *H*. Records *A–F* were formed by the superposition of about twenty faint traces. In *A–F* arrows indicate the approximate threshold levels of depolarization.

TABLE 1. Potentials and thresholds of motoneurones

Cell type	Resting potential (mV)	Spike potential (mV)	IS threshold (synaptic) (mV)	SD threshold	
				Synaptic (mV)	Antidromic (mV)
BST	−50	77	7	25	23
G	−60	75	5	19	21
BST	−56	74	7	25	23
BST	−60	80	7	25	25
G	−70	95	17	37	37
G	−67	75	9	20	24
BST	−80	87	18	29	32
Pl	−66	69	9	19	19
FDL	−70	77	15	28	32
FDL	−59	70	7	20	19
FDL	−68	84	16	28	29
Mean values	−64·2	78·5	10·6	25	26

BST = Biceps-semitendinosus, Pl = Plantaris, G = Gastrocnemius, FDL = Flexor digitorum longus.

the measured value for the membrane resistance. Likewise it has already been reported that, when an IPSP has been inverted to a depolarizing response by injecting Cl^- or NO_3^- ions, it caused the motoneurone to generate an impulse at the same level of depolarization as a monosynaptic EPSP (Coombs *et al.* 1955*b*), and that a similar threshold also obtained for monosynaptic and polysynaptic EPSP's (cf. Eccles, 1953). Bonnet (1956*a*, *b*) has reported experimental investigations on the effect of depolarizing currents on amphibian motoneurones and has also concluded that direct and synaptic depolarization are equivalent in evoking discharges.

A particularly effective test for the postulated equivalence of direct and synaptic depolarizations is proved by superimposing an EPSP on directly produced depolarizations of varying sizes up to threshold, as has been done in Fig. 11 *B–I*. It has been reported that under such conditions an impulse is generated by the EPSP when its addition to the direct depolarization brings it to the same total level of depolarization (Coombs *et al.* 1955*a*; Eccles, 1957, fig. 17). This is also illustrated in Fig. 12*A*, where the series partly illustrated in Fig. 11 *A–I* is plotted by the filled circles. The threshold level of depolarization is seen to increase a little (from 18 to 21 mV) with increase in the depolarizing current. Possibly this is attributable to accommodation (see Discussion) or possibly the coupling resistance between the two barrels was somewhat higher than the estimated value of 0·35 MΩ.

A more discriminative test can be applied by adjusting the size of the EPSP so that it is just at threshold when superimposed on each testing level of depolarization, as has been done in Fig. 6*B–F*. When allowance is made for the potential drop in the computed coupling resistance between the two barrels, the total depolarization is found to be virtually identical (16 mV) for all levels of the direct depolarization, as may be seen in the plotted points of Fig. 7. In Fig. 6*F* the depolarizing current was just below the threshold for initiating impulses, as was also indicated by the very small additional EPSP that was effective in generating impulses. It is preferable to perform this test, as in Figs. 6 and 7, without a compensatory circuit for neutralizing the initial capacitive artifacts, and to superimpose the EPSP after these artifacts have subsided. When plotting the threshold depolarizations, as in Fig. 7, it is then merely necessary to allow for the coupling resistance between the two barrels of the micro-electrode.

With antidromic propagation into a motoneurone, the IS spike is initiated by the depolarization produced by the activated nodes of the medullated axon. When recorded with an intrasomatic position of the micro-electrode, this depolarization (the M spike) is usually only 1–2 mV (cf. Coombs *et al.* 1957, Fig. 6 *H–K*). However, in contrast to direct or synaptic stimulation, where depolarization electrotonically propagates very effectively from the soma into the initial segment, an intrasomatic electrode is very unfavourably placed for

recording a depolarization electrotonically propagating antidromically into the initial segment from the medullated axon, as may be seen by reference to fig. 20*A* of the preceding paper (Coombs *et al.* 1957). On the rare occasions

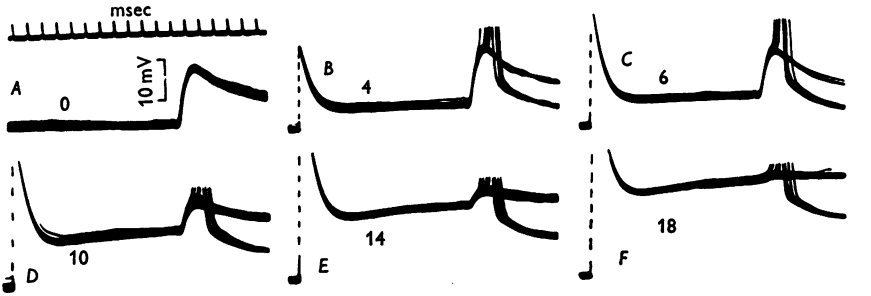

Fig. 6. Intracellularly recorded potentials from a biceps-semitendinosus motoneurone (resting membrane potential, −74 mV) which were evoked monosynaptically in *A*, and by monosynaptic activation that was applied 11·5 msec after the onset of a depolarizing pulse in *B*–*F*. The depolarizing pulse was progressively increased in strength from *B* to *F*, the respective currents being indicated in nA. Correspondingly, the monosynaptic activation had to be diminished in order that the combined stimulation would remain just effective in evoking a spike potential in about half of the trials. All records were formed by the superposition of about twenty faint traces. Since a compensatory circuit was not employed, the onset of the depolarizing pulse caused a large potential change due to capacitive coupling between the barrels. The spikes are truncated and their steep rising phases have been strengthened. At the onset of the current pulse there was a large artifact whose rising phase is shown by a broken line (see text).

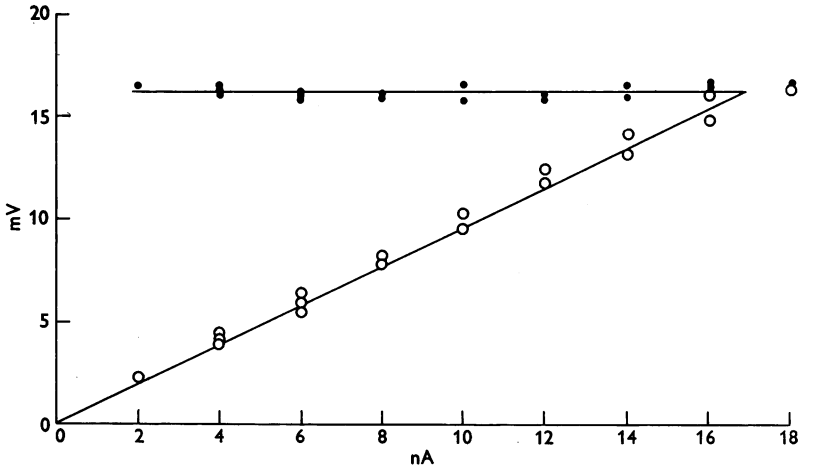

Fig. 7. Plotting of the membrane potentials (ordinates) against depolarizing current intensity (abscissae) for the series partly illustrated in Fig. 6, allowance being made for the potential drop in the coupling resistance between the two barrels of the microelectrode (0·35 MΩ), as measured from the series plotted in Fig. 12*B* (see text). ○, membrane potential just before the onset of the monosynaptic excitation; ●, the membrane potential that was just critical for initiating a spike. The line through the open circles has a slope corresponding to a membrane resistance of 0·96 MΩ.

when the large size of the IS spike relative to the SD spike indicated that the micro-electrode tip was located in the region of the axon hillock (cf. Coombs *et al.* 1957, figs. 4–6), the M spike potential was much larger, 7–10 mV. When the IS spike was almost as large as the SD spike, as in Fig. 8*D*, it may be assumed that the micro-electrode was recording from the IS segment almost as favourably as if it were actually inserted in this segment. It will be observed that the IS spike was then initiated by the M spike (Fig. 8*C*, *D*) at virtually the same level of depolarization as when it was initiated by the EPSP (Fig. 8*B*). Thus it can be concluded that all three methods of initiating IS spikes are dependent simply on the production of the same critical level of depolarization of the IS segment, which normally is in the range 7–17 mV.

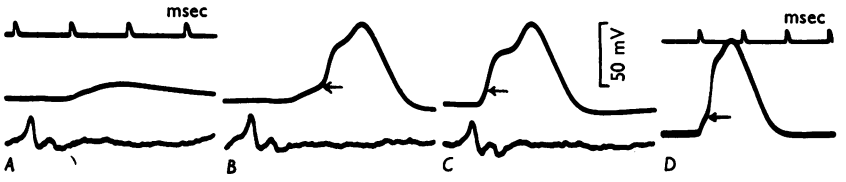

Fig. 8. Intracellular potentials in a biceps-semitendinosus motoneurone, the micro-electrode being presumed to be in the initial segment, as indicated by the large size of the IS spike relative to the SD spike (see text). *A*, *B*, monosynaptic activation by an afferent volley from biceps semitendinosus nerve. *C*, *D*, activation by an antidromic impulse from the biceps-semitendinosus nerve. In *A*–*C* the lower traces show the potentials recorded from the surface of the spinal cord.

The SD spike potential. With all methods of initiating impulses in motoneurones, synaptic, direct or antidromic, the SD spike potential is not initiated until the SD membrane has been very considerably depolarized by the IS spike potential (Fatt, 1957*b*; Fuortes *et al.* 1957; Coombs *et al.* 1957; Eccles, 1957). Since during the IS spike, the maximum extracellular potential relative to the indifferent earth lead is about 1 mV in the opposite sense to the intrasomatically recorded potential (Fatt, 1957*a*), the depolarization of the SD membrane will actually be about 1 mV larger than the IS spike as recorded intrasomatically, and not less, as has been previously assumed (Brock *et al.* 1953; Eccles, 1955, 1957). As illustrated in Fig. 9 the SD spike is taken to begin at the first sign of inflexion from the differentiated IS spike potential and is seen to be much earlier than the SD origin that would be derived by the earlier procedure of timing its origin by the inflexion in the potential records. As a consequence the threshold level of depolarization for the SD spike as measured in Fig. 9, and reported in Table 1, is considerably lower than has been previously reported (cf. Coombs *et al.* 1955*a*; Eccles, 1957).

With motoneurones in good condition the threshold depolarization has a value in the range 20–35 mV, and often is 1–2 mV larger with antidromic than with direct or synaptic stimulation (Table 1). Possibly this difference arises

because with antidromic stimulation virtually the whole of this depolarization has to be produced by the IS spike, whereas, with direct and synaptic stimulation, the IS spike has merely to increase the depolarization of the soma-dendritic membrane from 7–17 mV to 20–35 mV.

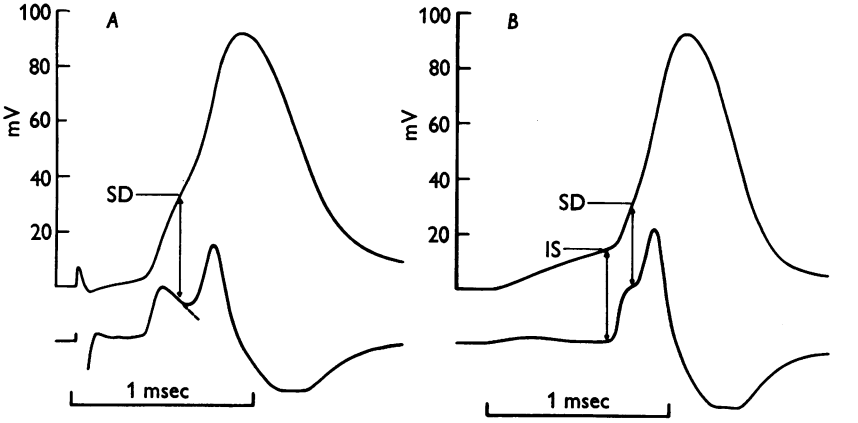

Fig. 9. *A*, *B*, tracings of intracellularly recorded spike potentials evoked by antidromic and monosynaptic stimulation of a motoneurone respectively, the original records being already illustrated (Coombs *et al.* 1957, fig. 9 *A*, *B*). The lower traces show the electrically differentiated records. Perpendicular lines are drawn from the origins of the IS and SD spikes, as indicated in the differentiated records, the respective threshold depolarizations being thus determined from the potential records, and indicated by horizontal lines labelled respectively IS and SD.

When the SD membrane is depolarized or hyperpolarized by the application of a brief current through one barrel of a double micro-electrode, an SD spike is generated at lower or higher levels respectively of the IS spike potential (Figs. 10, 11). When measurements from Figs. 10 and 11 are corrected for voltage-drop in the coupling resistance, they may be plotted as in Fig. 12*A* to show that the alteration in the threshold level for generation of the SD spike largely compensates for the initial change in the membrane potential.

The method for determining the coupling resistance is illustrated in Fig. 12*B* and is based on the assumption made by Frank & Fuortes (1956) that at the summit of the spike potential the membrane potential is altered by applying a current across the membrane only in so far as it is passively changed by the voltage-drop arising on account of the passage of the current through the membrane resistance. When the spike potential is plotted against the applied currents in Fig. 12*B* (filled circles), the slope of the curve gives a resistance of 0·40 MΩ, which is partly attributable to coupling resistance between the two barrels and partly to the resistance of the active membrane. If the active membrane at the spike summit is assumed to have a resistance of 5% of the resting membrane, which is relatively higher than the value determined for giant axons and muscle fibres (Fatt & Katz, 1951; Hodgkin & Huxley, 1952),

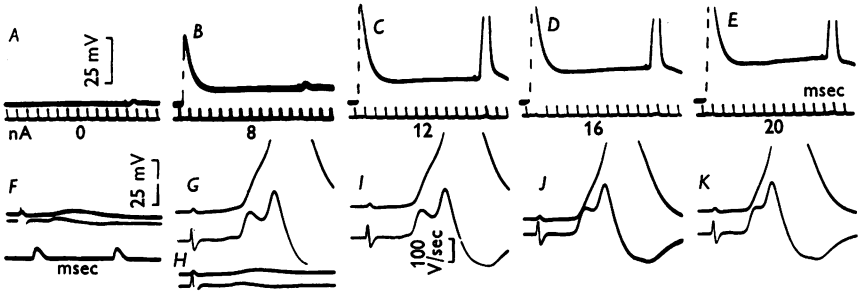

Fig. 10. Intracellular records with a double micro-electrode in a biceps-semitendinosus motoneurone (resting membrane potential −70 mV) which was activated by a depolarizing pulse applied 12·0 msec before an antidromic impulse. The lower records *F–K* give on a much faster time scale the antidromic responses corresponding to the records immediately above, the current strengths in nA being indicated between each set. A pulse of 20 nA was just below threshold for generating an impulse. The lower traces of *F–K* give the electrically differentiated records. At 8 nA the antidromic impulse sometimes gave only the M spike (*B*, *H*) as seen also with no conditioning current in *A*, *F*, and sometimes the IS–SD spike as well (*G*). Same potential and volt/sec scales for all records; also, as in Fig. 6, a compensatory circuit was not employed, hence the large initial artifacts in *B–E*.

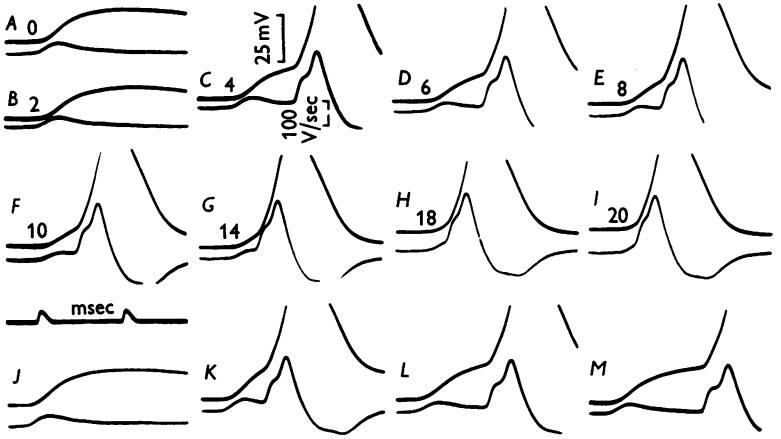

Fig. 11. Intracellular records obtained some minutes later from the same motoneurone as for Fig. 10, but in *B–I* evoked by monosynaptic activation that was applied at 12·0 msec after the onsets of depolarizing pulses, whose strengths are indicated in nA. Again no compensatory circuit was employed. The time courses of the potentials produced by the depolarizing pulses were similar to those illustrated in Fig. 10*B–E*. A pulse of 20 nA was just below threshold for generating a spike. A shows control EPSP in the absence of a depolarizing pulse. In *K–M* no depolarizing pulse was applied, but the EPSP was increased above threshold by post-activation potentiation after 15 sec tetanization at 680/sec, *K* showing record at height of potentiation and *L*, *M* during its decline. *J* is the subthreshold EPSP before potentiation. Lower traces give electrically differentiated records. Same voltage, volt/sec and time scales for all records. Note that spikes are truncated.

the coupling resistance between the two barrels must account for (0·40–0·05) MΩ, for the resting membrane resistance is shown to be about 1·0 MΩ by the slope of the resting membrane potential line (open circles in Fig. 12*B*). This slope gives a resistance of 1·36 MΩ, which is compounded of the coupling resistance 0·35 MΩ (as calculated above) and the resting membrane resistance, 1·01 MΩ.

The calculated threshold levels of depolarization for initiation of the SD spike are seen in Fig. 12*A* (open circles) to increase from about 30 to 32 mV with increase of the conditioning depolarizing current. Possibly this is a result of accommodation during the 12 msec application of the current, which would increase in its effect as the current was increased (Coombs, Curtis & Eccles, unpublished observations). Alternatively the slope could be due to too low a value being assumed for the coupling resistance.

When conditions are just critical for blockage of antidromic invasion of the soma-dendritic membrane, local responses of the soma-dendritic membrane are evoked instead of the full SD spike (Coombs *et al.* 1957). It is likely that

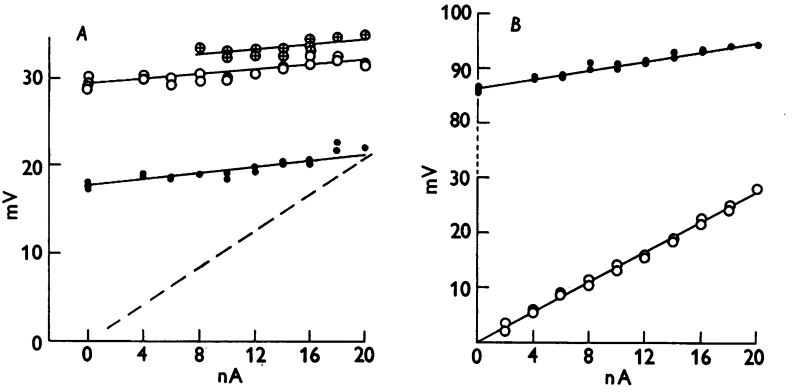

Fig. 12*A*. Measurements from the series partly illustrated in Figs. 10 and 11 have been made for the threshold depolarizations for generating the IS and SD spike potentials, the methods of measurement being illustrated in Fig. 9, and are plotted on potential-current co-ordinates as indicated. IS thresholds are plotted as ● points, while ○ and ⊕ points give the SD thresholds for synaptic and antidromic stimulation respectively. An allowance of 0·35 MΩ has been made for the coupling resistance between the two barrels, which has been derived as shown in Fig. 12*B*. The oblique broken line shows the membrane potential change calculated for a resistance of 1·01 MΩ as derived in Fig. 12*B* (see text).

Fig. 12*B*. The upper plotted points (●) give the recorded potentials at the summits of spike potentials generated by monosynaptic stimulation that was applied 12 msec after the onset of depolarizing pulses whose strengths are plotted as abscissae. Measurements are from lower amplification records photographed simultaneously with those of Fig. 11. The points (○) give the recorded potentials just before the synaptic stimulation, i.e. the difference between the lower and upper points for any current strength gives the total height of the spike potential evoked by the synaptic stimulation. The slopes of the lower and upper lines correspond respectively to resistances of 1·36 and 0·40 MΩ. Further description in text.

these responses arise in the soma-dendritic membrane adjacent to the IS segment, which will be more effectively depolarized by electrotonic spread from the IS spike potential (Coombs *et al.* 1957, fig. 20*A*). For this reason it is likely that this zone normally responds by a spike potential before the remainder of the SD membrane, and so would act as a type of intermediate response adding to the depolarization produced by the IS spike. If that is so, our measurements of the threshold level for the SD membrane would be too low by several millivolts. In effect the intrasomatic electrode picks up from the cell interior a weighted mean of the potentials across the various regions of the soma-dendritic membrane, and so virtually ignores the higher depolarization which the IS spike potential induces in the immediately adjacent SD membrane. If it be assumed that the whole SD membrane has the same threshhold level of depolarization (cf. Coombs *et al.* 1957), the initial part of the SD spike will arise in this local region and the generalized SD response will not begin until some millivolts higher depolarization is attained a little later on the inflected curve. This effect is particularly evident with antidromic activation of the SD membrane, where, as indicated in Fig. 9*A*, there is a much slower onset of the SD spike than with synaptic activation (Fig. 9*B*).

DISCUSSION

In Fig. 12*A* (● points) the threshold levels of depolarization for generating the IS spikes appeared to increase as relatively more of it was contributed by the applied current. This must not be taken to establish that a depolarization produced in this way is less effective than that synaptically evoked (cf. Frank & Fuortes, 1956), for the current was applied for 12 msec before the synaptic excitation was superimposed on it. Since motoneurones exhibit a significant accommodation in this time (Frank & Fuortes, 1956; Coombs, Curtis & Eccles, unpublished observations), the higher thresholds with larger currents could be explained in this way. However, a degree of uncertainty is introduced by the allowance for the coupling resistance. For example, if the coupling resistance in Fig. 12*A* was as high as 0·5 MΩ, which seems very unlikely (cf. Fig. 12*B*), the threshold depolarization would be virtually unaffected by current intensity. Likewise, if the coupling resistance in Fig. 7 were less than the assumed value, the threshold would increase with current intensity. It is desirable to repeat these experiments without double electrodes that have very low and stable coupling resistances.

Table 1 gives the IS and SD thresholds for motoneurones which were in reasonably good condition, as revealed by their resting and spike potentials. It has been assumed that motoneurones giving lower potentials have been injured by the micro-electrode and consequently no special significance should be attached to their low IS and SD thresholds. Possibly some of the thresholds reported in Table 1 have also been lowered by injury, and hence the very

large range of values would be explained. However, for the present it may be assumed that the mean threshold depolarizations for generating IS and SD spikes are 10 and 30 mV respectively. The SD threshold has been increased by 5 mV over the mean given in Table 1 in order to allow for the extrinsic potential and the zone of specially large depolarization adjacent to the IS membrane (cf. Coombs *et al.* 1957, fig. 20*A*).

It seems that this remarkable difference in threshold for two zones of the surface membrane of a cell cannot fully be accounted for by such extrinsic factors as the dense coverage of the soma-dendritic membrane by synaptic knobs or glial cells (cf. Eccles, 1957, pp. 51–2). Thus it is postulated that there is a difference between the membranes of the two zones. Such a difference is also indicated by the after-potentials, for the after-hyperpolarization is large after the SD spike and undetectable after an IS spike (Coombs *et al.* 1955*a*). A difference in the two zones is also indicated by cytological evidence, for the initial segment is distinguished from the soma and dendrites by the absence of Nissl substance and pigment granules (Chu, 1954) and by the sparseness of synaptic endings on its surface (Cajal, 1909; Hoff, 1932; Lorente de Nó, 1938; Barr, 1939).

By extrinsic recording of the potential fields produced during antidromic activation of motoneurones the velocity of impulse conduction in the dendrites has been measured as 2 m/sec (Lorente de Nó, 1947, 1953) or even as low as 0·7–1·0 m/sec (Fatt, 1957*a*). It is possible to account for these very slow conduction velocities, if it be assumed that the surface membrane along the whole length of the dendrites has the high threshold characteristic of the soma and proximal dendritic regions, which is the only region accessible to our methods of intracellular investigation.

It was shown above that with direct or synaptic activation of the motoneurone the impulse discharged down the motor axon was sometimes so early that it was probably initiated in the first node of the medullated axon and not in the IS segment. Since there are only a few microseconds between these events, a more discriminative investigation is required before these two possible origins can be distinguished. However, it should be pointed out that the impulse discharged from the Pacinian corpuscle arises at the first node (Diamond, Gray & Sato, 1956). Nevertheless, the situation here differs in that the impulse never invades the non-medullated axon of the Pacinian corpuscle, which by its depolarization provides the 'generator potential' for the first node. By contrast, if an impulse is generated in the first node of the medullated axon, it always invades the initial segment. There are no special difficulties in explaining the origin of the impulse in the first node, for it may be very close to the initial segment (within 200 μ, cf. Fatt, 1957*a*) and consequently the direct or synaptic depolarization would be but little more attenuated than in the IS segment.

The functioning of the motoneurone as an integrating unit is specially aided by the low threshold of the initial segment relative to the soma-dendritic membrane. As a consequence of this threshold difference, excitatory synaptic bombardment causes impulses to be generated in the initial segment and not anywhere over the whole soma-dendritic membrane. If these latter conditions obtained, a special strategic grouping of excitatory synapses (cf. Lorente de Nó, 1938) could initiate an impulse despite a preponderant inhibitory action elsewhere and a relative paucity of the total excitatory synaptic bombardment of areas remote from this focus. Such a breakdown of the integrative function of a motoneurone is prevented by the high threshold of the soma-dendritic membrane relative to the initial segment. Since synapses can thus be effective only in so far as they can depolarize the initial segment, those synapses remotely placed on dendrites will be functionally ineffective in view of the estimated space constant of 300 μ (Coombs *et al.* 1955*a*). Possibly this relative ineffectiveness of remote dendritic synapses may be correlated with their sparse distribution in such regions (Lorente de Nó, 1938; Barr, 1939; Bodian, 1952).

SUMMARY

1. By means of synaptic stimulation or by applying a depolarizing current through an intracellular electrode, motoneurones have been caused to discharge impulses which have been recorded in ventral root filaments.

2. This discharge was always associated with a spike in the initial segment of the motoneurone (the IS spike), but was quite independent of the soma-dendrite spike (the SD spike). Moreover, the impulse appeared in the ventral root so early after the onset of the IS spike that it must have been initiated either by the earliest part of the IS spike or even just before by a primary response of the first node of the medullated axon. It always arose before the onset of the SD spike.

3. When allowance is made for accommodation, it appears that depolarizations produced by synaptic action or directly by applied currents are equipotent in generating motoneuronal spikes.

4. The threshold depolarization for the initial segment was found to be in the range 5–18 mV (mean about 10 mV) for motoneurones relatively undamaged by the micro-electrode, while for the soma-dendritic membrane the range was 20–37 mV.

5. The relationship of these findings to the functioning of the motoneurone is briefly discussed.

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