PROGRESS IN BRAIN RESEARCH

VOLUME 12

PHYSIOLOGY OF SPINAL NEURONS

EDITED BY

J. C. ECCLES

AND

J. P. SCHADÉ

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PHYSIOLOGY OF SPINAL NEURONS

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Preface

The remarkable progress in basic neurophysiology over the last few years is illustrated by the fine collection of reviews in this volume. Gathered for this week of lectures and discussions were representatives from many of the leading schools of research into the properties of nerve cells and of their functional organization in the spinal cord, which has long been regarded as the simplest level of the central nervous system. However, after reading the complexities of neuronal interconnection here described, one may well wonder if this is an illusion!

A tremendous amount of integration is carried out at the spinal level — far more than Sherrington conceived in his classic book '*The integrative action of the nervous system*' — but, of course, this development in our concepts of spinal integration would have delighted him. One can predict that much more complexity of behaviour will be revealed as methods of investigation become more refined and are pursued with that systematic intensity that characterizes so much of present neurophysiology. Our ultimate hope undoubtedly is that, with increasing knowledge of neuronal interconnections, there will emerge clear ideas on basic patterns of neuronal organization, the same general type of pattern being employed in integrating the many different modalities of input.

Of great importance are the many modes of control exercised by the higher centres onto the spinal mechanisms. Though the wealth of descending pathways had long been revealed by anatomical investigations, there have until recently been only relatively crude concepts of the mode of operation of these pathways, both on the local mechanisms in the spinal cord, and on the relay of impulses up the ascending tracts to the brain. More than one third of this volume is devoted to the descending and ascending pathways, and a wealth of new information and ideas will be found in these important papers. We can anticipate many new developments in these attempts to understand the physiology of communication up and down the spinal cord.

I am going to be rash enough to predict that the centre of interest in the nervous system is now moving from the investigation of properties of the individual neurones and of the individual synapses to the much wider concepts of the patterns of functional organization, which give ultimate meaning to the individual neuronal and synaptic properties and subsume all these into the various levels of organization. In the context of these ideas we can appreciate that far more work is required on the way in which the nervous system handles inputs produced by carefully controlled adequate stimulation.

The cruder physiological methods of electrical stimulation are, of course, needed in order to define the patterns of connection and the modes of operation; but this understanding must be given functional meaning by rigorous investigations with adequate inputs and into the way in which these act and interact.

J. C. ECCLES

Contents

The Excitatory Responses of Spinal Neurones

J. C. ECCLES

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The body and dendrites of neurones are specialized for the reception and integration of information from other nerve cells, which of course occurs via the encrusting synaptic structures. During normal activity any one neurone is continuously bombarded by impulses impinging at its synapses, some of which are excitatory and tend to make this neurone in turn fire an impulse while some have an inhibitory action tending to prevent the initiation of impulses. My present task is to give an account of the way in which excitatory synaptic action causes spinal neurones to discharge impulses.

An initial brief reference must be made to the ionic composition of nerve cells and to the electrical charges on their surfaces because these are essentially concerned both in nerve impulse transmission and in synaptic action. As shown in Table I the surface

TABLE I

IONIC CONCENTRATIONS AND EQUILIBRIUM POTENTIALS FOR CAT MOTONEURONES

Outside mM	*Inside mM*	*Equilibrium potential (according to the Nernst equation) in mV*
Na 150	about 15	about +60
K 5.5	150	—90
Cl 125	9	—70

membrane of a nerve cell separates two aqueous solutions that have very different compositions. Within the cell sodium and chloride ions are at a lower concentration than outside, whereas with potassium there is an even greater disparity — almost 30-fold — in the reverse direction. Under resting conditions potassium and chloride ions move through the membrane much more readily than sodium. Necessarily the electrical charge across the membrane influences the rates of diffusion of charged particles in both directions between the interior and exterior of the cell. The potential across the surface membrane is normally about —70 mV, the minus sign signifying inside negativity.

As seen from the table the *equilibrium potential* for chloride ions is approximately

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the same as the resting potential, which signifies that under such conditions the inward and outward diffusion of chloride ions approximately balance. On the other hand the large electrochemical potential difference for sodium ions (130 mV) will cause the diffusion of sodium inwards to be more than 100 times faster than outwards. Fortunately the resting membrane is much less permeable to sodium than to potassium and chloride ions: but of course there must be some other factor concerned in balancing sodium transport across the membrane. Hence we have the postulate that there is built into the membrane a kind of pump that uses metabolic energy to force sodium ions uphill (up the electro-chemical gradient) and so outward through the cell membrane, as is diagrammatically shown in Fig. 1. This diagram further shows that there

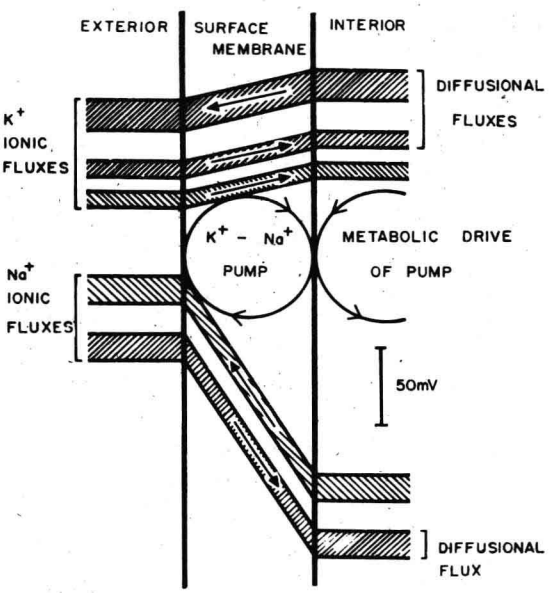

Fig. 1. Diagrammatic representation of K^+ and Na^+ fluxes through the surface membrane in the resting state. The slopes in the flux channels across the membrane represent the respective electrochemical gradients. At the resting membrane potential (—70 mV) the electro-chemical gradients, as drawn for the K^+ and Na^+ ions, correspond respectively to potentials which are 20 mV more negative and about 130 mV more positive than the equilibrium potentials (note the potential scale). The fluxes due to diffusion and the operation of the pump are distinguished by the direction of hatching. The outward diffusional flux of Na^+ ions would be less than 1% of the inward and so is too insignificant to be indicated as a separate channel in this diagram, because the magnitudes of the fluxes are indicated by the widths of the respective channels. (From Eccles, 1957.)

is an excess of diffusion outwards of potassium down the electro-chemical gradient of about 20 mV, and again the transport of potassium ions is balanced by an inward pump. In fact, as shown, the sodium and potassium pumps are loosely coupled together and driven by the same metabolic process, which is now fairly well defined (Hodgkin, 1958; Caldwell *et al.*, 1960a,b).

THE SPIKE POTENTIALS OF SPINAL NEURONES

Under resting conditions the surface membrane of the nerve cell and its axon resembles a leaky condenser charged at a potential of about —70 mV. If this charge is suddenly diminished, by about 20 mV, *i.e.* to —50 mV, it initiates an intense regenerative process adding to the depolarization and reversing the membrane potential as may be seen in Fig. 2A at —77 mV. It is postulated that this regenerative process is due to a high sodium permeability which occurs for only a fraction of a millisecond and is followed by a rapid development of a high potassium permeability with recharging of the membrane by outward movement of potassium ions (*cf.* Hodgkin,

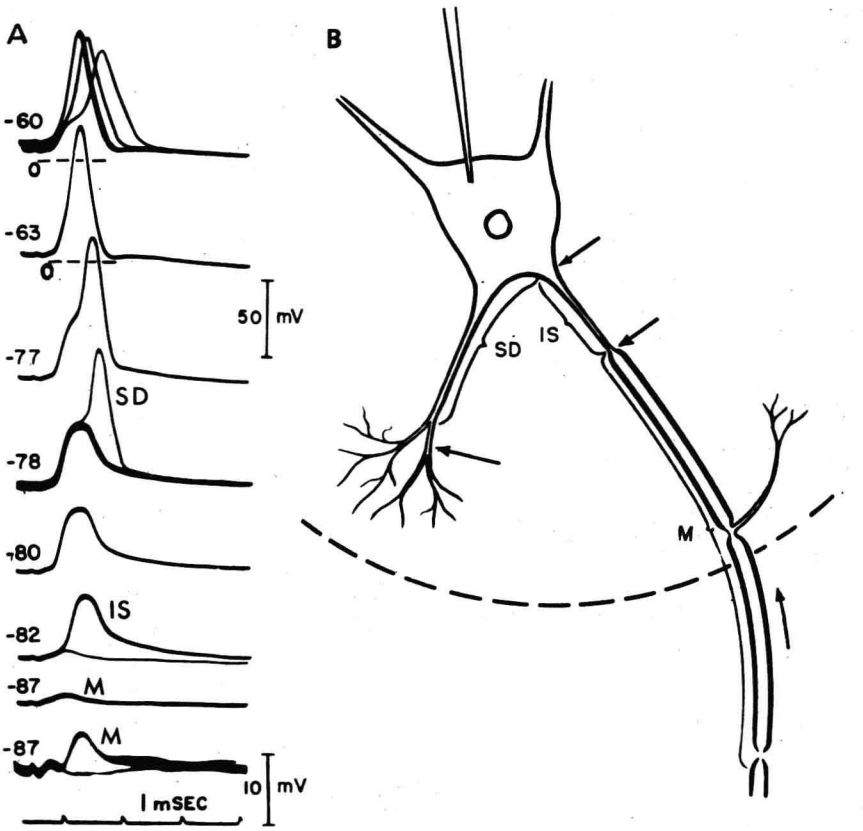

Fig. 2. (A). Intracellular responses evoked by an antidromic impulse, indicating stages of blockage of the antidromic spike in relation to the initial level of membrane potential. Initial membrane potential (indicated to the left of each record) was controlled by the application of extrinsic currents. Resting potential was at —80 mV. The lowest record was taken after the amplification had been increased 4.5 times and the stimulus had been decreased until it was just at threshold for exciting the axon of the motoneurone. (From Coombs *et al.*, 1955a.) (B). Schematic drawing of a motoneurone showing dendrites (only one drawn with terminal branches), the soma, the initial segment of axon (IS) and the medullated axon (M) with two nodes, at one of which there is an axon collateral. The three arrows indicate the regions where delay or blockage of an antidromic impulse is likely to occur. The regions producing the M, IS, and SD spikes are indicated approximately by the labelled brackets. (From Eccles, 1957.)

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1958). As a consequence the membrane potential rapidly is restored to normal. The potential change lasts for less than one thousandth of a second, its brevity earning it the name, *spike potential.* This general statement may serve as an introduction to a more detailed treatment of neuronal spike responses.

The complex morphology of a neurone is associated with a corresponding complexity in its excitatory responses. For example in Fig. 2B an antidromic impulse propagates in the direction of the arrow up to the motoneurone in which has been inserted a microelectrode, as shown diagrammatically. The full sequence of potential changes is illustrated in Fig. 2A in which current applied through one barrel of a double microelectrode changed the membrane potential from the resting level of —80 mV either up as far as —87 mV or down as low as —60 mV. This procedure (Coombs *et al.*, 1955a) shows that the antidromic spike potential has three distinct components, each of all-or-nothing character. There is first the very small spike (about 5 mV) that is seen alone sometimes at —82 mV and always at —87 mV, and which is shown by threshold differentiation (lowest record of Fig. 2A) to be generated by an impulse in the motor axon of the impaled motoneurone.

Second there is the larger spike of about 40 mV that is always set up at membrane potentials of —80 mV or less, and also sometimes at —82 mV. Finally, the full-sized spike is superimposed at membrane potentials of —77 mV or less, and rarely at —78 mV. Fig. 2B shows the antidromic pathway together with the regions of the motoneurone (M, IS and SD) in which the three components of the spike potentials are believed to be generated.

This identification was made originally (Brock *et al.*, 1953; Coombs *et al.*, 1955a) on the grounds that the large spike must be generated in that part of the motoneuronal membrane that is most closely related to the intracellular electrode, that is in the membrane of the soma and adjacent dendritic regions; and it was supported by an analysis of the extracellular field potentials generated by antidromic invasion of a single motoneurone (Fatt, 1957a) and of the action of an antidromic impulse on excitatory synaptic potentials. The conclusions from these earlier arguments have been fully confirmed by the very rigorous investigations of Terzuolo and Araki (1961) and Araki and Terzuolo (1962). The diagrammatic assignment in Fig. 2B of specific regions of the motoneurone to the three types of spike potentials in Fig. 2A, can therefore be regarded as firmly established, and it will be convenient to use the terms IS and SD spikes as indicated in Fig. 2.

By means of double microelectrodes Terzuolo and Araki (1961) recorded simultaneously from inside a motoneuronal soma and just outside it. The intracellular antidromic responses in Fig. 3A and B resemble either the large composite IS–SD spikes of Fig. 2A, with an inflection on the rising phase, or else the smaller IS spike, as with some responses evoked by the second antidromic impulse in Fig. 3B. The composite IS–SD spike is seen to be associated with a complex extracellular potential: firstly a negligible upward deflection (positivity), then a double downward negative wave, and a final large positivity. The second negative wave and the final positivity are shown in Fig. 3B to be associated with the SD spike, because, in the superimposed traces for the second antidromic response, these two waves are eliminated when the SD

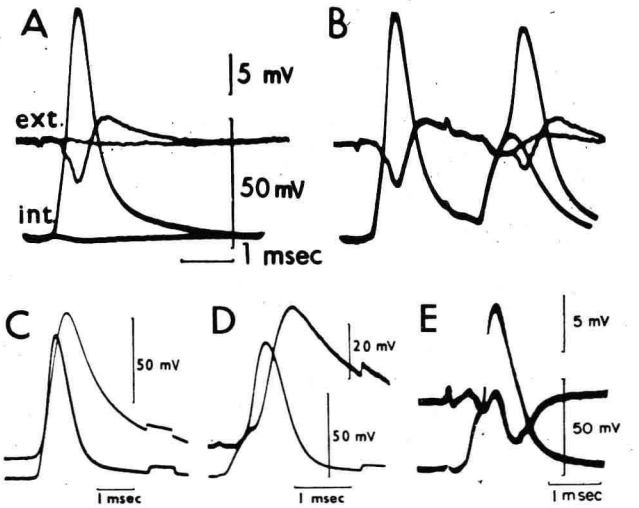

Fig. 3. (A, B). Potential changes recorded simultaneously, inside and outside the soma of spinal motoneurones with parallel microelectrodes. In A the stimulus was adjusted to activate the axon in the ventral root in approximately half of the superimposed traces of antidromic spike responses. In B the stimuli were applied to the ventral root at a short time interval, so that in some of the trials the soma–dendrite complex was not invaded. (C) and (D) show simultaneous intracellular traces of dendritic and soma spike potentials from the same motoneurone. In (E) there was simultaneous intracellular and extracellular recording as in A, but the extracellular recording was from a remote dendrite. (From Terzuolo and Araki, 1961.)

invasion fails. Furthermore, the onset of the second extracellular negative wave always occurs at the inflection between the IS and SD spike potentials.

Terzuolo and Araki (1961) point out that in interpreting these potential changes it is essential to recognize that the extensive radiating dendritic arborization of a motoneurone gives a closed field organization of extracellular potentials (*cf.* Lorente de Nó, 1947, 1953), as soon as the antidromic impulse invades the initial segment of the axon and the soma, which both lie approximately central to the dendritic arborization. The dendrites form the dominant source for extracellular currents flowing radially inwards first to the activated initial segment, then into the activated soma; hence an extracellular electrode in close proximity to the soma, and thus near to the centre of the closed field, would be expected to record negativity for both of these activations, as is seen to occur in Fig. 3A and B. The terminal positive wave only occurs after an SD spike potential, which is illustrated in Fig. 3B. This association indicates that the spike potential propagates from the soma out along the dendrites so that the soma becomes a source for extracellular current flowing into the sinks on the dendrites.

Conclusive evidence for impulse propagation along dendrites is provided by Fig. 3C–E. C and D give two examples in which simultaneously recorded spike potentials from the same motoneurone had quite different time courses, one was the typical intrasomatic potential as in Fig. 3A, the other had a slower rise and a much slower decline. In C the two spike potentials were about the same height, but there was 0.3 msec between summits, which must be attributed to dendritic conduction time.

In D the dendritic conduction time was almost 0.5 msec and the very small IS spike also demonstrated the remoteness of that intracellular electrode from the soma. A dendritic conduction time of about 0.4 msec for 0.3 mm propagation was observed by Fatt (1957a) in his elegant mapping of the extracellular field potential generated by antidromic invasion of a single motoneurone. In the extracellular record of Fig. 3E the second negative wave was much later than in Fig. 3A and B, the onset being simultaneous with the SD spike summit, and there was no subsequent positive wave. Evidently this extracellular recording must be from a region of the neurone that is rather more than 0.2 msec conduction time from the soma, and even so far out on the dendrites that the further dendritic invasion does not give opportunity for a reverse current flow in the outward direction. It can be concluded that the experiments here described, together with those of Fatt (1957a) and Lorente de Nó (1947) prove that an antidromic impulse propagates some hundreds of microns along the dendrites of motoneurones at a velocity of the order of 1 m/sec, but do not allow any statement about impulse conduction in fine dendritic branches. At least with the large dendrites we are justified in assuming that the surface membrane has the same excitatory properties as the soma membrane.

It was originally believed that an initial IS spike was observed only as a stage of

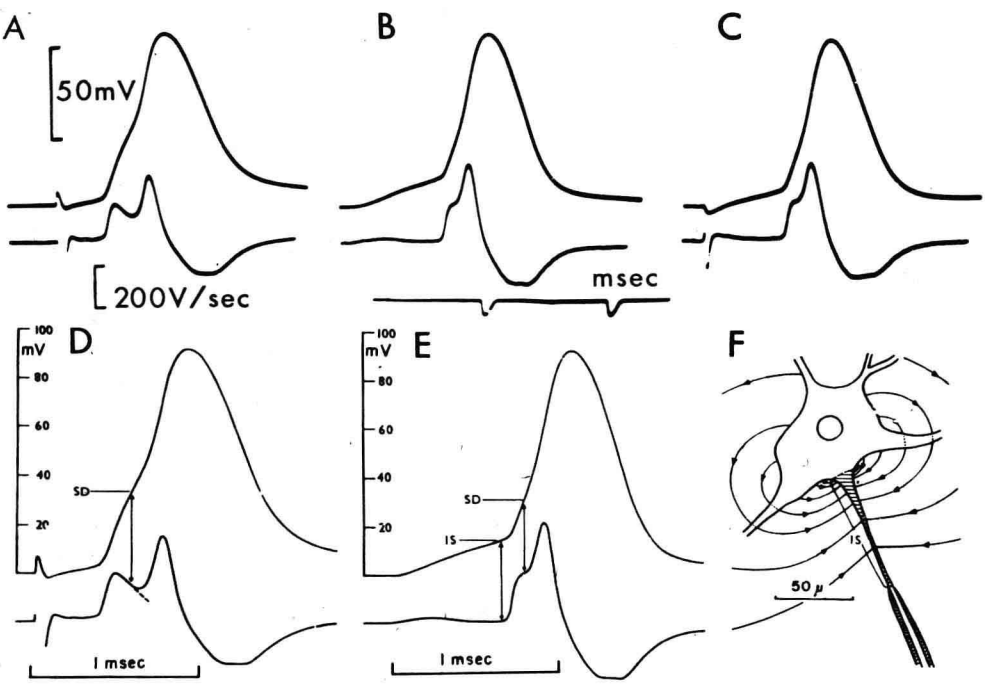

Fig. 4. Spike potentials evoked in a motoneurone with a membrane potential of —70 mV by three different modes of stimulation; A, antidromic; B, monosynaptic; C, by a depolarizing pulse through one barrel of the double microelectrode. The lower traces show the electrically differentiated records. D and E are tracings of A and B with perpendicular lines from the origins of the IS and SD spikes, the horizontal lines giving the respective thresholds. In F the lines of current flow are drawn as described in the text. (From Coombs *et al.*, 1957a.)

progressive invasion of a motoneurone by an antidromic impulse propagating sequentially from the medullated axon to the initial segment of the axon, then to the soma and dendrites. This explanation was refuted by the discovery that, with synaptic and direct electrical stimulation of a motoneurone, the IS spike also precedes the SD spike (Araki and Otani, 1955; Fatt, 1957b; Fuortes *et al.*, 1957; Coombs *et al.*, 1957a,b). The electrically differentiated records in the lower row of Fig. 4A, B and C show indubitably that with antidromic, synaptic and direct electrical excitation of the motoneurone, its first response is an IS spike. In B and C the stimulus applies the depolarization more directly to the soma than to the initial segment, where the spike is initiated; hence it must be concluded that the initial segment has a much lower threshold.

Comprehensive analytical investigations have led to the conclusion that with motoneurones in good condition the threshold depolarization of the initial segment is always less than half of that for the soma, the respective ranges being 6–18 mV and 20–37 mV with mean values of 10 mV and 27 mV respectively (Coombs *et al.*, 1957b). In Fig. 4 A–C potentials in the upper traces give virtually a record of the changes in soma membrane potential and show that the activation of the initial segment adds very effectively to the depolarization of the soma membrane so that its threshold is attained and the SD spike generated. The lines of current flow into the activated initial segment from the soma are shown in Fig. 4F. The levels of depolarization for synaptic generation primarily of an IS spike and secondarily of an SD spike are given in the drawings of Fig. 4E. As would be expected the IS spike generates an SD spike at practically the same level of depolarization for antidromic (D) and synaptic activation (E) of the motoneurone.

When SD impulse generation fails, the IS spike attains a peak of only about 40 mV and then declines as seen in Fig. 2A. It will be appreciated that it is merely the electrotonic extension of the IS spike to the soma that has this small value. With intracellular recording from the initial segment the spike potentials may be in excess of 80 mV (Coombs *et al.*, 1957a; Terzuolo and Araki, 1961). The relationship of depolarization to IS and SD spike generation is well shown in the voltage-clamp recordings of Fig. 5 (Araki and Terzuolo, 1962). By means of a feedback device the membrane potential is displaced to a desired level and held there by current applied through one barrel

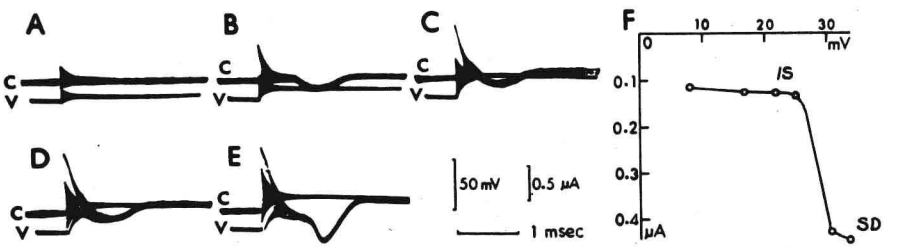

Fig. 5. Threshold difference between axon and soma. (A–E). Upper beam, membrane current; lower beam, membrane potential. (F). Relation between cathodal displacement of the membrane potential from the resting level (abscissa) and peak inward current (ordinate). Full explanation in the text. (From Araki and Terzuolo, 1962.)

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of a double microelectrode assemblage. In Fig. 5B a depolarization of about 10 mV was applied by the voltage-clamp, and, after the initial displacing current, a later inward current by the voltage-clamp (C trace) showed that the membrane depolarization had resulted in a spike-like inward current across it. Much larger depolarizations in C and D also resulted in the same brief inward current, but with a shorter latency. However, with a still further increase in depolarization (E), there was second large component of inward current. The plotted points of Fig. 5F show the step-like in-

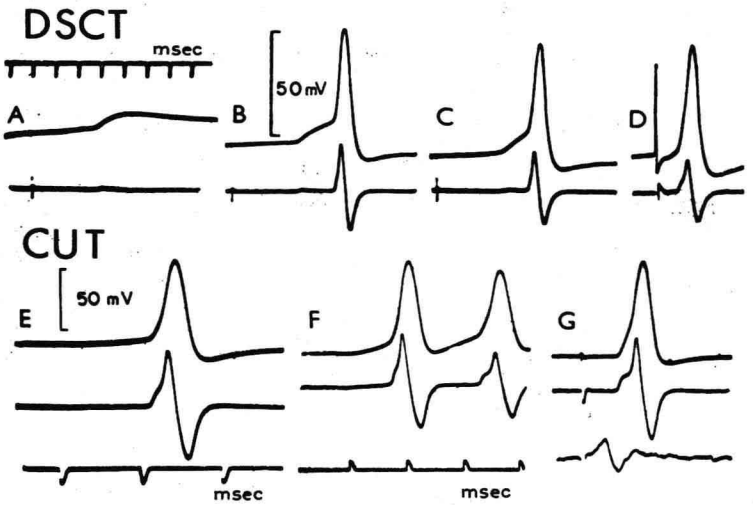

Fig. 6. (A–D). Intracellularly recorded responses of a neurone of Clarke's column with the electrically differentiated records immediately below. D is an antidromic spike potential evoked by an impulse descending the dorsal spino-cerebellar tract, A, B and C are responses evoked by progressively larger afferent volleys in the nerve to quadriceps muscle. The neurone was discharging spontaneously, hence the sloping base lines. (From Curtis, Eccles and Lundberg, unpublished observations.) (E–G). Intracellularly recorded responses of a neurone of the dorsal horn in the L7 segment, with the electrically differentiated records immediately below. G, the antidromic spike potential evoked by an impulse descending the lateral column on the ipsilateral side, the extracellular potential generated by the descending volley being seen in the lowest trace. E and F are responses evoked by a small and a large afferent volley from the superficial peroneal nerve. (From Eccles *et al.*, 1960.)

crement in inward current that occurred with a depolarization of 30 mV. There can be no doubt that the two components of motoneuronal excitability revealed in Fig. 5 are the IS and SD components of Figs. 2, 3 and 4. Of course under voltage-clamp conditions the inward current of the IS response cannot add to the depolarization of the soma membrane as in Fig. 4F. The SD membrane can be activated only when the applied voltage attains threshold level. The SD current in Fig. 5E had a later onset than the IS current solely because the applied voltage was just above the SD threshold, a long latency being observed under comparable conditions for the IS spike in Fig. 5B. With larger voltages the IS and SD spikes were synchronous (Araki and Terzuolo, 1962).

In the spinal cord there is much variation with respect to the threshold discrimination between the IS and SD regions of nerve cells. For example, the same large discrimination as with motoneurones is seen in the differentiated records from the