Electrophysiology of Supramedullary Neurons in *Spheroides maculatus*

1. Orthodromic and antidromic responses

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ABSTRACT This series of three papers presents data on a system of neurons, the large supramedullary cells (SMC) of the puffer, *Spheroides maculatus*, in terms of the physiological properties of the individual cells, of their afferent and efferent connections, and of their interconnections. Some of these findings are verified by available anatomical data, but others suggest structures that must be sought for in the light of the demonstration that these cells are not sensory neurons.

Analysis on so broad a scale was made possible by the accessibility of the cells in a compact cluster on the dorsal surface of the spinal cord. Simultaneous recordings were made intracellularly and extracellularly from individual cells or from several, frequently with registration of the afferent or efferent activity as well. The passive and active electrical properties of the SMC are essentially similar to those of other neurons, but various response characteristics have been observed which are related to different excitabilities of different parts of the neuron, and to specific anatomical features.

The SMC produce spikes to direct stimuli by intracellular depolarization, or by indirect synaptic excitation from many afferent paths, including tactile stimulation of the skin. Responses that were evoked by intracellular stimulation of a single cell cause an efferent discharge bilaterally in many dorsal roots, but not in the ventral. Sometimes several distinct spikes occurred in the same root, and behaved independently. Thus, a number of axons are efferent from each

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neuron. They are large unmyelinated fibers which give rise to the elevation of slowest conduction in the compound action potential of the dorsal root. A similar component is absent in the ventral root action potential.

Antidromic stimulation of the axons causes small potentials in the cell body, indicating that the antidromic spikes are blocked distantly to the soma, probably in the axon branches. The failure of antidromic invasion is correlated with differences in excitability of the axons and the neurite from which they arise.

As recorded in the cell body, the postsynaptic potentials associated with stimulation of afferent fibers in the dorsal roots or cranial nerves are too small to discharge the soma spike. The indirect spike has two components, the first of which is due to the synaptically initiated activity of the neurite and which invades the cell body. The second component is then produced when the soma is fired. The neurite impulse arises at some distance from the cell body and propagates centrifugally as well as centripetally.

An indirect stimulus frequently produces repetitive spikes which are observed to occur synchronously in all the cells examined at one time. Each discharge gives rise to a large efferent volley in each of the dorsal roots and cranial nerves examined. The synchronized responses of all the SMC to indirect stimulation occur with slightly different latencies. They are due to a combination of excitation by synaptic bombardment from the afferent pathways and by excitatory interconnections among the SMC.

Direct stimulation of a cell may also excite all the others. This spread of activity is facilitated by repetitive direct excitation of the cell as well as by indirect stimulation.

INTRODUCTION

Clusters of large neurons, lying close to, or on the dorsal surface of the medulla and spinal cord have been described in many species of teleost fishes (27). These supramedullary cells (SMC) were first seen in *Lophius* by Fritsch (20) and have recently been found also in a larval amphibian (25). The SMC's are generally considered to be homologs of dorsal root ganglion cells. In the Atlantic puffer or blowfish, *Spheroides maculatus*, and in some other fish, they attain diameters of several hundred micra. Thus, they are probably larger than any other vertebrate neurons, and are certainly the most easily accessible. The work reported here deals with an electrophysiological study, including microelectrode recording, of the various properties of these cells in *Spheroides*.[1]

[1] Dr. Crain, as a Grass Foundation Fellow during the summer of 1957, participated in the early and larger part of the present work, which was reported in abstract form (4, 13). Dr. Bennett held a Grass Fellowship during the summer of 1958 when additional work was done, some of which is included here. An earlier, unsuccessful attempt was made at a similar study by one of us (H.G.) with Dr. Ellis Berkowitz, who held a Grass Fellowship at the Marine Biological Laboratory in 1951. An account of responses and electrical properties of a Pacific puffer, *S. vermicularis*, has appeared recently (24).

The initial intent of the work was to utilize the advantages offered by the size and accessibility of the cells to study their passive electrical properties and the electrophysiological characteristics of the spike-generating mechanism. The investigation was extended, however, upon discovering that the SMC, rather than being primary sensory cells, form a hitherto undescribed variety of neurophysiological system. They are functionally interconnected and mutually excitatory. They are activated synaptically by various stimuli. All the cells then respond in synchrony, often repetitively. From each cell a single large neurite branches to form numerous large unmyelinated axons which emerge through a number of different dorsal roots. These are efferent fibers. The data on which the above statements are based and the electrophysiological manifestations of these properties are reported in the present series of papers.

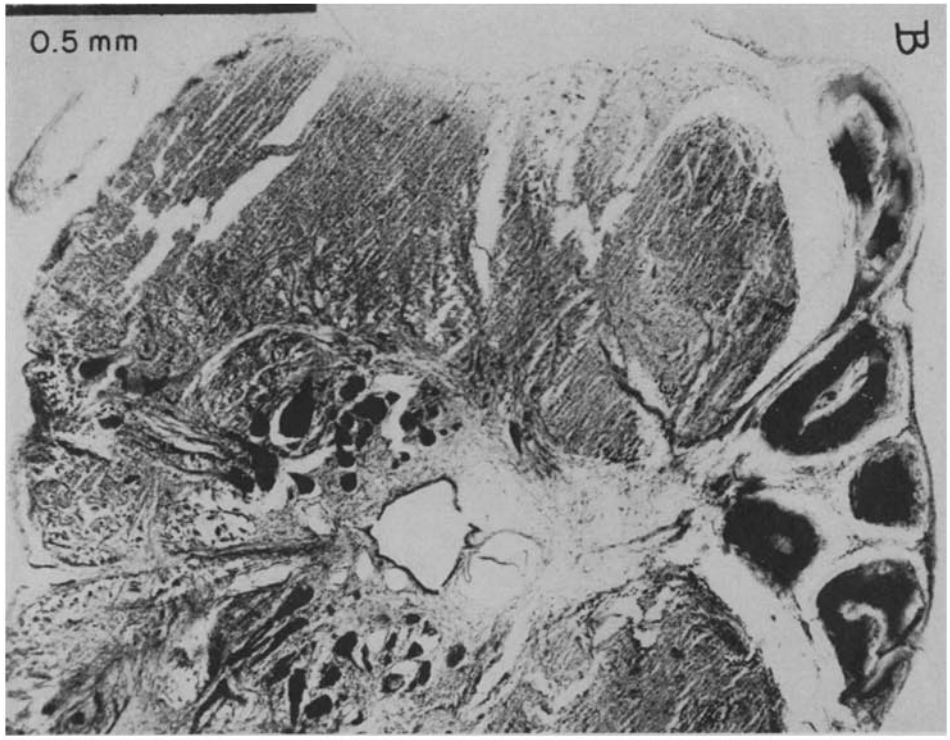
Part I characterizes the potentials that are recorded in the SMC's when the latter are excited by various means. The course of the axons and the properties of these fibers are identified. The potentials produced antidromically in the SMC's by stimulating their axons are also analyzed. Part II (5) describes the membrane properties of the neurons. Simultaneous extracellular and intracellular recordings demonstrate that the soma membrane of these cells is electrically excitable. They also identify the components of the intracellular spike. Part III (6) analyzes the synaptic connections and interconnections which are believed to be responsible for the repetitive indirectly evoked activity of the SMC and for their synchronized responses.

Anatomy of the SMC

In *S. maculatus* the SMC's lie in a cluster immediately behind the cerebellum, over the dorsal surface of the anterior 3 or 4 spinal segments (Fig. 1 *A*). Some 35 to 55 cells, spherical or slightly ovoid, and 200 to 300 μ in diameter, are visible through their covering of arachnoid coats (removed for the photograph). A few cells are usually obscured by the overhang of the cerebellum. A few more lie in the dorsal cleft of the spinal cord (Fig. 1 *B*), but the total of cells not visualized from the dorsal aspect is relatively small.

The arachnoid contains blood vessels, which also supply the neurons. Each cell has capillaries visible on its surface, and some appear to penetrate the cell surface (*cf.* reference 31). The SMC have many fine, but very short processes, thus lacking the extensive dendritic arborizations which in other cells appear to complicate interpretation of electrical recordings (11, 16, 17, 19). The neurites, about 30 μ in diameter, run ventrally in the dorsal fissure (Fig. 1 *C*), becoming progressively smaller in the process of branching.

The branches, passing laterally into the spinal cord as unmyelinated fibers 5 to 15 μ in diameter (Fig. 1 *B*, upper left cell), do not lie in a distinct tract, in contrast to fibers of some other species (*cf.* references 10, 14, 30). Therefore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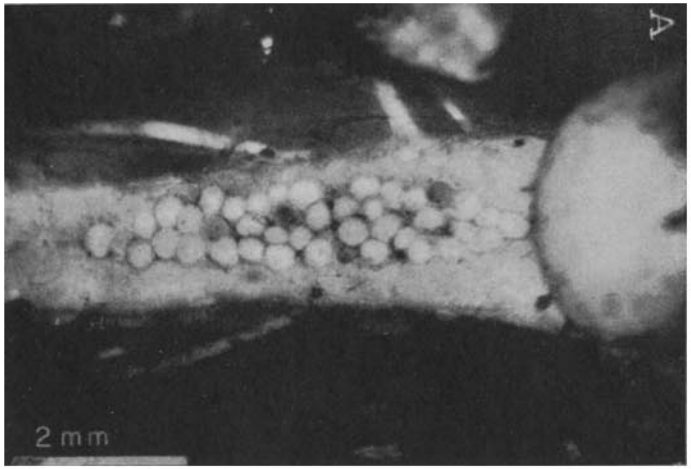
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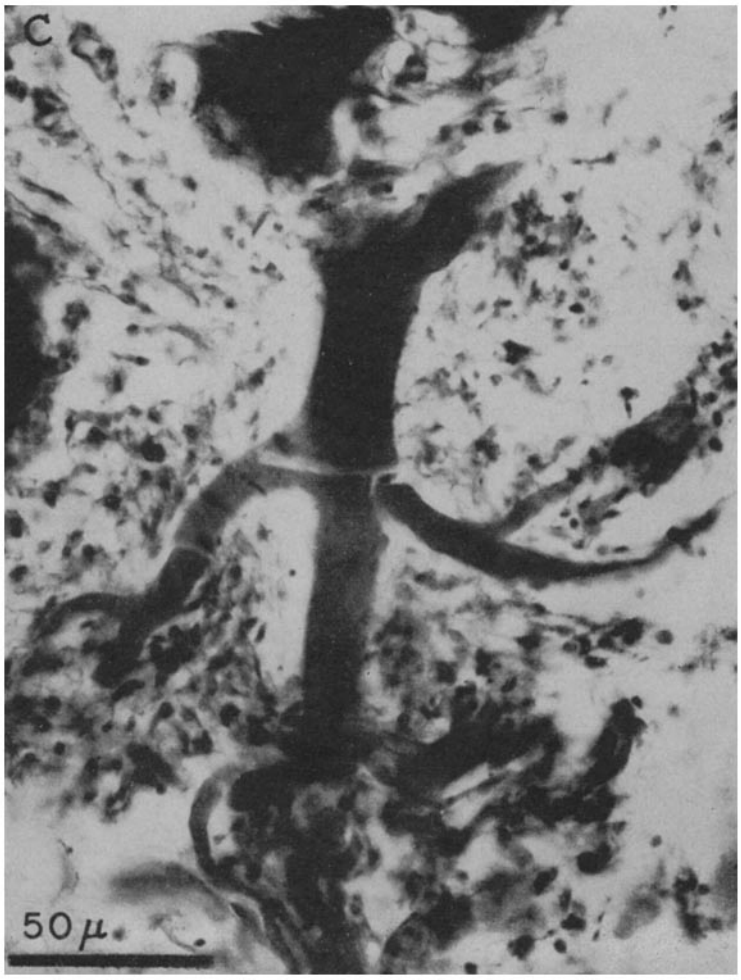
FIGURE 1. Anatomy of the SMC. *A*. Low power dorsal view of the spinal cord and cerebellum showing the supramedullary cluster. The arachnoid membranes have been removed and the fresh preparation lightly stained with toluidine blue. The cells, 250 μ in diameter, appear as a cluster of pale spheres on the surface of the cord. Several of the penetrated cells have taken up the dye more strongly. *B*. Cross-section of spinal cord showing five cells of the cluster. One neuron lies entirely within the dorsal cleft and would not be visible from the exterior. The neurite of the upper left hand cell is seen descending in the dorsal cleft and then turning into the dorsum of the cord where it branches several times. Preparation stained with hematoxylin and eosin. *C*. High power photograph of a neurite leaving a supramedullary cell and sending out at least five branches soon thereafter (hematoxylin and eosin preparation). The axon hillock region of the cell is penetrated by capillaries.



the individual fibers become difficult to follow. However, at all levels of the spinal cord large unmyelinated nerve fibers are clearly seen passing laterally in the dorsal, but not the ventral half. Large (5 to 10 μ) unmyelinated axons are also found in the dorsal roots, but not in the ventral. In the dorsum of the cord, from the cluster down to the roots, there is no indication of cells which could form a synaptic relay between the axons of the SMC and the large unmyelinated root fibers. Electrophysiological data to be presented below, clearly show that the axons of the SMC emerge in the dorsal roots as the fibers of slowest conduction, and form a distinctive elevation in the compound action potential.

The connections rostrally have not yet been examined anatomically in *S. maculatus*, but in *Lophius* (20) and *Ctenolabrus* (30), axons from the SMC pass into the trigeminal and vagus nerves. In *Tetraodon* (26), the axons have been traced to the vagal and trigeminal nuclei. The present electrophysiological data show that the axons from SMC of *S. maculatus* leave the cranium in several nerves of the facial trigeminal group.

The SMC have usually been considered by anatomists (*cf.* reference 27) to be sensory, members of the group of large intramedullary sensory cells that have migrated to the surface. However, it has been pointed out a number of times (*cf.* references 25, 30) that this homology has in no case been demonstrated. Indeed, it is not clear whether or not SMC of various species are themselves homologous. This problem is aggravated by the fact that the function of the supramedullary cells has not been established.

In a number of respects the present electrophysiological data are at variance with the views previously derived from anatomical studies. The SMC are excited synaptically from all the various afferent pathways tested, and therefore they are not homologs of dorsal root ganglion cells or of intramedullary primary sensory neurons. In response to various kinds of afferent stimuli the axons of the SMC send out impulses in the dorsal roots and therefore they are efferent, not afferent fibers. The striking peculiarity of the supramedullary cluster, the capacity of all its cells to discharge in synchrony, suggests that the cells are mutually interconnected.

The responsiveness of the cells to indirect stimulation, and their synchronized discharges indicate that the SMC are endowed with complex synaptic organization. Nevertheless, synaptic contacts have not been described in relation to these cells. The electrophysiological data suggest that the synaptic junctions are located distant from the cell body on the neurite. However, this newly raised anatomical problem has not yet been studied with adequate techniques.

Morphological conditions in *S. maculatus* are particularly favorable for electrophysiological studies. Because of the accessibility and large size of the cells, several microelectrodes can be placed in known relations to a single

neuron. Limited only by the available equipment and the small surface of the cluster (about 5 mm. × 2 mm.; Fig. 1 *A*), a number of cells of the cluster may be studied simultaneously. As in other plectognaths, the spinal cord is greatly shortened, the vertebral canal being largely filled with cauda equina. Therefore, sufficient lengths of dorsal and ventral roots could be obtained in the present work for both stimulation and recording. The fish tolerate well acute and chronic operative procedures.

Methods

The techniques and procedures described in this section apply to all three parts of this series of papers.

PREPARATION The fish was held rigidly fixed between a double set of tapered stainless steel rods, the tips of which pressed against the body. A jet of sea water, directed into the mouth, sufficed for respiration. The cerebellum and spinal cord were exposed (Fig. 1 *A*), and in some experiments the intracranial portions of the facial trigeminal nerve groups were also made available by rostral extension of the opening. Dorsal and/or ventral roots were dissected free in many of the experiments for recording and stimulation.

Spinal section and division of most of the cranial nerves were carried out in a few preparations to immobilize the fish. In most experiments, however, *d*-tubocurarine was injected intramuscularly (8 to 10 mg./kg.). The high concentration required to immobilize the animal probably reflects not only the route of administration, but also the relative insensitivity of fish to this drug (*cf.* reference 8). Its use did not appear to affect the responses of the SMC.

The cells were made accessible to microelectrode penetration by pulling aside and tearing or cutting the overlying arachnoid membranes. The blood vessels in the membranes were left intact insofar as was possible. When they were to be divided, prior clamping with fine forceps usually prevented bleeding. The skin around the operative exposure was sometimes pulled up at the sides to hold a pool of *Lophius*-Ringer solution (35) or mineral oil. The latter has the advantage that glass microelectrodes are more readily seen in it. The operation required 1 to 2 hours and the preparations usually survived 6 to 10 hours.

One series of the present experiments was carried out in New York City. The fish survived very well in an aquarium with circulating seawater.[2] About seventy preparations contributed to the data reported here. Many, or most of the cells of the cluster were examined during the course of an experiment. The temperature ranged from about 20 to 26°C.

STIMULATION AND RECORDING Stimulating electrodes applied to nerves, roots, the spinal cord, or the skin were 100 μ Teflon-insulated silver wires exposed at their tips. Square pulses of various durations were obtained from a battery of independently

[2] These fish were supplied through the kindness of Mr. John Poole, Aquatic Biologist, New York State Conservation Department. We hereby express our thanks for this cooperation, and for the advice and help of Mr. Robert Mathewson, Science Curator of the Staten Island Institute of Arts and Sciences.

controlled stimulators. They were connected to the preparation through low capacity *r–f* coupling units (32). Recording electrodes for pick-up from roots or nerves were also Teflon-insulated silver wires. Two high-grain D.C. amplifiers were available for recording from nerves.

Microelectrodes for intracellular stimulation and recording were conventional KCl-filled glass micropipettes (28, 29). The tip diameters of these electrodes were not of critical importance, since the SMC are large cells. They ranged, however, below 1 μ. For extracellular recording close to the surfaces of the cells (5), similar microelectrodes were filled with 4 M NaCl; alternatively, large (10 to 40 μ tip) Ringer-filled pipettes were used.

An especially convenient type of micromanipulator with a magnetically coupled micrometer drive[3] permitted concentration of a number of independently controlled microelectrodes within the small area of the cluster. The electrodes were positioned under 15 to 25 magnification with a long working distance binocular microscope.

Transistorized, neutralized capacity, low grid current amplifiers (2)[4] were connected between the recording microelectrodes and the oscillograph. The latter had four channels, so that this number of cells could be examined simultaneously when desired. In many experiments the frequency response was monitored continuously by inserting a calibrating square pulse between the preparation and ground. However, as will be seen below, the cells respond with relatively slowly rising potentials. Furthermore, since the electrodes did not need to be of extremely high resistance, the feedback correction of the neutralized capacity amplifier was not critical.

In experiments which utilized intracellular stimulation the applied currents were also monitored. Large currents (10^{-7} A, or more; *cf.* Fig. 2) are required for intracellular stimulation of the cells, and this tends to cause non-linear electrode properties. The use of a single electrode for stimulating and recording in bridge arrangement (3, 18) was thereby precluded. Since penetration was under visual control, separate electrodes proved convenient, and this reduced capacitative coupling to a greater degree than would have been possible with a two barreled electrode (7, 12, 15, 34).

RESULTS

A. Varieties of Intracellularly Recorded Potentials in SMC

RESTING POTENTIAL Uncorrected for junction artifacts, the resting potentials in different cells ranged from 50 to 80 mv., inside negative (Fig. 2 *A*, *B*, and Part II, Table I). Since the microelectrodes used in this work had relatively large tips, spurious potentials reported to occur with very fine tipped microcapillaries (1) were probably negligible. This had also been found in previous work with electrodes of similar size used for impaling and injecting squid giant axons (23). The smaller values were probably due to

[3] Designed and manufactured by Andrew Pfeiffer, Box 450, RFD 1, Old Lyme, Connecticut.
[4] Available from Bioelectric Instruments, Box 204, Hastings-on-the-Hudson, New York.

injury of the cells or to faulty penetration. The latter was sometimes indicated by gradual development of the maximal resting potential.

DIRECTLY EVOKED SPIKES An intracellularly applied depolarizing current produced a spike which will be designated as "direct" (Fig. 2 *A*), since it differed in certain respects from the "indirect" spike elicited by a neural volley (*B*). The direct spike was evoked by rheobasic currents of 1 to 5 $\times$ 10^{-7} A (*cf.* Table I, Part II), and, near threshold, there was first produced a local response (Fig. 2 *C*). Stronger stimuli progressively shortened the latency. The maximum amplitude of the spikes recorded in this work was about 110 mv., but 80 to 100 mv. were the magnitudes usually observed (5, Table I). The minimum duration of the spikes was about 3 msec.

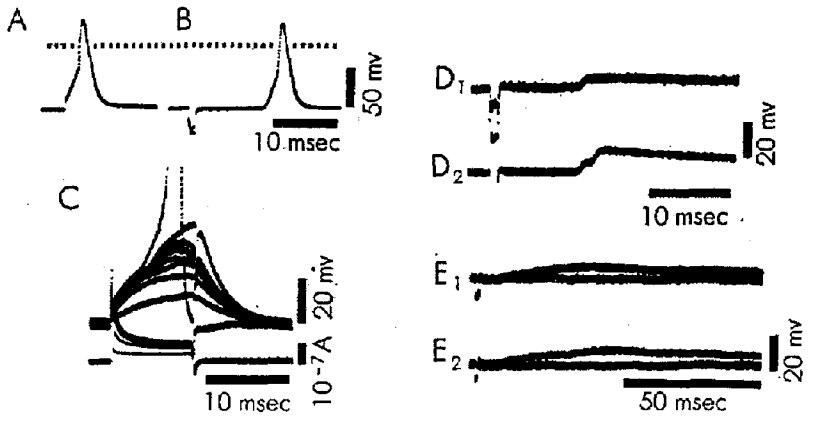


FIGURE 2. Varieties of potentials recorded intracellularly in SMC. *A*. Direct spike, produced by intracellularly applied 2 msec. depolarizing stimulus. Dotted line is the zero reference. The resting potential was 80 mv., inside negative. *B*. Indirect spike of the same cell evoked by stimulus to cauda equina. *C*. Graded local responses produced by increasing intracellular depolarizations. Eight sweeps superimposed, the strongest stimulus evoking a spike. Simultaneously recorded amplitudes of the 10 msec. depolarizing current pulses shown on lower trace, positivity of the intracellular electrode produced an upward deflection in this and subsequent figures. *D*. Antidromic potentials (ADP) produced by stimulating a dorsal root at two strengths. The weaker stimulus evoked a single potential (D_1), but the stronger (D_2) also activated another axonal branch of the same cell. *E*. Postsynaptic potentials (p.s.p.'s) of shorter (E_1) and longer (E_2) durations. A superimposed trace without stimulation shows the base line.

INDIRECT SPIKES The details of this response will be described and analyzed in Parts II and III (5, 6). Several features may be noted here. The responses to a brief stimulation of a dorsal root, of the spinal cord itself, or of the cranial nerves began after a long delay which represents not only conduction time, but a latency that was shortened with stronger afferent stimu-

lation (Fig. 3). Frequently the response was repetitive (Fig. 4), the number of spikes increasing with stimulus strength up to ten or more. The maximum number of repetitive responses occurred when the indirect stimuli were applied at intervals of no less than 20 or 30 sec. apart. More frequent stimulation decreased the number of repetitive spikes. Tactile stimulation of any area of the skin also produced spike activity which was usually repetitive. Scratching the skin was the most effective stimulus. Adaptation to tactile stimuli was rapid.

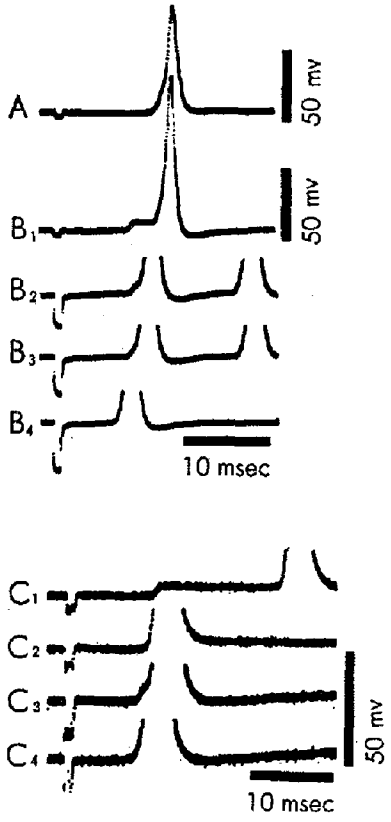


FIGURE 3. Indirect spikes and ADP. *A*, *B*. Simultaneous recordings from two SMC responding to stimulation of cauda equina. Spike in B_1 preceded by ADP. Stimulus strength was increased successively for B_2–B_4: This produced repetitive discharge as well as an earlier spike. The latency of the ADP changed relatively little (B_2–B_3). Indirect spike in B_4 occurred at a shorter latency than the ADP in previous records and presumably obscured the latter potential. C_1–C_4: Another experiment, showing altered response to increasing stimulus strength. The ADP arose about 1 msec. earlier in C_3 than in C_1. However, the latency of the indirect spike varied by approximately 20 msec. The spike occurred early enough in C_2 and C_4 to obscure the ADP.

The indirect spike had two components (5). A slowly rising "first component" denoted by an inflection (Figs. 2 *B*, 3 *A*) was probably due to activity of the neurite, the impulse being synaptically evoked some distance from the cell body. After a delay probably due to the geometric conditions (propagation from the fiber into a large volume, the cell body; *cf.* reference 15), the discharge of the cell body gave rise to a second component. Under various conditions (*e.g.* during repetitive activity or hyperpolarization of the cell) the second component was absent (*cf.* Figs. 15, 17).

ANTIDROMIC POTENTIAL (ADP) Frequently, stimulation of nerves or of the cord evoked a small potential that preceded an indirect spike (Fig. 3 *B*), or appeared without the latter (Fig. 2, D_1, D_2). This potential attained amplitudes of 2 to 10 mv., the peak occurring at 1 to 2 msec. The duration was usually about 10 msec. Sometimes two of the potentials summated (D_2). In section C of this paper, it will be shown that this potential is the pick-up of an antidromic impulse from the axons of the SMC. The impulse is blocked before it reaches the cell body, only one case of complete invasion having been seen in all the experiments. The antidromic origin was corroborated by the relatively fixed latency of the response compared to the variable latency of the synaptically evoked indirect spike (Fig. 3), and by the fact that in repetitive responses to a single stimulus, spikes following the first (B_2, B_3) lacked this component.

POSTSYNAPTIC POTENTIAL A potential similar in magnitude to the ADP, but much longer lasting, and of variable form was also observed (Fig. 2 E_1, E_2). This potential rose to its peak in from 20 to 50 msec. and fell somewhat more slowly. As the strength of the indirect stimulus was increased, the amplitude of the potential was continuously graded, presumably by summation of different small components. The long duration, gradedness, and summative capacity suggest that this response is a postsynaptic potential (p.s.p.). Further evidence on this matter will be presented in Part III (6).

B. Activity and Efferent Relations of SMC

SYNCHRONIZED AND REPETITIVE ACTIVITY OF INDIRECTLY EXCITED SMC Simultaneous recording from two cells (Fig. 3 *A*, B_1) showed that both were always activated together by a neural volley. This proved always to be the case when observing from two or more cells simultaneously and it may therefore be concluded that all the SMC's discharged synchronously. The synchronization of activity in all the cells of the cluster also obtained in multiple spike responses, as will be described more fully in Part III (6).

EFFERENT DISCHARGE OF THE SMC An efferent discharge in the axons of SMC which resulted from the simultaneous activity of the entire cell cluster was recorded from the dorsal roots (Fig. 4) and from the cranial nerves. However, no efferent discharge related to the activity of the SMC emerged in the ventral roots (Fig. 5). A volley that emerged in all the dorsal roots examined at one time, was associated with each discharge in a train of repetitive activity of the SMC (Fig. 4*B*, *C*). The shape of the discharge in a given root was not constant and probably depended upon the relative times of firing of the different cells in the cluster. However, the form of the efferent discharge was sufficiently characteristic so as to be unmistakably recognizable as the activity involving the SMC.

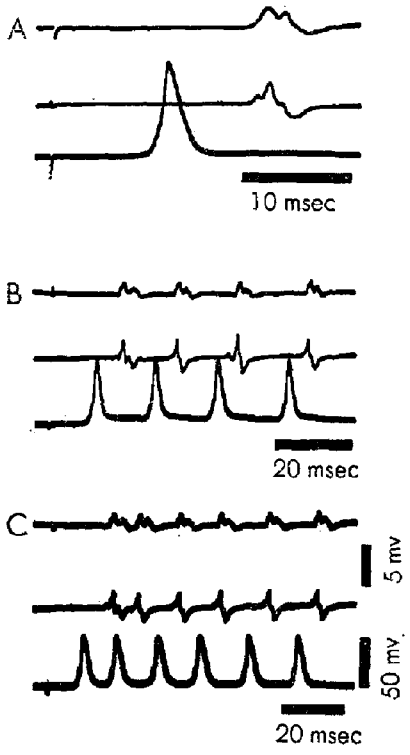


FIGURE 4. Efferent discharges in two dorsal roots (two upper traces) in correlation with spike responses of SMC to stimulation of cranial nerves. *A*. Single discharge recorded at high sweep speed. An efferent volley follows the spike by 10 msec. *B, C*. During repetitive activity each discharge of the cell was associated with efferent activity in both dorsal roots. Note small changes in form of the efferent discharge, particularly in the dorsal root of the middle trace.

The multifibered repetitive discharge in a dorsal root, resulting from activating the SMC by stimulating a cranial nerve, occurred without the accompaniment of activity in the ventral roots (Fig. 5 *A*). Therefore, the fibers

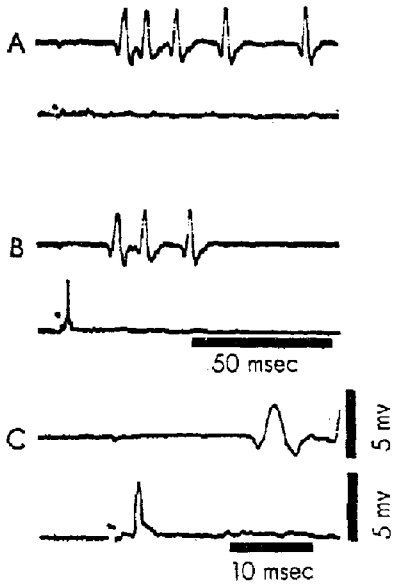


FIGURE 5. Absence of efferent discharge from supramedullary cluster in a ventral root. *A*. Cluster discharge, evoked by stimulating cranial nerves, produced repetitive efferent activity in a dorsal root (upper trace), but not in the ventral (lower trace). *B, C*. Short latency reflex response produced in the ventral root by stronger stimuli to the cranial nerves occurred independently of the dorsal root discharge.

efferent in the dorsal roots did not themselves set up reflex activity in the ventral root of the same segment. A strong stimulus to the cranial nerves produced an early spike in the ventral root (Fig. 5 *B*, *C*). This reflex response had no effect upon the activity of the cluster, and stimulation of the ventral root did not excite the SMC. The efferent activity in the ventral root had a shorter duration than did the efferent response in the dorsal root (*C*), and appeared to be composed of briefer spikes.

THE COMPOUND ACTION POTENTIALS IN THE DORSAL AND VENTRAL ROOTS

Analysis of the efferent discharge involves prior knowledge of the fiber composition of the action potentials of the roots. Three prominent elevations comprise the action potential in the dorsal roots (Fig. 6). The conduction

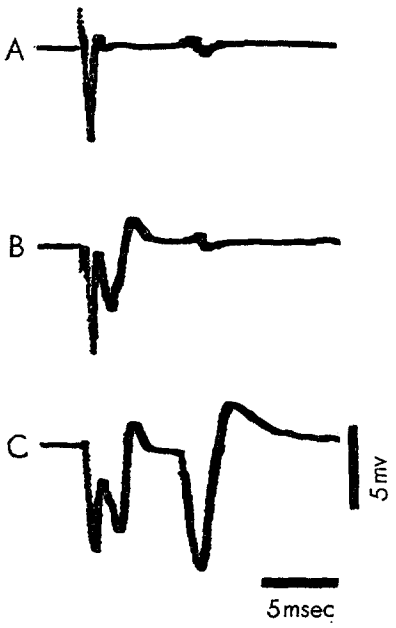


FIGURE 6. Components in the action potential of a dorsal root. *A*. Weak shock, but maximal for an early component, conducted at highest velocity. A dorsal root reflex emerged about 8 msec. after the stimulus. *B*. A stronger stimulus excited a more slowly conducting fiber group. *C*. Very strong stimulus brought in a group of slowest conduction. The distance to the proximal recording electrode was about 2 cm.

velocity of the fibers in these three groups was 30 to 40 m.p.s.; 15 to 20 m.p.s.; and 2 to 3 m.p.s. respectively. There was also a dorsal root reflex (33) which was evoked by the lower threshold, more rapidly conducting, fibers. The action potential in the ventral roots, on the other hand, had only one prominent component (Fig. 7), indicating that one fiber group predominated. These fibers had about the same range of conduction velocity as did the fibers of the most rapidly conducting group in the dorsal roots. A few fibers conducting at about 15 to 20 m.p.s. were also present. Thus, the obviously large difference between action potentials in the dorsal and ventral roots indicates an equally large divergence in fiber composition. Histological preparations confirmed this difference. As noted above, the dorsal roots contain a promi-

nent component of large unmyelinated axons which is absent in the ventral roots. However, counts of the fibers in the roots were not done.

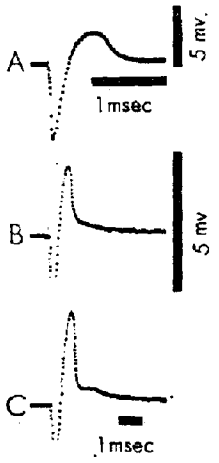


FIGURE 7. Action potential of a ventral root. *A, B.* Response to same strength of stimulation at two sweep speeds. *C.* Response to a supramaximal stimulus.

FIBERS THAT CARRY THE DISCHARGE OF SMC IN THE DORSAL ROOTS The volley that was recorded in a dorsal root when the whole supramedullary cluster was activated (Fig. 8 *A*) was not affected collisionally by eliciting a

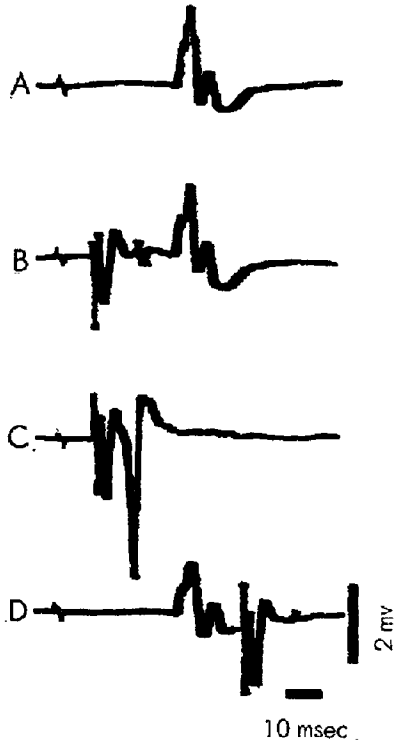


FIGURE 8. Identification of efferent axons of the SMC. *A.* Stimulation of cranial nerves produced characteristic efferent discharge in a dorsal root due to activity of cell cluster. *B.* A stimulus to the root activating the first and second groups of dorsal root fibers (and the dorsal root reflex) did not affect efferent discharge. *C.* A strong stimulus activating the third component of the dorsal root action potential blocked efferent discharge by collision. *D.* The same strong stimulus to the dorsal root as *C*, but following soon after an efferent discharge, failed to activate the third group of fibers.

prior centripetal volley of the first two fiber groups in the root (*B*), but when the third group was activated (*C*) the efferent discharge was eliminated. The conduction time in the root was such that the block must have been caused by collision. Likewise, when the discharge preceded the direct stimulus to the dorsal root, the third group of this response was blocked (*D*). Thus, not only are all the dorsal root efferents of the SMC contained in the group which conducts most slowly, but all the fibers of that group are axons of the SMC. The difference in the areas of the efferent and the directly elicited responses was due to the greater degree of the dispersion of the former, due both to differences in conduction time and in the time of excitation of the different cells.

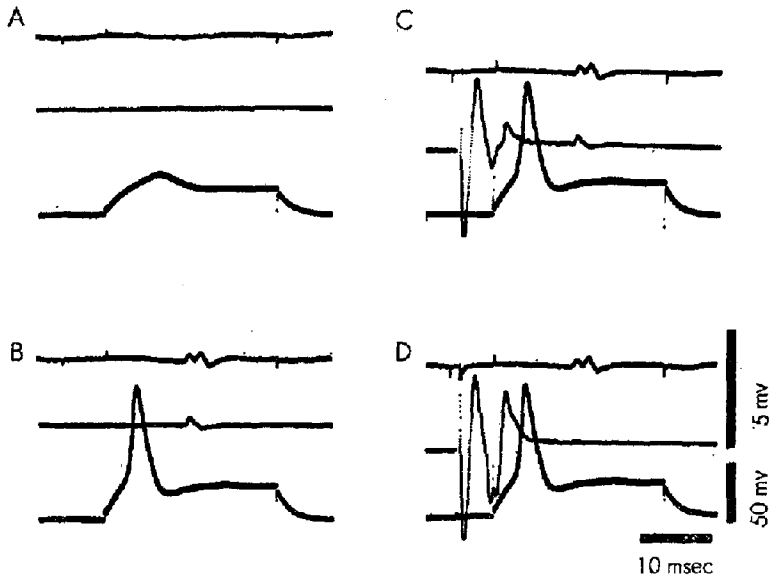


FIGURE 9. Activity in two dorsal roots (upper traces) produced by intracellular stimulation of an SMC (lower trace). *A*. Weak stimulus (2×10^{-7} A) produced only local response. *B*. Stronger stimulus (2.8×10^{-7} A) which evoked spike also caused efferent activity in both dorsal roots. Response in upper trace probably was that of two axons. *C*. One root was stimulated to produce the first two elevations of the compound action potential and also to fire a small number of third group fibers. The efferent discharge was not blocked. *D*. An increased root stimulus, activating about half the fibers of the third group, blocked the efferent discharge in this root, but not in the other.

SINGLE CELL ACTIVATION AND EFFERENT DISCHARGE Stimulation of a single SMC by intracellular depolarization evoked efferent activity in many dorsal roots. On repetitive stimulation of a cell the efferent discharges could be elicited at frequencies greater than 100/sec., nearly at the maximal rate of activation of the cell body by intracellular stimuli. Fig. 9 shows simultane-

ous recordings from two roots. A very weak stimulus produced a local response in the cell, but no efferent activity (*A*). A stronger stimulus (*B*) which evoked a spike, also produced discharges in both roots. In one (upper trace) the response appeared to be double, and probably represented the activity of two axons of the cell running parallel in the same root.

A stimulus to the other root, strong enough to activate some of the fibers of the third group did not block its efferent impulse (*C*). A stronger stimulus, involving about one-half the total of the third group, eliminated the efferent activity by collisional blockade (*D*). As may be expected, with different cells, or with the same cell projecting to different roots, collisional blockade required different proportions of the third group fibers. It will be noted that blockade in one root did not affect the efferent discharge in the other root (Fig. 9 *D*). The significance of this will be discussed below.

PATTERNS OF PROJECTION OF SINGLE CELLS A pattern of organization was observed in the efferent responses of different SMC. The axons of the hindmost cells tended to emerge in the most caudal dorsal roots. The most anterior SMC did not send axons to these roots. These axons presumably emerged in the cranial nerves, but the rostral projections were not studied with single cell activation. However, in correlation, antidromic potentials to stimulation of the dorsal roots occurred most frequently in the caudal cells, and on stimulating the cranial nerves they were more frequently observed in the rostral cells.

A single SMC sends its axons into a number of dorsal roots of both sides. The largest number observed was seven. In this experiment, the 5th, 7th, and 8th right dorsal roots and the 4th, 6th, 7th, and 8th left dorsal roots were found to possess efferent fibers activated by discharge of one SMC. However, this number may not represent fully the complement of axons that a single cell may supply to the dorsal roots. While gross injury during the dissection of the roots was excluded by testing for the presence of the massive efferent discharge, it was not possible to rule out damage to any single root fiber.

Two, or more, distinguishably different efferent impulses were often observed in one dorsal root when a single SMC was directly excited with only one brief intracellular stimulus. Two impulses are seen in the uppermost trace in Fig. 9. The less frequent appearance of three impulses is shown in Fig. 10, from a different experiment.

The efferent responses were not affected by a prior stimulus to the root (*A*) which elicited activity only in the first two groups of root fibers. The smaller of the two peaks in the efferent discharge was eliminated (*B*) when a stronger stimulus brought some of the third group fibers into activity. A still stronger stimulus which activated more of the third group fibers (*C*) decreased the remaining elevation. Maximal stimulation of the third group of fibers (*D*, *E*) eliminated the last efferent impulse.

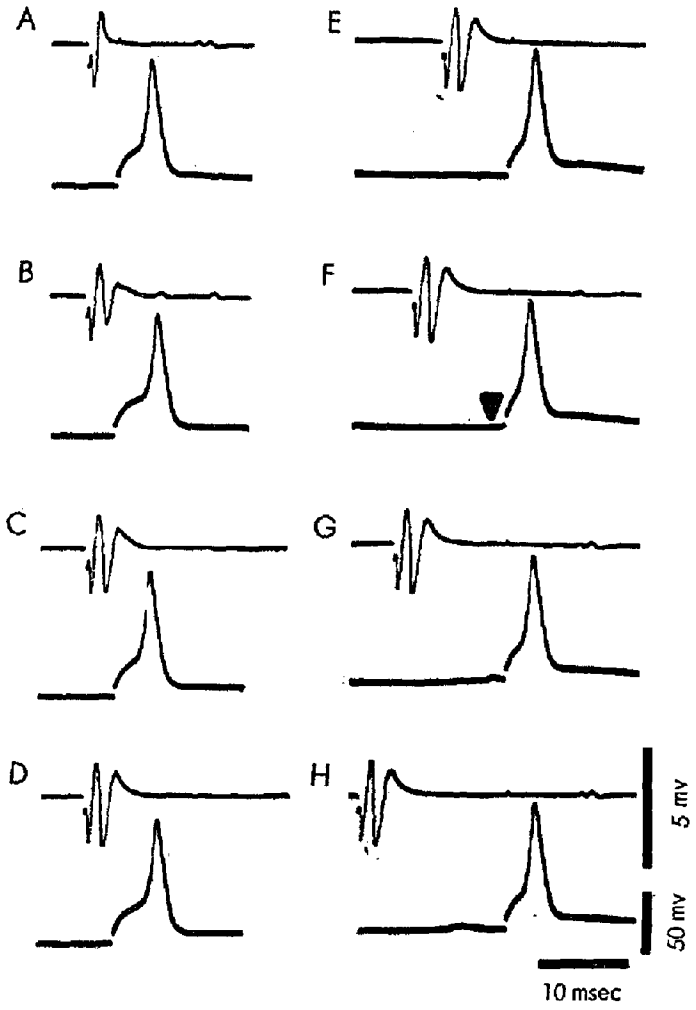


FIGURE 10. Efferent activity in three fibers of same dorsal root produced by stimulating a single SMC. *Upper trace*, dorsal root recording; *lower*, response of cell to a direct stimulus ending after trace was completed. *A*. A weak stimulus to dorsal root did not interfere with two small efferent spikes evoked by activity of SMC. The second, and larger, was due to summated activity of two axons. *B*. A stronger root stimulus, bringing out activity in part of the third group, eliminated the small earlier efferent spike. A dorsal root reflex occurred in this case. *C*. A stimulus almost maximal for the third group diminished the amplitude of the second efferent root spike revealing its double origin. *D*. A maximal stimulus to the root also eliminated this efferent activity. *E*, *F*. As the interval between maximal root stimulation and the direct response of the cell was increased, one efferent spike appeared (*F*) and then also the second (*G*). Note small antidromic potential ahead of intracellular response in *F* (marked by arrow), multiple antidromic potential slightly earlier in *G*, and still earlier in *H*.

EFFERENT ACTIVITY WITHOUT SPIKES IN THE CELL BODY Direct stimulation of an SMC on occasion was seen to evoke efferent activity before a spike was generated in the cell body. This denotes that the neurite or its axons may be more excitable than is the cell body. An experiment of this kind is shown in Fig. 11, with simultaneous registration from two dorsal roots. A stimulus which caused only a small depolarization in the cell body (*B*), produced an efferent discharge in one dorsal root. A somewhat stronger stimulus, yet still not enough to elicit the cell spike (*C*), also excited a discharge in the other root. Full excitation of the cell (*D*) introduced no further efferent activity in these roots. Stimulating one root so as to elicit a centripetal discharge involving part of the fibers in the third group did not block the efferent discharge (*E*), but stronger stimuli caused blockade (*F* to *I*) in this root.

Several points are of interest in the data of this experiment. (*a*) The cell body was less excitable than were its axons. (*b*) There probably was also a higher threshold region in the part of the neurite separating the two axons, for otherwise the response would have arisen in the neurite and might then have spread to all the axons and, perhaps, also to the cell body. (*c*) A spike originating in one axonal branch of the neurite did not spread to another branch. This will be dealt with in more detail in section C below. (*d*) The additional depolarization in the cell body that resulted when an efferent impulse was produced in the axon was not much greater than the ADP (Fig. 11 *B*, *L* and Fig. 14). This fact suggests that the site of impulse initiation was about as distant from the cell body as was the site of failure of antidromic invasion. (*e*) The different thresholds for the two axonal branches may indicate that like the cell surface, different axons may have different excitabilities. However, were one of the sites of impulse initiation closer to the cell body than the other, the excitatory current at the proximal site would have been greater and more effective. The latter possibility is supported by evidence derived from the ADP's, if it is assumed that antidromic invasion failed at approximately the same points at which the orthodromic impulses were initiated by the direct stimulus. The ADP should be somewhat the larger for the impulse that invades farther, and this axon should have a lower threshold to depolarization applied in the cell body. The axon with the lower threshold (*B*, upper trace) was also the one which produced the larger ADP (*L*). Thus, it may be inferred that the lower threshold was at least in part due to the more proximal location of the site at which the impulse was initiated.

C. The Antidromic Potential

THRESHOLD FOR INITIATION OF THE ADP Adequate stimulation of a dorsal root to which a cell projected always resulted in the intracellular potential (Fig. 2 *D*) which has been designated as an antidromic potential (ADP).

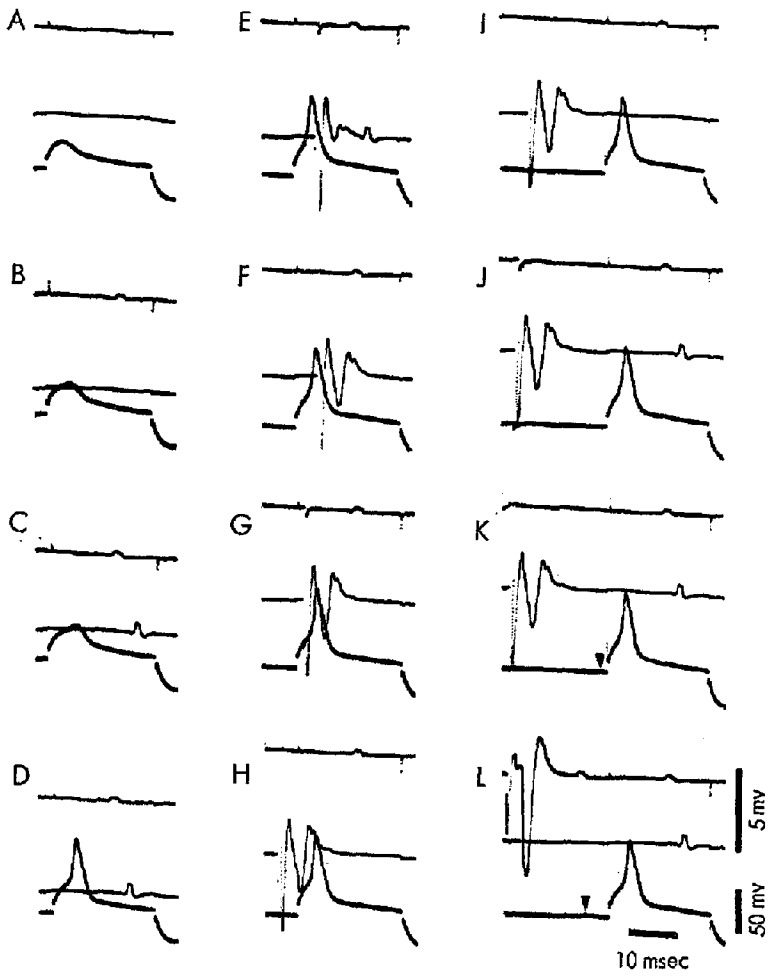


FIGURE 11. Efferent activity in different roots evoked by stimulation of one SMC. Three simultaneously recorded traces in each set: first two register from two roots, lowest records from a SMC excited by an intracellular stimulus. The stimulation was effected through a large resistance paralleled by a capacitor which accounts for the form of the deflection of the intracellular trace. *A.* A weak depolarizing stimulus produced only a small depolarization of the cell. *B.* A somewhat stronger stimulus caused an action potential propagating out into one dorsal root without producing a spike. *C.* Activity now propagated out into both dorsal roots, but the cell still did not produce a spike. *D.* Soma spikes developed without further increase in the efferent dorsal root discharges. *E.* A stimulus to the dorsal root of middle trace excited only a few of the third group of fibers and did not block the efferent spike. *F–I.* Exciting more of the third group fibers blocked appearance of the efferent spike in this root, but not in the other. The interval between the dorsal root stimulus and that to the cell was increasing in *F–I* and in *J* and *K* was sufficiently long to permit reappearance of the efferent spikes. Arrow points to the ADP. *L.* A strong stimulus to the other root also evoked an antidromic potential in the cell, as well as a dorsal root reflex.

The threshold antidromic stimulus for an ADP was also the minimum stimulus required to block an efferent impulse of the same cell by collision with an ascending impulse (Fig. 12). Further evidence on the nature of the ADP is presented in this section.

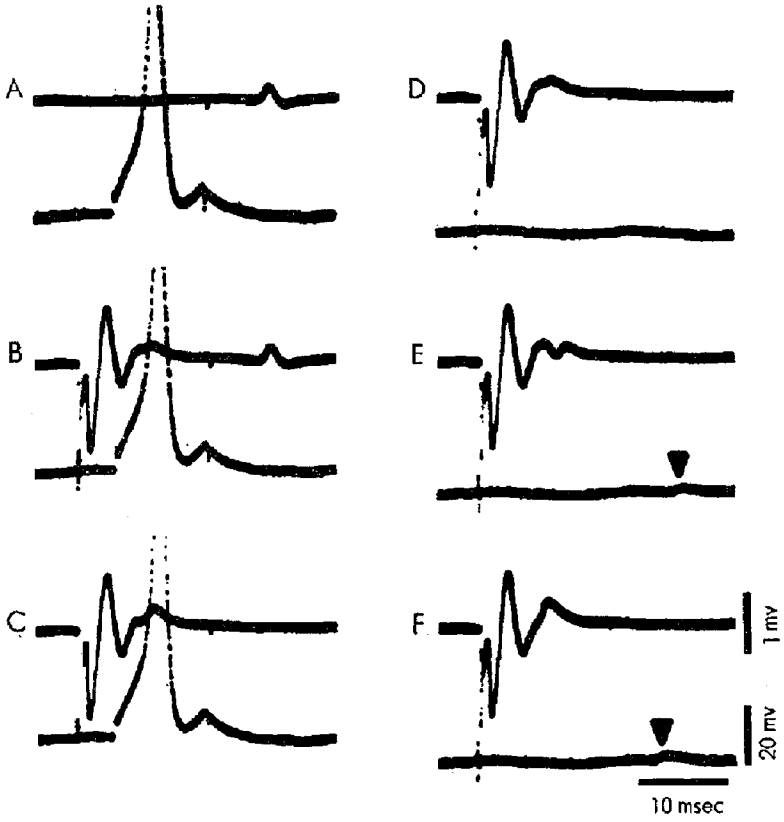


FIGURE 12. Identity of DR threshold for blocking efferent activity and for producing ADP. Simultaneous records from a dorsal root (upper trace) and an SMC (lower). In *A–C*, the cell was stimulated through another microelectrode. The terminal artifact of the 10 msec. pulse appears on both traces. The discharge of the cell caused an efferent response in the DR (*A*). This was not blocked by prior stimulation of the dorsal root which excited only a small part of the third group (*B*). The same DR activity (*D*) produced a p.s.p. in the SMC. *C*. A stronger stimulus to the DR, which evoked a larger third group response, blocked the efferent discharge from the cell. This stimulus (*F*) evoked an ADP in the cell (marked by arrow), as well as an earlier p.s.p. When the stimulus was slightly weaker (*E*) the fiber of the cell was excited later and the ADP (arrow) was correspondingly late.

TIME COURSE OF COLLISIONAL BLOCK OF EFFERENT ACTIVITY BY ANTIDROMIC IMPULSES The maximum interval between the DR stimulus and the direct stimulus to the cell which still permits blockade of an efferent volley

should be the sum of the antidromic conduction time and the period during which the neurite is refractory. That this timing obtains is seen in Figs. 10 and 11. In the latter, as the stimulus to one root was made progressively earlier (*G*—*K*), the collisional blockade was lifted (*J*, *K*), and in *K* the intracellular record carried a small ADP (arrow) which indicated that the ascending impulse had arrived in the neurite near the cell body. The duration of absolute refractoriness in the axon was short, as may be determined by comparing records *J* and *K*, and as will be shown for the soma in Part II (5). At no interval did stimulation of the first root affect the efferent activity in the second root. An ADP, somewhat larger and arising slightly earlier, also occurred when the latter was stimulated (*L*), but this excitation caused no interference with activity in the first root. The absence of interference between impulses in separate axons of the same cell was a consistent finding, and indicates that the antidromic impulses failed before invading a common portion of the neurite.

This separation of different antidromic impulses also holds for axonal branches of one cell in the same root, as seen in Fig. 10. When a maximal stimulus to the dorsal root was delivered progressively earlier in relation to the direct stimulus to the cell (*D*—*H*), blockade of antidromic invasion occurred when the ascending impulses arrived in the vicinity of the SMC. This was denoted by the appearance of ADP's in the intracellular records (*G*—*H*), ahead of the intracellular stimulus. The different axonal branches of the cell which conducted descending impulses at different rates, as denoted by the distinct peaks of the efferent impulses (*A*), also conducted the ascending activity at different rates. This is seen in the appearance of a dual ADP in *G* and *H*. In these records the impulses arrived sufficiently ahead of the direct stimulation of the cell to permit recovery from refractoriness, certainly in two, and probably in all three axonal branches, and the efferent responses were similar to those seen in *A*. However, when the antidromic impulses arrived at a shorter interval before the direct stimulus (*F*), only one of the axonal branches had recovered sufficiently to permit the initiation of an efferent discharge. This fiber was that in which the conduction velocity was highest, since it was the one responsible for the early peak in the efferent discharge (*A*, *G*, and *H*).

BLOCK OF ADP BY EFFERENT ACTIVITY Collisional blockade can be produced by an efferent impulse which is initiated before the antidromic, or after it, during the whole time that the first impulse of either sequence occupies the conducting pathway. The collision results in elimination of both the efferent discharge and the ADP. When the efferent discharge occurs, the ADP is also blocked by refractoriness of the axon to subsequent stimulation. The total duration of blockade therefore is the sum of the anti- and ortho-

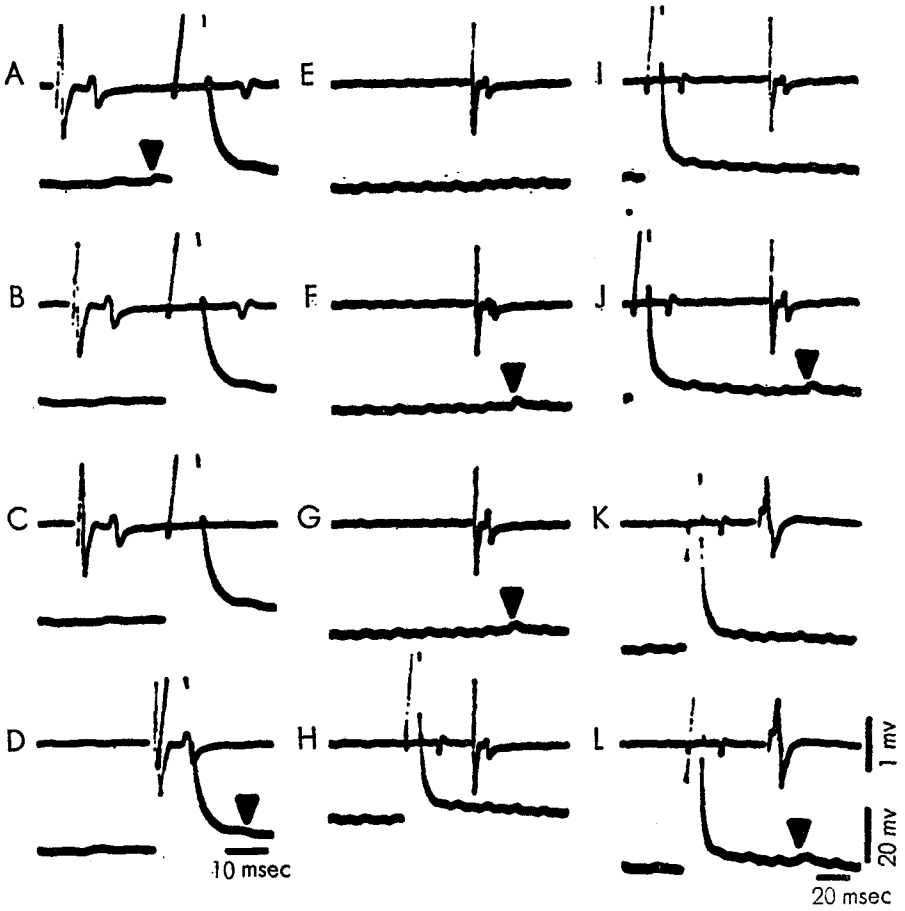


FIGURE 13. Block of ADP by collision and by refractoriness of the axon. Simultaneous recordings from dorsal root (upper trace) and SMC. *A*. A stimulus to the root which excited a few third group fibers evoked an ADP in the cell, seen just preceding a direct spike (marked by arrow in this and other records). The latter caused an efferent discharge. *B, C*. Reducing the interval between the root stimulus and the direct response by 2 msec. sufficed to eliminate the efferent discharge by collisional blockade. *D*. The conduction time of the antidromic impulse was about 20 msec. An ADP which could have appeared after the direct spike (at the time indicated by the arrow) was absent, blocked by the collision of the descending and ascending impulses. *E–G*. Subthreshold and threshold volleys in the DR for producing an ADP. The third group fiber which evoked the ADP responded with a longer latency to a just threshold stimulus (*F*) and earlier to a slightly stronger one (*G*). Prior efferent activity made this one axon refractory (*H, I*), the ascending volley in the dorsal root losing this component, and the ADP disappearing. Relative refractoriness lasted about 60 msec. (*J*) after the efferent discharge. *K, L*. When the root was stimulated maximally the refractory block of the ADP was shortened to about 30 msec. The first and second fiber group responses in the dorsal root were lost in the large stimulus artifact.

dromic conduction times and the refractory period. These components are shown in Fig. 13, block of the ADP by collision in *C* and *D*, and by refractoriness in *H*, *I*, and *K*. The relatively refractory period to threshold stimulation of the axon in the dorsal root was about 60 msec. (*I*, *J*), but was shortened to about 30 msec. when the stimulus was maximal (*K*, *L*). The duration of refractoriness in this experiment was much longer than in others (*cf.* Figs. 14 to 16). The dorsal root had been dissected out for a long distance, shown by the long conduction time for the ADP (20 msec., Fig. 13 *A*), and deprivation of the blood supply may have been responsible for the prolonged relative refractory period.

A stimulus which produces an efferent discharge without a direct spike also blocks the ADP. In the experiment of Fig. 14, a neural stimulus evoked

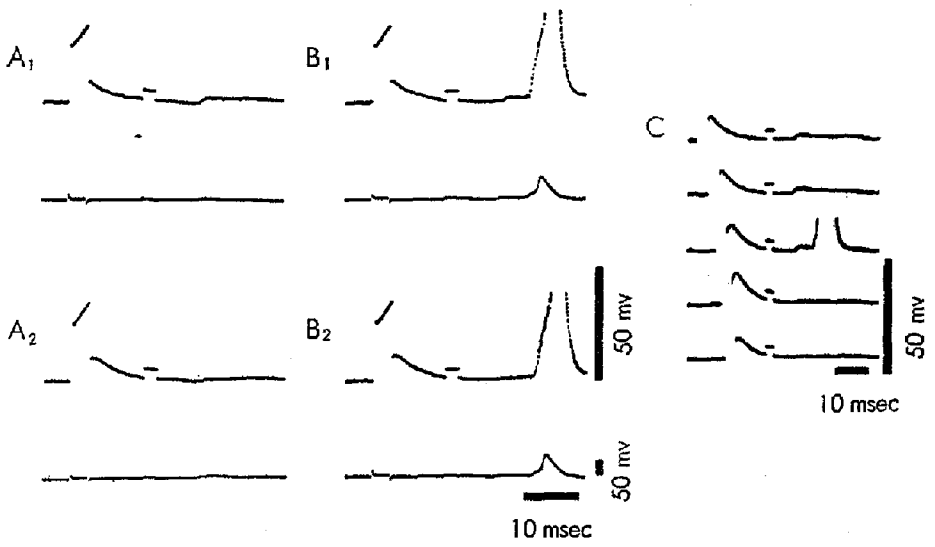


FIGURE 14. Block of ADP by intracellularly initiated axonal impulse without soma spikes. Simultaneous recording from two cells, that registered on upper trace also impaled with a stimulating electrode. A_1, A_2. A weak stimulus to the cauda equina (shock artifact a square pulse on both traces) evoked ADP's in both cells. A preceding weak intracellular stimulus to one cell did not block the ADP (A_1), but a stronger stimulus (A_2) caused an axonal spike which blocked the ADP by colliding with the ascending impulse. The ADP of the other cell (lower trace) was not affected. B_1, B_2. The indirect stimulus sometimes initiated a cluster discharge, denoted by the spike activity in both cells. Note the greater latency of the indirect spike over that for the ADP. A weak intracellular stimulus, which did not evoke a response of the neuron (B_1, upper trace), did not block the ADP. A stronger stimulus (B_2) blocked the ADP of the stimulated cell only. It did not affect the synaptically evoked indirect spike. *C*. Time course of collisional blockade of ADP. A weak neural stimulus evoked an ADP and occasionally also an indirect spike (third record). In three upper traces a weak intracellular stimulus, which evoked efferent axonal impulse, was delivered too early to block the ascending spike that gave rise to the ADP. On the fourth trace, an intracellular stimulus delivered 2 msec. later than on the third, blocked the ADP.

ADP's in each of two cells impaled on microelectrodes (A_1, B_1). The stimulus sometimes also evoked indirect spikes of both cells, this response then appearing after the antidromic potential (B_1). When one of the cells was excited with an intracellular stimulus sufficiently strong to produce a small potential in the soma (A_2, B_2), the ADP was eliminated in that cell only, not in the other. This did not affect the synaptically evoked and synchronized spike discharge of either cell (B_2).

The time course of the blockade is seen in Fig. 14 *C*. An ADP could appear at the cell body when the latter had been stimulated to produce subthreshold activity more than 15 msec., but not less than 13 msec. before the antidromic

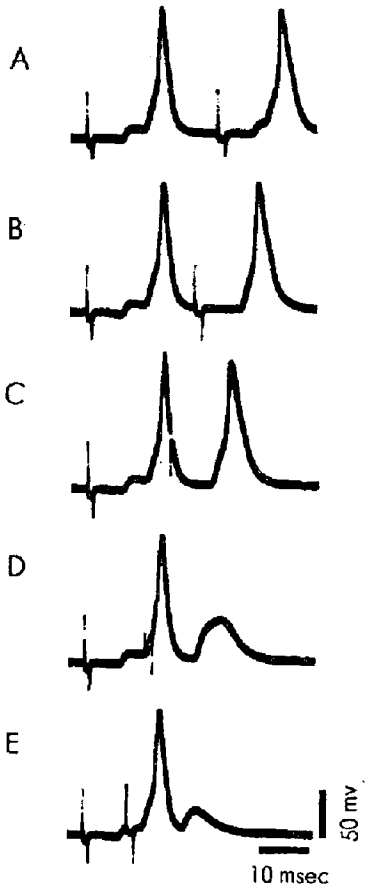


FIGURE 15. Different time relations in block of ADP and of soma spike. Two stimuli were applied to the cauda equina at progressively briefer intervals. *A*. Each indirect spike is preceded by ADP. *B*. ADP of second response was blocked by the efferent impulse which resulted from first spike in the cell. *C*. The second component of the indirect spike, developing during relative refractoriness of the neuron, began later on the initial component. *D*. Still earlier in refractoriness the second component disappeared, leaving the initial component and a small local response. *E*. The initial component was diminished by its occurrence soon after the end of the preceding spike (*cf*. reference 5).

stimulus. Since the antidromic and orthodromic conduction times are equal in this preparation (Figs. 10 to 13), the antidromic impulse (conduction time, 10 msec.) must have been initiated without an appreciably long refractory period. That the disappearance of the ADP was caused by refractori-

ness in the cell body is unlikely, since only small direct responses were evoked and, furthermore, the indirect spike was unaffected (B_2).

An indirect spike may also block an ADP (Fig. 15). The indirect spike arising 10 msec. before the next stimulus, eliminated (*B*) the antidromic po-

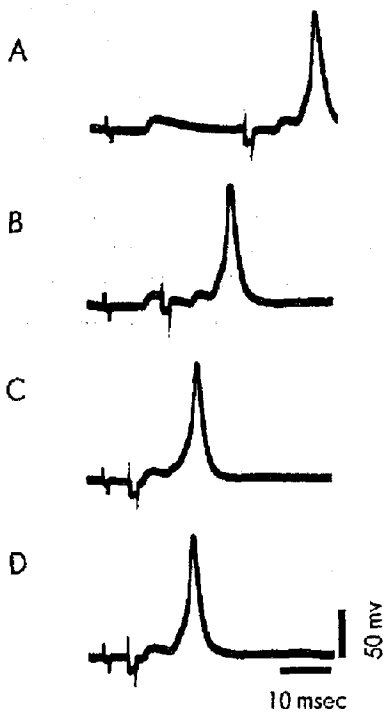


FIGURE 16. Summation of ADP's. Two stimuli were applied to cauda equina at progressively shorter intervals (same cell as in Fig. 15). The second of the pair was stronger and evoked a spike which followed the ADP (*A*). *B*, *C*. The second stimulus evoked an ADP which summed with the ADP produced by the first stimulus. In *C* the indirect spike developed slightly earlier on the ADP than in *A* or *B*. *D*. The second stimulus applied 5 msec. after first occurred during refractoriness of the axon and failed to develop an ADP.

tential that preceded the second neurally evoked spike at longer intervals (15 msec., *A*). Only at much shorter intervals (*C*—*E*) was the second spike blocked. Since conduction time in this experiment was 8 msec., refractoriness of the axon was more than 2 msec. and less than 7 msec. The refractory period, determined directly (Fig. 16) by two stimuli to the dorsal root, was between 5 and 6 msec. The same experiment also shows that following each other, the ADP's of the same axon, far from blocking one another, may summate their depolarizations. However, since the spikes causing the two potentials cannot occur simultaneously, the summation was not as great as was that observed when the separate axonal branches were active simultaneously, or nearly so (Fig. 2 D_2). ADP's could be elicited at the same high frequencies as efferent discharges (above 100/sec.).

SITE OF BLOCKADE OF ANTIDROMIC INVASION The data indicate that block must take place at some distance from the cell body. At the point of failure, the invading impulse should have an amplitude which is about equal to the threshold of the membrane in that region. Assuming that this threshold

is the same as that of the cell body (5) the activity that generated the ADP was reduced five to ten times by electrotonic decrement, and must therefore have been nearly two space constants distant from the cell body. The site of initiation of directly evoked efferent activity without spikes in the cell body must also be distant, since the impulses associated with the activity of the axons produced only a small increase in the intracellularly recorded subthreshold depolarization (Fig. 11 *A*, *B*, and Fig. 14).

Consistent with the inference based on size of the potential, large hyperpolarizations were required to affect the ADP (Fig. 17). The indirect spike was also affected, but by small amounts of hyperpolarization. The inflection on the rising phase was first exaggerated and then the main part of the spike failed, leaving the initial component. Further hyperpolarization reduced the initial component somewhat, but not to the extent of the decrease of the ADP. These observations will be discussed more fully in the subsequent papers (5, 6).

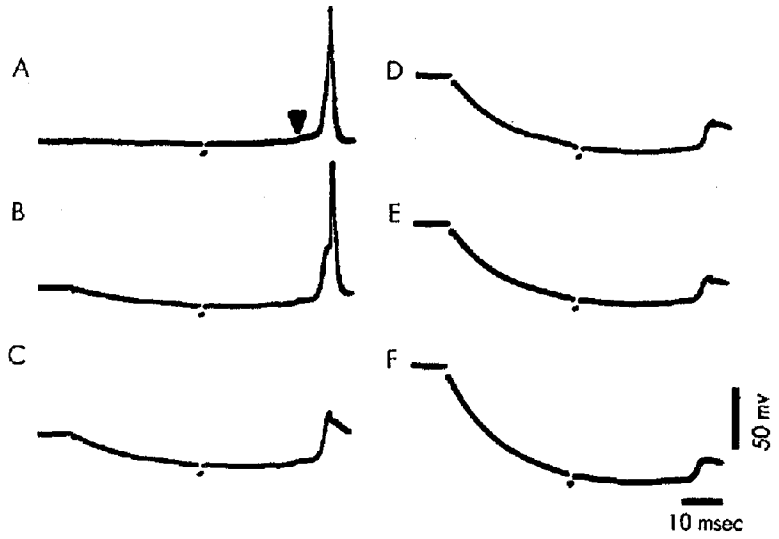


FIGURE 17. Effects of intracellular hyperpolarization on the ADP and on the components of the indirect spike. A brief stimulus to the cauda equina evoked an ADP (arrow) and a spike (*A*). Gradually increasing hyperpolarization delayed (*B*) and eliminated the second component of the spike (*C–F*). The ADP was unaffected until the hyperpolarization was greater than 50 mv. (*E*, *F*) when it was reduced to the noise level. The first component of the spike was somewhat reduced.

COMMENTS

The afferent and efferent relations of the SMC, derived in the present study chiefly from electrophysiological data, but also supported by anatomical work, provide detailed information that has not hitherto been available regarding these cells. The supramedullary neurons participate in a complex

synaptic organization, both with various afferent pathways in the spinal cord and the brain stem, and amongst themselves, as will be shown in detail in Part III (6). Thus, the fibers which they project out of the neuraxis are efferent although they emerge in the dorsal roots.

It has not, as yet, been possible to designate the function of the cells. The axons project to the skin, but probably not to the musculature (Bennett, unpublished observation). Fish deprived of the neurons for up to 2 months showed no defects and this suggests that the efferent actions of the SMC are relatively subtle, perhaps of some autonomic variety. The absence of obvious peripheral effects is particularly surprising since the SMC are so organized that even local stimulation of the skin may cause a synchronized repetitive discharge. However, autonomic efferents emerging in the dorsal roots of the lower vertebrates have been described (27). Comparative electrophysiological studies may clarify the function of the SMC.

Electrophysiological data should also help to resolve the question whether the SMC in various fish are homologous (25, 30). Preliminary work indicates that at least in several species of African fresh water puffers the SMC have essentially the same properties as they do in *S. maculatus.* The work on *S. vermicularis* (24) suggests that synaptic activation occurs also in this form, but none of the unique characteristics of the organization of the SMC in *S. maculatus* was investigated.

That the restriction of the axons of the SMC to a rather uniform group of unmyelinated fibers indicates some primitive function is an attractive hypothesis. These fibers, though unmyelinated, are relatively large and have no counterpart in the nervous system of adult higher vertebrates (22). It is of some theoretical interest to note that the existence of such large unmyelinated fibers argues against the view (*cf.* reference 21) that myelination occurs as a result of attainment of a critical diameter which is generally estimated at about 1 μ. In the ventral and dorsal roots of *S. maculatus* there are many myelinated fibers much smaller in diameter than the large unmyelinated axons of the supramedullary cells.

Another unique feature of the efferent fibers is their profuse branching within the spinal cord and the exit of these separate axons in many roots. Presumably this branching and a consequent reduction in the safety factor for antidromic propagation hinder the invasion of an antidromic impulse into the cell body and it is likely that failure occurs at these forks. Also, the higher threshold of the cell body and neurite (demonstrated in Figs. 11 and 14) must be a factor in preventing invasion. Centrifugal, efferent propagation, on the other hand, must be facilitated by the lower threshold of the axons. The threshold differences may be considerable since several axons can be successively excited by depolarization in the soma without development of either soma or neurite spike.

The potential produced in the cell body by an antidromic impulse is comparable in size to the "antidromic spikes" seen in the somata of lobster cardiac ganglion cells (9). However, in the crustacean cells the somata are incapable of electrically excitable activity. As will be shown in Part II (5), the spikes of the SMC involve activity of the soma membrane.

Antidromic or orthodromic activity in one of the axonal branches may proceed without leading to, or affecting activity in another branch (Figs. 9–11). This finding indicates that centripetal propagation of the impulse in any branch fails before invading a portion of the neurite which can be also invaded by an impulse in another branch of the same cell. This is confirmed by the finding that ADP's from different branches can sum almost completely in the cell body, whereas repetitive ADP's from the same branch sum to a lesser degree.

Different axonal branches of the same neurite may differ with respect to their threshold not only to root stimulation, but also to intracellular depolarization of the cell body. Also, it is not always possible to produce efferent activity in a given root by a stimulus which is subthreshold for the cell body (*cf.* Fig. 9 *A*). One reason for the differences among cells may be inferred from the anatomical data. The branches of the neurite of the upper left cell in the preparation of Fig. 1 *B*, were much more distant from the cell body than they were in the cell of Fig. 1 *C*. In the former case depolarization of the soma would be a less effective excitant to the axons.

The depolarization produced by an antidromic impulse in one branch presumably increases the excitability in the other branches. It is, indeed, likely that the depolarization in the latter is greater than that in the cell body. In the subsequent papers of this series it will be shown that antidromic impulses are excitatory both for the synchronized discharge of the cluster and for direct excitation of a single cell. However, the effect of an antidromic impulse on the threshold of other axonal branches has not been tested.

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