Electrophysiology of Supramedullary Neurons in *Spheroides maculatus*

**III. Organization of the supramedullary neurons**

M. V. L. BENNETT, S. M. CRAIN, and H. GRUNDFEST

**INTRODUCTION**

This part, which concludes the present series of papers (2, 3) on supramedullary neurons, describes various phenomena that are associated with synaptic activation of the SMC, including the mechanisms of the synchronized responses which characterize indirect excitation of the cells. The data and those of the preceding parts are sufficient to formulate a schema of the functional organization of the SMC, which takes account not only of the interrelations of the cells and of their interconnections, but also of the geometric and physiological differences between different parts of the same cell. In the second paper (3) it was shown that the p.s.p. is too small to initiate impulses in the cell body, its indirect spike arising from a stronger depolarization, the initial component. The data indicated that the latter resulted from the response of the neurite.

**RESULTS**

**A. The P.S.P. and Discharges of SMC's**

**FIBER GROUPS WHICH EXCITE THE SMC** In the most excitable preparations cluster discharges were evoked (Fig. 1 A) by stimulating only the lowest threshold fibers of a single dorsal root (DR). Although the discharge could be repetitive, further excitation of the cluster by a second weak stimulus was elicited only if the interval between stimuli was long, 10 to 20 sec. When the stimulus was increased to include activity of the second group of DR fibers (B) the discharges of the cluster could be evoked by more frequent stimulation. A maximal stimulus to the DR, which evoked activity of the third fiber
group, permitted indirect discharge of the cells as frequently as once every 2 sec. However, the efferent discharge, as recorded in the dorsal root, was then modified by the refractoriness left behind by the ascending impulses. In less excitable preparations, single maximal stimuli even when delivered to several roots simultaneously sometimes failed to activate the cluster.

**Figure 1.** Activation of efferent discharges of SMC's by stimuli involving different fiber groups of a single dorsal root. A. Weak stimulus to root excited only first fiber group, but elicited repetitive discharge of cluster denoted by the two large efferent volleys. B. A stimulus which evoked submaximal activity in the second group of fibers was capable of discharging the cluster at shorter latency. C, D. A maximal afferent stimulus could discharge the cluster still earlier, but the efferent volley was diminished and dispersed by the prior activation of the third group fibers. The second discharge of the SMC in D was not affected.

**Gradation of p.s.p.'s** Stimuli to a dorsal root which did not discharge the SMC's, nevertheless, evoked p.s.p.'s in the cells (Fig. 2) and these were graded with the size of the afferent volley. When fibers of the third group were also activated (C', D') the briefer ADP was superimposed on the long lasting p.s.p.'s in the cells projecting to the stimulated root, with little or no change in the p.s.p.'s.

The p.s.p. elicited by a volley of afferent impulses was prolonged (Fig. 3) in comparison with the p.s.p. evoked by a single stimulus. A tetanus about half-maximal for the first group (B) produced a small, long lasting p.s.p. The amplitude was increased by exciting also the second group of DR fibers (C, D). The p.s.p. rose to its peak slowly and lasted throughout the period of the sweep, more than 250 msec. The train of maximal second group volleys (D) evoked a cluster discharge near the peak of the p.s.p. and an efferent volley in the third group fibers of the root.

Gradation of p.s.p.'s and accompanying change in the response of the SMC's were also produced by tactile stimulation (Fig. 4). Different regions of the body could be so stimulated to produce activity in the SMC. The cells discharged approximately when the p.s.p.'s had reached their peak. However, it must be emphasized again that the depolarizations recorded in the
cell body (Figs. 2 to 4) during the p.s.p.'s were much too small to discharge the cells, which have a critical firing level of approximately 20 mv. (3).

HOMOSYNAPTIC FACILITATION Two stimuli to the same root could activate the SMC when neither afferent volley by itself was effective. In the ex-
Figure 3. Summation of p.s.p.'s evoked by a train of four stimuli of different strengths to DR fibers. Upper trace, recording from SMC; lower, from a DR. 

A. Weak afferent volley, involving only part of first group of fibers. B. Maximal stimulation of first group. C. Submaximal stimulation of second group. D. Maximal stimulation of second group. This caused a discharge in the cell (upper trace) and also in the cluster, as indicated by the later, large and long lasting potential in the DR.

Figure 4. Gradation of activity in SMC evoked by tactile stimulation of the skin: 

Left, over the right eye; right, on the right side of dorsal fin. In lower trace of C and C1, recordings from DR; other traces show intracellular responses of one cell. A, A1. Weak stimuli evoked small p.s.p.'s. Stronger stimuli evoked larger p.s.p.'s and spikes associated with discharges in DR.
Experiment of Fig. 5 A–E, a maximal volley in the dorsal root (A) did not produce activity of the SMC. However, when the root was previously stimulated so as to activate only its first two fiber groups the cluster was discharged after the second stimulus (B, C). When the two stimuli were separated by more than 50 msec. (D) the facilitatory interaction ceased. Thus, the weaker conditioning stimulus, itself incapable of exciting the SMC's, nevertheless initiated excitatory processes which were capable of facilitating a second, ineffective stimulus. In this experiment it may be noted that both the centripetal and the centrifugal action potentials contributed by the third group were typically large. Therefore massive antidromic invasion of the cluster, which should have been produced by the strong stimulus to the root, did not fire the SMC. Apparently, though richly supplied with efferent fibers from the SMC this root had a weak inflow of afferents to the cluster.

The conditioning stimuli need not be subthreshold to facilitate responses to
Facilitation of discharges with two stimuli to the same root with one or both adequate to produce discharges. Three simultaneous traces registered (from above downward) from two dorsal roots and from an SMC. A pair of stimuli were delivered to the root of the upper trace every 10 sec. (A -- E) or every 2 sec. (F -- J). Note faster time base in the latter series. The second stimulus of the pair in this series was also weaker. In isolation, it did not cause a discharge of the SMC, but a long lasting p.s.p. was evidence of the facilitatory capacity of the afferent volley. The facilitation that occurred in both series increased the frequency of the discharges as well as their number. In E, the second afferent volley arrived at the SMC early enough to change the frequency of the discharges caused by the first stimulus alone which had remained unaffected in A -- D. The antidromic component of the testing volley was interfered with in B, C, D, I, and J and probably in E by refractoriness after an efferent volley in group three fibers or by collision with a volley descending in these fibers.
a subsequent stimulus. In Fig. 6 A each of two maximal stimuli to a root produced a pair of discharges of the SMC. As the interval between the stimuli shortened (B - E) a total of five responses was evoked and these discharges tended to occur at higher frequencies. In B and C the preceding efferent volley had reduced the third group component in the response of the root to the second stimulus. Facilitation was nevertheless present. In D the timing was such that an efferent volley probably blocked impulses ascending in the same fibers excited by the second stimulus to the root. In E the first efferent discharge was reduced by the preceding afferent volley, but at this interval between the two stimuli the facilitatory interaction was largest.

Complete characterization of the time course of homosynaptic facilitation requires evaluation of facilitatory effects on latency, number and frequency of repetitive discharges, and must be based upon a large sampling of responses. However, maximal synaptic responsiveness was obtained only when intervals of 30 sec. or more had elapsed between stimulations. In the time required to obtain a large group of data at this rate, conditions and responsiveness of the preparation were prone to change. Therefore, detailed analysis of the time course and the factors which affect it was not attempted in the present work.

The effects of the rate of stimulation may be seen by the comparison of the responses in Fig. 6 A - E, evoked at intervals between stimuli of 10 sec., with those in F - J, in which the stimuli were delivered every 2 sec. Applied at the higher frequency, the maximal conditioning stimulus now caused only a single discharge of the SMC (F) which rose out of a long lasting p.s.p. A weaker testing stimulus did not evoke a discharge, although a p.s.p. developed in the penetrated cell (G). Delivered 60 msec. after the conditioning stimulus, the testing stimulus produced no discharge (H) although at the same interval (B) between the two stimuli facilitation was evident when the SMC were excited less frequently. At shorter intervals between the stimuli (I - J), facilitation occurred. The facilitation in this case, too, developed despite block of the third group fibers in the testing volley (I) and their collisional encounter (J) with the first efferent volley.

Thus, while an excitatory influence from the third group fibers was sometimes required to discharge the SMC (as in Fig. 6 F, G), long lasting excitatory effects were not dependent upon this group of fibers, but were produced by afferent activity in the more rapidly conducting lower threshold fibers. Since the third group of fibers is composed of the axons of the SMC, the excitatory influence which they contribute is that caused by the ADP (2) and its duration is relatively brief. Two kinds of facilitation may be produced in this way. The depolarization contributed by the ADP may sum with the depolarization by p.s.p.'s, and also the two successive volleys may generate two ADP's that sum. Facilitation of the first kind was involved in the ex-
periments of Fig. 5 $A - E$ and Fig. 6 $F - J$. In Fig. 6 $A - E$, both volleys were maximal and the facilitation might also have involved summation of the ADP's in some cases.

Figure 7. Excitation of SMC by heterosynaptic facilitation of volleys afferent in different dorsal roots. The upper and middle traces from two dorsal roots ($a$ and $b$). Note that the amplification of the middle channel is three times that of the upper. The lowest trace records from a cell of the SMC. $A$. Stimulus to dorsal root $a$ only. The afferent volley comprised the maximal responses of fibers in groups 1 and 2 and a small component of group three fibers. This stimulus failed to discharge the SMC. $B$. Dorsal root $b$ was stimulated 60 msec. before dorsal root $a$, also without affecting SMC activity. The stimulus to root $b$ activated only the fibers in first group. $C - F$. Closer approximation of the two stimuli evoked activity in the observed cell and efferent discharges in both dorsal roots. Facilitation occurred when either afferent volley preceded the other, but latency was briefer when the weaker afferent volley in root $b$ was the immediate excitant ($E, F$).
The longer lasting action of the afferent fibers which must arise from
synaptic effects also has two components. The facilitative interaction between
two afferent volleys, which lasted about 60 msec. (Fig. 5 C, D, and Fig. 6 B),
indicates a relatively simple pathway of synaptic bombardment of the SMC.
However, superimposed upon this excitatory influence were longer term
actions, predominantly diminishing the effectiveness of synaptic excitation if
the latter occurred more frequently than once in 10 sec. This indicates the
possibility that very complex pathways were involved, but the matter has not
been studied in further detail.

HETEROSYNAPTIC FACILITATION  Stimuli applied in appropriate timing
to two different roots evoked discharge of the SMC (Fig. 7 C - F), although
the individual afferent volleys were not effective by themselves (A, B). This
type of excitation involves different pathways which converge into a common
path. However, if the afferent pathways have any degree of complexity, and
this appears to be likely, the difference between homosynaptic and hetero-
synaptic types of facilitation which may be expected in simpler situations
(13) would be blurred in the activation of the SMC. Heterosynaptic facilita-
tion was also observed between afferent volleys in cranial nerves and the cauda
equina.

The facilitatory interaction need involve only the low threshold, afferent
fibers and therefore can be produced solely by summation of p.s.p.'s. How-
ever, as noted in the preceding section and in Part II (3), one component of
facilitation of the SMC is the depolarizing contribution of the ADP. Although
two different roots have efferent axons from the same SMC (2) it is unlikely
that all, or even the majority of the third group fibers in one root derive
from the same neurites as do the fibers in another root. For most cells, there-
fore, the excitatory contribution of ADP's in heterosynaptic facilitation is
thus principally by summation of single ADP's with p.s.p.'s, whereas in homo-
synaptic facilitation an additional component is the further contribution
from a second ADP.

In the example illustrated in Fig. 7, the latency of the discharge is also of
interest as indicating the synaptic nature of the facilitatory interaction. When the dorsal root of the middle trace (root b) was the conditioning path-
way (C), the SMC discharged in response to the additional stimulation from
the dorsal root of the upper trace (root a) at a somewhat shorter latency than
when the two volleys were nearly simultaneous (D). In the inverse order
of presentation, application of the afferent volley in root b, after the volley
in root a, the discharge came earlier (E, F) than in either of the other com-
binations. The greater potency of this afferent sequence is shown further by
the repetitive discharges which could then be produced (E).
B. Synchronization of Responses in the SMC

Synchronized responses to different afferent stimuli The preparation of Fig. 8 is that photographed in Fig. 1A of Part I (2). Four cells that are shown in the diagram (Fig. 8D) were impaled with microelectrodes for simultaneous registration. Electrical stimuli were applied to a cranial nerve trunk (A1 – A3) or to the cauda equina (B1 – B3).

Weak electrical stimuli evoked a single spike in each of the four cells (A1, B1). These threshold stimuli occasionally failed to evoke responses at all. In that case the spike was absent in all four simultaneously observed cells. The most rostral of the cells responded earliest to cranial nerve stimulation (A1), the other cells responding later, in the order of their distance from the rostral cell. The reverse order of latencies occurred in response to the caudal stimulation (B1). The differences in latency were diminished with stronger stimulation (A5, B5), and were occasionally reversed, as is seen in the response of the two rostral cells (upper traces A5; cf. Fig. 9B5).

No matter what kind of stimulus was applied, the form of the response of each cell was rather constant and distinct. Thus, the two most rostral cells had a pronounced neurite spike which was less conspicuous in the two more caudal cells. On the other hand, both of the latter manifested ADP's ahead of the spikes in B1 and B2. As was already noted (2) no ADP was produced in the caudal cells in response to stimulation of the cranial nerves.

Repetitive, synchronized activity was produced when the stimuli were stronger (A5, B5). The latencies of the later spikes in a repetitive response did not show consistent differences. Not every cell responded with a spike, but when the latter failed to develop there was always an elevation due to the neurite spike. There was also a pattern in this respect. In the four sequences shown, the most rostral cell failed to develop full spikes 6 out of 16 times, the other cells 2, 1, and 0 times respectively. Since the late onset of a soma spike on the neurite spike indicates a low safety margin for invasion of the cell body (3), it is not surprising that the most rostral cell failed to produce spikes most frequently.

Tactile stimulation of the skin also produced synchronous repetitive responses in these four cells (C). The forms of the spikes in each of the cells were characteristic and identical with those produced by the electrical stimulation of afferent pathways. In this experiment, however, prolonged spikes (3) developed in three of the four cells responding repetitively to the tactile stimulation. In many experiments spontaneous, frequently repetitive discharges were observed. These were also synchronously present in all the cells under examination at the time.
Figure 8. Synchronous activity in four cells of supramedullary cluster evoked by different pathways (A, B, C) and by different strengths of the stimuli (A1 – A3 and B1 – B3). The cluster is shown diagrammatically in D. Lines symbolize microelectrodes penetrating the four cells to which they point. In each sequence of simultaneous recordings, the activity in the most rostral cell is on the top trace, the others below in order of their position in the cluster. Calibration pulses (50 mv., 1 msec.) occur at the beginning of each of the four traces. A1 – A3. Stimuli were given to cranial nerves. A1, weak stimulus; A2, stronger stimulus; A3, same stimulus strength, but slower time base. B1 – B3. Stimuli were applied to cauda equina. B1, weak; B2 and B3, stronger stimuli. The stimulus artifacts are discernible following the 50 mv. calibration pulses in A1 – A3 and B1 – B3. C. Responses to tactile stimulation.

The maximum frequency of the repetitive bursts calculated from the intervals between responses was about 200/sec. (A3, B3). The maximum firing frequencies in response to tactile stimuli were comparable to those produced by indirect electrical stimuli. However, the intervals between responses varied considerably. Thus, to the strongest rostral stimulus the cells responded, at first, with high repetitive rates (A3) and then more slowly, the fifth spike occurring about 50 msec. after the fourth. The strong caudal stimulus (B3) on the other hand, evoked a rather uniformly spaced train of five impulses. The
pattern of repetitive activity in the responses to tactile stimuli usually showed the greatest variation, probably reflecting the irregular nature of the stimulation and the dispersed nature of the synaptic excitation of the SMC. In Fig. 8 C the first response of three of the cells was followed immediately by a small elevation which represents a neurite response as described above.

The occurrence of synchronized responses to any form of indirect stimulation suggests either that the afferent excitation operates through some "synchronizing center" which then excites the SMC, or that the SMC are linked together so that excitation of one tends to excite the others. The data presented in the subsequent sections confirm the latter alternative.

![Figure 9](image)

**Figure 9.** Distribution of excitatory effects to different cells of the cluster. Left, cranial nerve stimulation; right, of the cauda equina. Simultaneous recordings from a rostral cell (upper trace) and a caudal cell (middle trace); a reference line is also shown (lower trace). From above down, three strengths of afferent stimulation. Further explanation in text.

**Distribution of excitation from different afferent paths** As already noted, the latencies of the responses in the rostral cells were shorter than those in caudal cells upon weak stimulation of the cranial nerves ($A_1$) and longer when the caudal nerves were excited ($B_1$), but the differences were reduced when the stimuli were stronger and during repetitive responses. The p.s.p.'s show some correlation with these findings (Fig. 9). A weak stim-
ulus to cranial nerves evoked a larger p.s.p. in a rostral cell (upper trace) than a similar stimulus to the cauda equina \((A, B)\). The reverse was true for the p.s.p.'s evoked in a caudal cell by the same stimuli, but the difference was not as marked.

**SITE OF THE SYNCHRONIZING MECHANISM** The effects of acute sections of the spinal cord and of the cluster indicated that the synchronization takes place within the immediate confines of the cluster. On this basis alone, it seems likely that the cells themselves participate in the processes which lead to synchronization.

![Figure 10](image-url)

**Figure 10.** Responses evoked in a pair of cells of the cluster after transection of the cluster and dorsal half of the spinal cord. Upper trace records from a cell in rostral half of divided cluster, lower trace from a caudal cell. \(A\). Each cell responded only to the stimulation of its side of the division (stimulus to cord appears earliest on the trace). \(B, C\). Cranial nerve stimulus also produced a single delayed response in caudal cell. \(D\). Each stimulus evoked activity across the section. These responses were late and were not synchronized with responses of the cells on the other side of the section.

Transection of the neuraxis immediately above the cluster did not affect the synchronized responses of the cells to stimuli from the caudal part of the neuraxis. Similarly, transection of the spinal cord immediately caudal to the cluster left intact the synchronized responsiveness of the cells to stimulation of cranial nerves. A complete transection through the middle of the cluster left intact the synchronized responsiveness of each half of the cluster.
to stimulation on its own side of the transection. These results indicate that the synchronizing mechanism occurs at both ends of the cluster of SMC.

In one experiment with histological verification, a cut was made which divided the cluster and the dorsal quarter of the spinal cord. This left intact the synchronized responsiveness of all the cells to stimulation both of the spinal roots and of the cranial nerves. From this experiment and a similar one without histological verification, it may be concluded that synchronization does not necessarily involve interconnections close to the cell bodies themselves.

When the cord section was carried down to the level of the spinal canal (three experiments, two with histological verification) synchrony between the two half-clusters was disrupted. Stimuli delivered to pathways on one side of the cut still produced activity on both sides (Fig. 10). However, the responses were later and fewer in number in the half-cluster across the section, but within each part activity was always synchronous.

C. Spread of Directly Evoked Impulses

SYNCHRONIZED ACTIVITY FOLLOWING A DIRECT STIMULUS TO ONE CELL

If, as was indicated in the preceding section, connections between the SMC are responsible for synchronized activity in the cluster, intracellular stimulation of only one cell might be expected to spread and thus fire all the cells. Spread did occur in about 25 per cent of the cells in the present experiments, one of which is illustrated in Fig. 11. The cell of the lower trace which was directly excited lay caudal in the cluster to the cell of the upper trace. To stimulation of a cranial nerve (F), both cells responded (and presumably the rest of the cluster also), although with the marked difference in latency that has already been described in connection with Fig. 8. The other records of Fig. 11 show examples of responses to stimulation of the caudal cell with an intracellular electrode. In about half the stimulations, a small potential followed the direct spike (B - E). When this potential occurred, a spike also followed in the cell of the upper trace. Invariably in this and other experiments, whenever the small potential occurred in a directly excited cell, a discharge also occurred in other cells under observation, and the characteristic efferent volley could also be seen in the dorsal roots. Therefore, synchronous activity of all the SMC was involved. The responses differed in an important respect from the spike in the directly excited cell which initiated the repetitive

1 If the medial blood vessel of the cord was cut in performing the transection, the indirectly evoked activity of the caudal portion of the divided cluster disappeared rapidly. Even when the vessel was preserved by carefully sectioning the cord around it there tended to be diminution of responsiveness in the caudal cells, marked by absence of repetitive activity. This reduced excitability probably was due to injury of the cells when deprived of blood supply. However, interruption of "tonic" excitatory afferents from a higher level cannot be excluded.
discharge. They resembled in form an indirect spike, being preceded in all cases by a neurite spike.

The records in Fig. 11 were arranged in order of increasing latency of the appearance of the small potential in the directly excited cell, although, in the experiment, the potential occurred at random intervals and was absent in half of the records (A). In only this experiment was the additional potential late enough and large enough to permit reexcitation of the refractory directly excited cell (E). Thus, this potential appears to be identical with the neurite component of the indirect spike, and shows the same reduction in amplitude as did that component, when it occurred during refractoriness of the cell (cf. reference 3, Fig. 12).

Presumably, the late potential arose by reexcitation of the directly stimulated cell by the cells which had been discharged by its activity. This indicates that some kind of mutual interconnections exists between the cells of the clus-
ter, and further evidence for this conclusion will be presented below. At this juncture, however, it is appropriate to note the time relations of the responses in the two cells of Fig. 11. The interval between the direct spike and the response of the rostral cell varied, but between the small potential and the discharge of the rostral cell the interval was nearly constant. It seems likely therefore that spread of excitation from the directly stimulated cell occurred by exciting first the adjacent cells and then spreading outward from this local center of activity. This order of activation was confirmed by experiments such as that of Fig. 16.

**Figure 12.** Potential recorded in one cell when an immediately adjacent cell was excited directly. Simultaneous recordings from two cells in upper and middle trace. The second cell was also impaled with a stimulating microelectrode. The intracellular current delivered by the stimulus is shown on the third trace. A. The stimulus evoked a spike. A small, slowly rising and falling potential appeared in the adjacent cell. B. The same stimulus evoked a subthreshold response. There was no potential change in the adjacent cell.

**MECHANISM OF SPREAD** On recording from two immediately adjacent cells a small potential was frequently observed in one cell, when the other was excited directly, although a cluster discharge did not ensue (Fig. 12 A). The potential did not occur when the directly excited cell developed only a local response (B). The potential was small (ca. 5 mv.) and lasted about 15 msec. It was found only in contiguous cells, and not in all of these. It is probable that this potential is associated with the mechanism of spread of excitation from one cell to another in the cluster, and it has been designated as the "spread potential." Its small size suggests that, like excitation by afferent pathways, excitation by spread is initiated at some distance from the cell body. A local response in a SMC was never observed to produce spread.

**FACILITATION OF SPREAD BY REPETITIVE DIRECT STIMULATION** The probability of spread occurring from directly excited cells varied considerably. At low rates of stimulation (1 in 2 sec.) direct stimulation of some cells might never cause spread of activity. Stimulation of other cells at the same rates invariably caused spread (Fig. 14). Thus, the probability of spread ranged from 0 to 1 for different cells.

In cells with low probability of spread, it was frequently possible to facili-
tate involvement of the cluster by repetitive stimulation, but once produced spread was not thereby established for subsequent stimuli. Intervals at which facilitation was observed ranged up to 100 msec. This is long compared to the time constant and suggests that the facilitation was not due to summation of spread potentials. An example of an experiment in which it was difficult to obtain spread is shown in Fig. 13 B. In more than twenty excitations of one cell (lower trace) with trains of stimuli at various frequencies, only the one case of spread that is illustrated was observed. When spread occurred the

![Image](image_url)

**Figure 13.** Spread of activity during repetitive direct stimulation of one cell. Synchronized discharge of the cluster is denoted by activity produced in the cell registering on the upper trace. A1, A2. Direct stimuli to a cell registering on lower trace were at 50/sec. B. Stimulation at 70/sec. in a different experiment. Note that a small potential appeared after the directly evoked spike whenever the other cell also discharged in records A and B. A gradual prolongation of the direct spike is also seen in B.

directly excited cell developed a small additional potential similar to that seen in Fig. 11.

In other experiments, cells were found capable of causing spread at relatively high frequencies for brief periods (Fig. 13 A1, A2). Modification of the
spread from two such cells in the same cluster is shown in Fig. 14. Single excitation of one of the cells always caused response in the whole cluster (firing probability = 1). The firing probability was only 0.3 for stimulation of the other cell. When trains of five stimuli at 50/sec. were applied to each cell, the firing probability of the second cell (which was that of Fig. 13 A) increased to nearly 1 at the third stimulus of the train, while that of the first cell decreased. On the fourth and fifth stimulations the firing probability of both cells decreased nearly identically. On the same graph, a plot for the cell in Fig. 13 B would indicate zero firing probability.

Facilitation of Spread by Interaction of Direct and Indirect Stimuli

Direct excitation of a single SMC which did not cause spread could do so when it was preceded by indirectly evoked activity of the cluster. The response of the directly excited cell was then followed by a small potential like that in the responses which produced activity of the cluster in Figs. 11 and 13. The excitatory effects of the indirect volley which led to spread of the directly evoked response in this case were complex. The indirect stimulus probably produced excitation both synaptically and by ADP's. However, that due to the latter should not outlast cluster discharge. In addition, the interconnections which led to the synchronized discharge of the cluster may also have been facilitated by their activation as shown in the preceding section. These different factors have been analyzed in other experiments.

![Graph showing probability of spread of activity during repetitive direct stimulation.](image-url)
Spread was facilitated frequently by afferent stimuli which included only first group fibers. It occurred more frequently when strong stimuli activated the second and third group fibers. Firing of the cluster was not necessary for facilitation of spread. The cell of the upper trace in Fig. 15 excited the cluster frequently when it was stimulated at a low rate. However, when it was stimulated at 15/sec., as in the illustration, spread was prevented. Every 2 sec. during the sequence of direct stimulations a stimulus was also delivered to the spinal cord. This did not discharge the cluster, but produced an antidromic potential in another cell (lower trace). The sequences of Fig. 15 are arrayed according to increasing intervals between the indirect stimulation and one of the direct stimuli. When both were delivered nearly at the same time (A) the antidromic impulse arrived at the cluster nearly 15 msec. after the cell of the upper trace had been excited directly and nearly 60 msec. before the next direct stimulus. No spread occurred. When the direct stimulus was delivered at intermediate times, as in B and C, the depolarizations...
produced by ADP might have aided spread. However, in D the ADP ended considerably before the next direct stimulus produced spread. Thus, the indirect stimulation facilitated spread for more than 50 msec. (D), but less than 60 msec. (A). Assuming that the ADP has the same duration in all cells, the facilitation developed in D must have resulted only from the synaptic bombardment of the cluster by the afferent volley. The duration of facilitation following a direct stimulus was always shorter (A, D) and may be correlated with the short duration of the spread potential (Fig. 12).

As may be expected from the finding (Fig. 9) that both anterior and posterior cells of the cluster developed p.s.p.'s when stimulated by either cranial or caudal afferent pathways, spread from directly excited cells at either pole of the cluster was facilitated by both afferent paths. This is shown for direct stimulation of a posterior cell in Fig. 16. Spread occurred first to cells in the middle of the cluster (B, F) and then to those at the rostral pole. Although the rostral cell (upper trace) was farther from the middle cell (middle trace) than was the directly excited caudal cell (lower trace) most of the delay in the spread took place between the latter two. This response pattern also occurred when the spread resulted from repetitive direct stimuli without facilitation by p.s.p.'s (E). On the other hand, in the synchronized responses to cranial nerve stimulation (D) the caudal cell always responded later.

D. Invasion of an Antidromic Impulse

That the SMC's respond synchronously to indirect stimuli itself suggests that antidromic invasion does not occur. Otherwise, at intermediate stimulus strengths some axons would have been excited to produce spikes in some cells, but not in others. The not infrequent absence of cluster activity on maximal stimulation of a dorsal root (Figs. 2 and 5) confirms the data presented earlier (2) that antidromic invasion does not normally occur.

However, if antidromic invasion did occur, spread of the excitation to other cells would also tend to be produced, since the response would develop on a background of synaptic excitation by the lower threshold afferent fibers, which would facilitate spread (Figs. 15 and 16). These points may be noted in the one observed instance of antidromic invasion (Fig. 17). In this experiment a stimulus to the spinal cord on occasion fired a cell which responded with activity very similar to an indirect spike, except that it did not always involve other cells of the cluster (A, C). Frequently the cell failed to produce a spike (A) and there remained a potential similar to the ADP, but of larger amplitude. At the same time (upper trace in B, B) a small ADP was seen in another cell. That the large prefatory potential was an ADP was demonstrated by its collisional blockade with a directly excited efferent impulse.
Facilitation of spread from a caudal cell by rostral and by caudal stimulation. Diagram (H) of cluster with cells indicated from which recordings were made; dotted region obscured by blood vessels. Upper, middle, and lower traces: rostral, middle, and caudal cells respectively. Single direct stimulation of caudal cell did not result in spread (A). Cranial nerve (C) or cauda equina (G) stimulation, each itself subthreshold, facilitated spread (B, F) which first reached the cell nearest the directly excited cell. Repetitive activation of cell also facilitated spread (E) which occurred with the same pattern of latencies. Suprathreshold cranial nerve stimulation (D) fired rostral cells earlier.

(B2). In the cell of the upper trace the smaller ADP was not affected. The ADP, though unusually large, barely reached the critical firing level of the cell (C). Sometimes, a synchronous discharge of the cluster was evoked by the stimulus which produced the ADP. Then, the spike occurred after the
peak of the ADP ($A_2$) as well as near it ($A_4$). In the former case the spike could not have been produced by the ADP and was probably caused by spread of activity from other cells. In the latter case, the ADP might have initiated the spike and the synchronous activity might then have resulted by spread from this cell. Spread was not observed when the cell was directly stimulated ($B_4$, $C$, right).

Further evidence to support the conclusion that the prefatory potential was an abnormally large ADP was obtained from the ease with which it was diminished markedly by even small hyperpolarization of the cell ($C$, left). The reduction occurred in an all-or-none manner, and left behind a smaller potential, similar in size to ADP's usually observed. The neurite component of the indirect response, it will be recalled (3), is rather insensitive to hyperpolarization. The normal ADP also is relatively insensitive, but less so than the initial component.

![Figure 17](image)

**Figure 17.** Antidromic invasion of an SMC. $A_1 - A_4$. Recording from two SMC's, stimulation of the cauda equina. A spike occasionally occurred in the cell of the lower trace without activity of the other cell. $B$, $C$, upper trace, a third cell; second trace, same as lower trace in $A$; third trace ($C$ only), current through intracellular stimulating electrode in cell of second trace. Explanation in text.
DISCUSSION

INTERACTIONS OF AFFERENT CONNECTIONS

Several varieties of evidence have been presented which show that the SMC are excited indirectly by many different afferent pathways. The full synaptic complexity of the afferent paths is not known. However, antidromic impulses, which travel in fibers of slowest conduction (2), frequently generated ADP’s before the start of synaptically evoked activity. Since synaptic excitation of the cluster is evoked by more rapidly conducting fibers, it is therefore likely that the delay is caused by synaptic delays at the numerous relays. Our histological preparations do not show a prominent cellular component in the dorsal half of the cord in the vicinity of the SNC’s. However, the interneurons might be located elsewhere in the neuraxis.

The synaptic excitatory actions probably involve chains of various lengths and this complexity could give rise to varieties in the patterns of the discharges such as were shown in Figs. 8 and 9. The slow rise and fall of the p.s.p. and its long duration, as well as the long duration of facilitatory effects are consonant with this interpretation. The intervention of pathways of greater complexity is also indicated by the alterations in the relative latencies of different SMC’s on stimulation of caudal and cranial afferent nerves with different intensities. The shortened latencies could result from reduced synaptic delays due to more vigorous inflow of afferent impulses, but it is more likely that they would result from a by-passing of interneurons in a chain of synaptic connections. Combinations of brief and long lasting synaptic bombardments which result in cat cortex from a single stimulus to the caudate nucleus have been recently described (20).

It seems likely that the central nervous activity which excites the SMC also develops inhibitory influences. This is suggested by the effects of frequent stimulations, as noted in connection with Figs. 6 and 15, and also by the decline of the probability of spread in Fig. 14. The inhibitory effects to account for the data of Figs. 6 and 15 need not have developed at the final path represented by the SMC’s themselves. Indeed, no hyperpolarizing p.s.p.’s were ever seen in these cells, although they have been observed in spinal neurons of the puffer (Bennett, unpublished data). On the other hand, it seems likely that inhibitory influences which might depress the probability of spread of excitation would have occurred in some relatively direct relation to the SMC’s, since spread is itself due to interactions amongst these cells themselves. However, inhibition is a process which can develop without hyperpolarization and even without changes in the resting potential (4, 7, 14).
INTERACTION BETWEEN P.S.P.'S AND ANTIDROMIC IMPULSES  Antidromic impulses did not, except in one experiment (Fig. 17), invade the cell body. Nevertheless, the ADP's which these impulses produce must add their depolarizing excitatory effects to the excitation produced by synaptic means. This is indicated by the fact that sometimes maximal afferent activity in the first and second groups of dorsal root fibers could be facilitated by the addition of antidromically ascending impulses in the third group of the same or different roots. The same facilitatory action of the ADP may also aid spread of excitation from intracellular stimulation of a single SMC. The antidromic impulses probably do not initiate synaptic activity in the spinal cord, since they produce little or no increases in the p.s.p. and the efferent discharge in the dorsal roots is not associated with activity in the ventral roots (2) or in other fiber groups of dorsal roots.

SITE OF SYNAPTIC CONNECTIONS  Only indirect evidence is available concerning the sites of the synaptic connections on the cells. They do not appear to be on the soma. Since the p.s.p. is small, and the cellular depolarization which it produces is far below the critical firing level for the spike, the fact that synaptic excitation of the latter does occur indicates that, at least some of the synapses and the sites at which the impulse is initiated are distant from the cell body (cf. reference 3). Electrotonic losses would then diminish the potential recorded in the cell and slow its rise and fall.

Identification of p.s.p.'s by direct tests. Since p.s.p.'s are electrically inexcitable they exhibit a constellation of characteristic properties which differentiate them from electrically excitable responses (11). However, the p.s.p.'s of the SMC's generated at sites distant from the cell body are not readily accessible to tests for a number of these properties. Nevertheless, the duration, gradation, and summation of the p.s.p.'s (Figs. 2 to 4) indicate that these potentials are generated in electrically inexcitable membrane.

SYNCHRONIZATION  Two structural factors appear to play the major role in synchronizing the activity of the SMC. The synaptic connections of afferents are widespread (Fig. 9) and tend to involve many, if not all, the cells. The latency differences seen in Fig. 8 A, B, and the tendency for these differences to disappear with stronger afferent stimulation and in later responses of a train of spikes indicate that the delay is probably not simply a matter of conduction time in the afferent fibers, but is dependent on synaptic behavior. The evidence for the participation of interneurons in activity of the SMC is presumptive, as discussed above.

However, it is not likely that the p.s.p.'s evoked by the afferent volleys are alone responsible for synchronization among the SMC's. The p.s.p.'s are long lasting, relatively flat-topped depolarizations. While they probably contribute excitatory effects they would not lead to the same number of spikes at only slightly different latencies in all the cells. Synchronizing drive must come from discrete excitatory impulses. As noted above in connection with Fig. 10,
synchronization must reside within the confines of the cluster of SMC's. Therefore it appears likely that the cells themselves are endowed with a synchronizing mechanism in the form of interconnections. These connections are further indicated by the occurrence of spread of discharges from a directly excited cell.

Thus, two cooperative processes are probably involved in the synchronized, indirectly evoked discharge of the SMC. Each SMC is depolarized to some degree by afferent bombardment, one or more sufficiently so as to produce a spike. Those cells that are discharged tend to fire adjacent cells that are already excited subliminally by the synaptic depolarizations. The discharge spreads to involve the whole cluster, in a regenerative and possibly reverberatory manner, since the reciprocal interconnections could result in a second excitatory depolarization of the earlier firing cells (cf. Figs. 11, 13, 15, 16).

Reciprocal excitation has been found in the functional relations of septate giant axons and other neurons in earthworm and crayfish (17, 18). It may occur as well between motoneurons and excitatory interneurons via recurrent collaterals (8, 21).

**Nature of the Synchronizing Interconnections** Considerable data indicate that the connections between the SMC's are synaptic and that extrinsic currents (ephaptic excitation (1, 13)) do not mediate synchronization. (a) The form of the spread potential (Fig. 12) is more characteristic of synaptic than of ephaptic potentials. (b) The maximum interval at which a single cell may be repetitively excited to facilitate spread is about 100 msec., a period too long for the persistence of extrinsic currents generated by the spike of the cell. (c) The spread of excitation from a directly excited cell is a relatively rare event and usually a variable one. (d) Its dependence on frequency of stimulation and (e) its ready fatiguability also indicate that the relatively invariant effects of extrinsic currents cannot be involved.

The electrophysiological data do not, however, offer clear evidence regarding the complexity of the interconnecting links. The increase in the probability of spread (Fig. 14) seen early during repetitive stimulation may be due to homosynaptic facilitation and summation of potentials and need not indicate synaptic relays. The uniform decline of the index on continued stimulation of cells with initially high or low indices suggests some inhibitory influence, which might be due to participation of interneurons as discussed above. On the other hand, exhaustion of a transmitter store in the terminals of the excitatory collateral interconnections or the desensitization of the postsynaptic membrane (19) could accomplish the same result.\(^1\)

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\(^1\) *Note Added in Proof* Recent experiments (Bennett, unpublished) disclose that both hyperpolarization and depolarization of one SMC may cause polarization in adjacent cells. This potential is of the same sign, but much reduced and slowed, as would be expected from electrotonic conduction. These observations suggest the existence of electrical connections between cells which might be responsible for the synchronization of SMC. However, synaptic connections cannot as yet be excluded.
The duration of interaction of direct and indirect stimuli may be explained by electrotonic summation of the short and long depolarizations respectively that they produce in the cell. However, the duration of facilitation of direct stimuli is much longer than the spread potential and therefore this potential must itself be facilitated either directly or through the action of interneurons.

SITE OF INTERCONNECTIONS The evidence of Figs. 11 and 12 indicates that the synchronizing connections among the SMC involve parts of the nerve which lie in the depth of the dorsal half of the spinal cord, and are distinct from the cell bodies. The interconnections have not been described by anatomists.

However, an indication of the site is the size of the spread potential, which approximates that of the p.s.p. Both are too small to excite the cell directly, yet, both are capable of producing a spike in the neurite. The region in which the spread potential is generated therefore may also be the site of the synaptic connections with afferent fibers, and it is tempting to consider the interconnections as collaterals which cross-link adjacent SMC’s. The closeness of the sites of production of the two kinds of potentials is further indicated by the facilitatory interaction of direct and indirect stimuli.

Since the spread potential is seen only in cells immediately adjacent to an active one, and since excitation spreads first to adjacent cells (Fig. 16), the interconnections between cells are probably relatively short, and this would account for their having been missed in anatomical studies. The spread potentials are briefer than the p.s.p.’s, but this need not denote that they are produced by different kinds of excitable membranes. Since the p.s.p.’s probably involve complex chains of neurons they may represent the response to a long lasting bombardment. The spread potential, in contrast, is the effect of a single discharge of one SMC.

THE STRUCTURE OF A SMC AND ITS CONNECTIONS AS SYNTHESIZED FROM ELECTROPHYSIOLOGICAL DATA The information now available on the electrophysiological properties of the SMC, which has been presented in this series of papers, is sufficient to construct a simplified schematic representation of the SMC and their connections, afferent and efferent (Fig. 18).

Dorsal root fibers of the first and second groups both contribute an excitatory influx, but the probable existence of synaptic relays in these excitatory paths has already been noted. Since all the fibers in the third group are axons of SMC’s, their excitatory action on the cluster, as disclosed by facilitation of the action of the other groups, is explicable as antidromically produced depolarization of the neurite. It is unlikely that these efferent fibers also make connections on their passage along the neuraxis (2). On the efferent side, numerous large unmyelinated axons branch out of the neurite, and a single SMC supplies axons to many dorsal roots on both sides of the body and the
cranial nerves. They certainly do not mediate motor activity, and they may be part of the autonomic system. They have been recently traced to the skin (Bennett, unpublished data). The initial part of the neurite and the proximal portion of its branches are shown in the diagram as the sites of synaptic junctions with afferent fibers and of collateral connections with other SMC's. As noted above, the latter must be essentially reciprocal connections. For diagrammatic purposes the synchronizing connections have been shown as separate collaterals. However, they may well occur in other ways.

The cell body has electrically excitable membrane (3) in contradistinction to that of some neurons (15, 23; cf. also references 9 and 10), but like others (5, 6, 22) there seem to be differences in the excitability of different regions (3). The threshold of the neurite and of the cell body is higher than that of the axons (2, 3). This factor, and the branching of the neurite result in the failure of antidromic invasion (2). The sites of failure are separate for the different axonal branches and the transition to higher threshold membrane (lined area, Fig. 18) may well occur at the neurite forks. It might therefore be expected that the refractory period of the axons would also be shorter. The distal site of impulse initiation and the low safety factor for excitation of the soma engender the possibility that synaptic excitation and reexcitation could produce repetitive volleys in the neurite without involving the cell body. The persistence of an initial component potential when the soma spike fails during repetitive discharges denotes that this event does occur. The late
initial component in directly excited cells which had caused spread also indicates this.

Granted that the synaptic connections are distal with respect to the cell body and the initial portion of the neurite, the initial component must be a synaptically evoked spike invading toward the cell body, but blocked at the neurite-soma junction (3). However, the synaptic sites may be spread out along the bases of the axonal branches, the afferent volley initiating activity approximately simultaneously in all the axons. In that case part or all of the initial component may represent summated, synaptically generated ADP's. Histological evidence, not now available, should help to resolve this matter. Hagiwara and Saito (16) designate the initial component in the SMC of the Japanese puffer as a p.s.p. However, they do not give their reasons for that conclusion and apparently have made that identification merely by analogy with responses of other kinds of cells. As has been shown in the present series of papers this analogy is not justified.

Each of the different potentials recorded in the SMC may be ascribed to some specific structural and physiological characteristic of the SMC. The low excitability in the neurite proximal to the axons and the low safety factor at the forks are responsible for block of the antidromic spikes, and this gives rise to small ADP's in the cell. Synaptic excitation also developing in this distal region, the p.s.p.'s recorded in the cell body are small, themselves incapable of directly exciting the soma. However, the p.s.p.'s evoke spikes in the nearby membrane, perhaps that of the lower threshold axons, and this activity propagates to the soma where it elicits the soma spike. The spread potential may be another variety of p.s.p. generated by the reciprocal interconnections of the SMC's. The synaptic and reciprocal excitations probably develop near to each other. Together, they provide the basis for a remarkable characteristic of the SMC's, their synchronized, repetitive activity. Peculiarly, and for unexplained reasons, the SMC's can produce prolonged spikes.

CONCLUSION

In closing this series of papers it is pertinent to stress some of the features of the data and of their use in analyzing the physiological and anatomical properties of the SMC's. Many varieties of these results were obtained on the same cell, and sometimes simultaneously on a number of cells. In general, they confirm electrophysiological findings already available from studies of other types of cells. However, they have also disclosed some new phenomena which are probably of general physiological importance.

Several kinds of data have shown that the different parts of the neuron membrane are differently excitable. Together with specific anatomical
features this physiological factor operates to prevent antidromic invasion of
the cell body. This blockade occurs in the SMC's despite the demonstrated
electrical excitability of the cell body. Thus, failure of antidromic invasion
alone cannot always be considered demonstrative of electrical inexcitability.

The same and related features give rise to direct and indirect spikes of
different configurations. The indirect spike bears a remarkable resemblance
to the spikes of motoneurons. Nevertheless, the properties which underlie
these two-component spikes are fundamentally different. This emphasizes
again the difficulty in interpreting underlying processes from consideration
of form of potentials alone (12).

The synchronizing mechanism of the SMC's, though probably not unique
in central nervous systems, has been studied in this case more completely than
elsewhere. With the electrophysiological data as a guide, anatomical studies
can now be directed toward testing the deductions regarding various synaptic
connections postulated in the present paper. The new data on structure
should in turn spur further electrophysiological work. The large cells are
particularly convenient for study of the kinetics of excitability and electo-
genesis, as has been indicated in some of the present data. The discovery of
the prolonged spikes of the SMC suggests that such studies may be well
worth while in the possibility that they will throw new light on bioelectric
phenomena.

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