*AN EFFECT OF POSTSYNAPTIC NEURONS UPON PRESYNAPTIC TERMINALS**

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Abstract.—Centrifugal ("antidromic") discharges in cat sensory fibers are observed consistently in a variety of experimental preparations and with many different surgical and recording techniques. As is well known, they can be either "spontaneous" or induced by afferent volleys in other sensory fibers. In addition, it is shown here that they can be elicited by antidromic motoneuron activation when the latter is conditioned by natural sensory stimuli or by shocks to the dorsal roots. The latency of the centrifugal dorsal root response to ventral root stimulation is shorter than that of the monosynaptic reflex mediated by the same fibers. An "antidromic" coupling, probably of an electrical nature, between motoneurons and presynaptic terminals is postulated.

The presence of nerve impulses leaving the spinal cord via dorsal roots (centrifugal sensory discharges) was discovered almost 80 years ago[10] and has been repeatedly confirmed by later investigators.[2, 5, 6, 9, 12, 18, 19] Nevertheless, in spite of all the evidence encountered, it may be said that at present these discharges are still considered by neurobiologists to be a most uncommon type of sensory fiber activity. Although the reasons for this attitude are varied,[1, 11] one of the most important probably is the disturbing nature of the phenomenon vis-à-vis classical neuronal theory. However, even if one does not accept the existence of this type of dorsal root (DR) activity as a normal physiological phenomenon, the question about the mechanism producing it remains perfectly valid.

Dorsal root volleys generate a long-lasting depolarization, the dorsal root potential, in the activated as well as in neighboring DR fibers.[3, 15] During the beginning of the depolarization, a mass discharge of "antidromic" sensory impulses, the DR reflex, can often be observed.[19] Because of its relation to the DR potential, this type of efferent DR activity has been proposed to be a direct result of the presynaptic depolarization.[4, 20] A marked increase in electrical excitability, maximal at the terminals and with a time course similar to the DR potential, has also been found in these afferent fibers.[21]

Although the concept of depolarization of presynaptic terminals has been linked with presynaptic inhibition, the mechanism by which the DR potential is generated still remains unclear. Eccles[7, 8] has proposed a multisynaptic neuronal chain ending in axo-axonic synapses (chemical) on the primary afferent fibers. On the other hand, Barron and Matthews[3] proposed that the depolarization that involved both stimulated and adjacent passive fibers was due to the extracellular accumulation of some ionic substance liberated by the excited terminals; some type of ionic interaction would then be present inside the cord.

This communication is concerned with some aspects of the centrifugal dis-

charges observed in cat sensory fibers and with the description of a new mechanism affecting the excitability of presynaptic terminals inside the central nervous system.

Materials and Methods.—All experiments were performed on adult cats. The preliminary surgical procedures were performed under ether anesthesia in all cases except two, in which Nembutal was used. The first series of experiments was designed to study the "antidromic" discharges of sensory fibers in a number of experimental preparations (see *Results*). In experiments in which the centrifugal activity was recorded in the central stump of DR filaments, a lumbar laminectomy was performed. The dura was opened, one dorsal root (usually L7) was severed distal to the cord, and single fiber filaments were prepared for recording. The skin flaps of the lumbar incision were lifted and the pool thus formed was filled with warm mineral oil. To eliminate the possibility of artifacts due to surgical trauma to the cord, the laminectomy was not performed in a number of experiments, in which all the recordings were carried out in peripheral (cutaneous) nerves, either in the main trunk or in one of its branches.

The second group of experiments was designed to study the effects of motoneuron activation upon presynaptic fibers. The preparations used in all experiments were of the high spinal type (anemic destruction of the brain plus spinal section at the level of C1). After a standard lumbar laminectomy had been performed, the ventral root (VR) and dorsal root of one or two segments (L7 and S1 usually) were cut distal to the cord. A thin DR filament, generally about $^1/_4$ to $^1/_5$ of the total root, was isolated and mounted on bipolar silver electrodes for recording. In experiments in which the DR potential was recorded, one of the electrodes was placed on the filament very close to its junction with the cord (Figs. 1 and 3). In the cases where the main interest was recording of action potentials (Figs. 4 and 5), the recording electrodes were placed farther from the DR-cord junction. The stimulus producing the DR potential, and thus changing the excitability of primary afferent fibers, was delivered to the main portion of the dorsal root; it will be referred to as the conditioning stimulus. As is the standard practice in this type of experiment,[7, 8] a volley of three or four stimuli at a frequency of 300 to 400 Hz was often used instead of a single stimulus in order to obtain a stronger DR potential. Stimulation of the central stump of the ventral root of the same segment, or an adjacent one, provided the antidromic excitation of the motoneurons, which served as the test stimulus. A diagram of the stimulating and recording arrangement is shown in Figure 1.

The electrical activity picked up by the recording electrodes was conventionally amplified and stored on magnetic tape. After the experiment, the tape was replayed and the activity displayed on the screen of a Tektronix 565 oscilloscope and photographed with a Grass kymographic camera. In experiments designed to measure latencies, the oscillographic tracings were photographed during the actual experiment. Throughout the experiments, the temperature in the body and cord was maintained between 37.5°C and 39°C by radiant and conductive heat. All animals were immobilized with Gallamine triethiodide and kept under artificial respiration at the time of the experimental measurements. Recording was carried out at least two hours after the animal had been with-

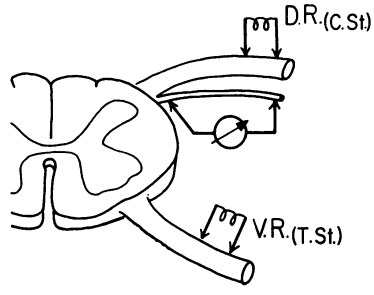


FIG. 1.—Diagram of the recording and stimulating electrodes on the experiments designed to study the effects of motoneuron activation on presynaptic fibers. *C.St.* and *T.St.* designate the conditioning and test stimuli, respectively.

drawn from ether. Further details of the experimental techniques are presented with the results.

Results.—"Antidromic" firing of primary afferent fibers: A number of different experimental preparations were used to test for the presence of centrifugal sensory discharges. The results shown in the upper part of Figure 2 are from a cat under Nembutal anesthesia in which all supraspinal centers were left intact. The recordings were obtained from the central stump of a thin DR filament; the filament belonged to DR L7, which was the only dorsal root severed in this experiment. Figure 2*A* shows an example of the "spontaneous" centrifugal firing at a time when no experimental stimuli were being applied to the animal. Figure 2*B* was taken while the ipsilateral paw was pressed; the considerable in-

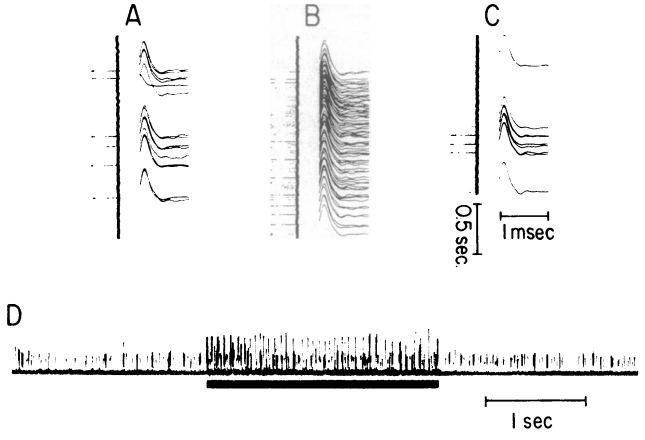


FIG. 2.—*A*, *B*, and *C* are recordings from the central stump of a thin DR filament (L7). On each picture the efferent sensory discharges are recorded in a stationary beam at the left and on a fast time base at the right (where the action potential itself triggers the sweep). *A* and *C* are the controls before and after the ipsilateral paw was pressed (*B*). The recording of *D* is from the central stump of the Suralis nerve in another experiment. The thick black bar below the record marks the time during which an electrical tetanus (300 Hz) was being delivered through two small pin electrodes implanted subcutaneously on the inner side of the knee.

crease in "antidromic" activity triggered by this natural stimulus is seen very clearly. Figure 2*C* shows the activity of the filament immediately after the termination of the sensory stimulation. Although the stimulus was applied almost at the beginning of the continuous tracing on *B* and then maintained during the whole record, the firing frequency of the DR fiber slowly declined after an initial maximum. This phasic component of the centrifugal DR discharge was a common finding in these experiments (see also Fig. 4). Figure 2*D* also shows the "antidromic" discharge of sensory fibers recorded this time in the central stump of a cutaneous nerve (Suralis). The experiment of Figure 2*D* was carried out in a decerebrate cat (midcollicular section under ether anesthesia); laminectomy had not been performed, and the surgical intervention involved only decerebration, dissection of the Suralis nerve, and preparation of an oil pool in the leg.

Very little severing of nerve fibers was done, and the recording is therefore of almost the whole Suralis nerve. The figure reveals clearly the increase of the centrifugal sensory activity during the stimulation, electrical in this case, of another sensory pathway. This experiment thus shows that surgical trauma to the cord cannot account for these effects, a finding that is not in accord with previously published reports.[1, 11] Besides the excitatory effects illustrated in Figure 2, electrical and natural stimuli could also produce an inhibition of these "antidromic" sensory discharges.

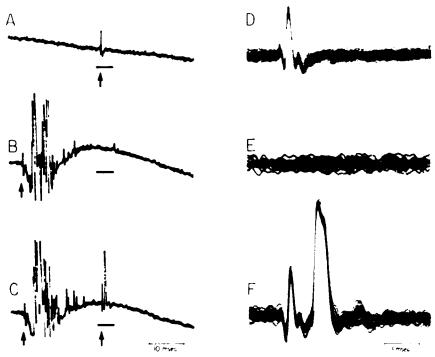
The "spontaneous" centrifugal discharges in sensory fibers were found in acute and chronic spinal, midcollicular decerebrate, and anemic decerebrate animals,[16] and in two animals under Nembutal anesthesia with their supraspinal centers intact. The fact that centrifugal sensory discharges were found in such a variety of experimental conditions indicates that, regardless of their actual functional role, these discharges in sensory fibers cannot be attributed to the surgical intervention or to the condition of the experimental preparation. Analysis of some of the mechanisms producing this type of sensory fiber activity could then be considered one of the first steps for understanding the functional role of these centrifugal sensory discharges.

Motoneuron-presynaptic interaction: The working hypothesis originally used in the second part of the investigation considered that the system controlling presynaptic inhibition, being the powerful control system it is purported to be,[8] should likely receive some information concerning the performance of the postsynaptic element (the motoneuron). The experimental design was simple: investigation of changes in the substratum of presynaptic inhibition, the DR potential, when the DR volley producing it was paired at different time intervals with an antidromic stimulation of motoneurons. However, no obvious modification of the DR potential was found to occur as a result of motoneuron activation. On the other hand, there were certain surprising results with regard to the excitability of the DR fibers.

Antidromic stimulation of the ventral root in the mammal does not produce any active signs of nervous activity recordable in DR fibers.[3, 13] However, Figures 3*A* and *D* show a complex, polyphasic deflection in the dorsal root recorded following antidromic stimulation of the ventral root. This complex electrical potential proved to be an entirely passive phenomenon, as it persisted after the DR filament had been crushed between the DR-cord junction and the recording electrode.

The effects of stimulating the main part of the DR L7 (the conditioning stimulus) are shown in Figure 3*B*. The first clear deflection is a positive variation (DR IV of Lloyd and McIntyre[15]) immediately followed by an asynchronous discharge, the DR reflex which is observed riding on the beginning of a slowly rising negative wave. This negative deflection, the DR potential (DR V of Lloyd and McIntyre), attains maximum value about 20 msec after the shock and then slowly decays. The fast time base of Figure 3*E* shows that there are no "antidromic" discharges between 18 and 23 msec after the delivery of the conditioning stimulus.

Figure 3*C* presents the results from stimulation of the dorsal root (condition-



A and *D:* Antidromic stimulus of VR S1 (test stimulus alone).

B and *E:* Stimulation of the main part of DR L7 (conditioning stimulus alone). Note the lack of any "antidromic" activity in the delayed sweep (*E*).

C and *F:* Same as *B*, but this time the test stimulus has also been delivered at approximately 20 msec after the conditioning stimulus (is in the same position as in *A* and *D*). The "antidromic" discharge driven in this circumstance by the VR stimulation is well seen. Note the extremely short and fixed latency of the discharge.

FIG. 3.—All records were obtained from the central stump of the DR filament (L7). The pictures at the right were obtained from photographic superposition (each is about 20 sweeps) at twice the gain and ten times faster time base than the tracings at the left. Owing to the delayed sweep circuit used, they display the activity present in the DR filament at the time marked by the thick black bar under the records at the left. Conditioning and test stimuli (*arrows*) were delivered at a frequency of 4 Hz.

ing stimulus) followed, after a 20-msec interval, by a stimulus to the ventral root (test stimulus). A large discharge driven by the test stimulus can be seen. The superimposed sweeps (Fig. 3*F*) show the constancy of both amplitude and latency (0.8 msec) of the response in successive stimulations. This effect of motoneuron activation upon DR fibers was observed when stimulation and recording were in the same spinal segment (Figs. 4 and 5) as well as when they were in adjacent segments (Fig. 3). The response could also be seen in cats in which all the ventral roots had been distally cut with recording performed in the peripheral, and functionally de-efferented, muscle nerves. The conduction velocity observed in these cases indicates that the DR fibers mediating this phenomenon belong to the fast myelinated fiber group (group 1).

Because natural stimuli are also known to induce presynaptic depolarization,[3] the question arises whether such natural stimuli may also be employed for conditioning in the present experimental situation. In the experiment illustrated in Figure 4, some "spontaneous" centrifugal activity is initially present in the DR filament, but the test stimulus by itself cannot drive any efferent sensory discharge. However, when the skin of the contralateral flank is lightly touched, a large increase in activity appears in the filament (as in Figs. 2*B* and *D*). During the application of this natural stimulus, the action of motoneurons (the test stimulus) can induce an "antidromic" discharge in DR fibers. The presence of the response at times when there was very little background activity (e.g., near the termination of the natural stimulation), as well as its constant latency, indicate that this discharge is triggered by the activation of the motoneurons and is not a chance occurrence.

The latency of the "antidromic" DR response when measured from the VR artifact varied between 0.7 and 1.0 msec. Among other things, this variation in latency reflects the variation in the lengths of both ventral and dorsal roots in different experimental animals. It is therefore important to compare the latency of these "antidromic" discharges with the duration of the shortest known tran-

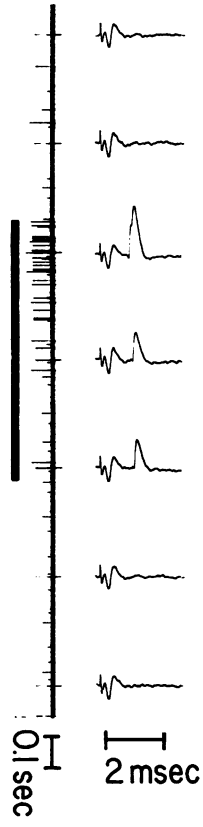


FIG. 4.—Recording from the central stump of a DR filament (L7). The test stimulus was applied to the central stump of VR L7. The tracing at the left shows the continuous activity of the DR filament as recorded in a stationary beam of the oscilloscope. The thick vertical bar marks the time when the skin of the contralateral flank was being touched. Note that the increased "antidromic" discharge thus triggered lasts only during part of the stimulus application. The sweeps at the right show the activity directly evoked by the test stimulus (the positive-negative deflection at the beginning of each sweep is the passive effect induced by ventral root stimulation). An "antidromic" action potential was driven by the test stimulus only during the period of natural stimulation.

synaptic response in the cord, the monosynaptic reflex. In this type of experiment (Fig. 5), the latencies were measured in the same VR-DR filament pair simply by switching the stimulating and recording electrodes; in Figure 5, the latencies were 1.2 msec for the monosynaptic reflex and 0.9 msec for the DR response. Even in cases where the monosynaptic reflex was previously conditioned by another DR volley, thereby reducing its latency,[17] the DR centrifugal response was always earlier by at least 0.2 msec. Inasmuch as both the motor and sensory fibers involved in the monosynaptic reflex are among the fibers with the highest conduction velocity in mammals, it is difficult to explain the different latencies by conduction velocity differences. However, it is now generally accepted that the monosynaptic reflex is mediated through a chemical transmitting synapse, and the minimum delay for such a synapse in the cat has been

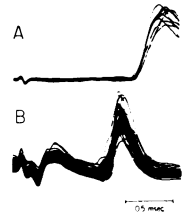


FIG. 5.—This picture was made by alternately stimulating and recording from a thin DR filament. When the DR filament is being stimulated (*A*), the recording is from the whole L7 VR. This picture (*A*) shows the monosynaptic reflex obtained by photographic superposition of 10 sweeps at a rate of 1 Hz. In *B* the situation is reversed, and the recording is now in the DR filament (picture obtained by photographic superposition of 40 sweeps). A conditioning shock was given to the main part of DR L7 15 msec before the beginning of the sweep, and then a shock to VR L7 was delivered, which drives an "antidromic" discharge in the DR filament.

reported to be between 0.2 and 0.3 msec.[7] This transmission delay agrees well with the observed latency differences between the monosynaptic reflex and the DR "antidromic" discharge. The extra amount of time needed to obtain a monosynaptic reflex suggests that a nonchemical, faster mechanism is involved in the case of the motoneuron-presynaptic interaction described in this communication

A preliminary interpretation of these findings can be made if one postulates some type of "antidromic" coupling between motoneurons and presynaptic terminals of primary afferent fibers. The speed with which this "antidromic" interaction occurs strongly suggests that an electrical mechanism is involved, since all known types of chemical synapses are too slow to account for the observed delay of this phenomenon. The postulated electrical coupling would be present all the time that motoneurons discharge, but it is only during periods of heightened presynaptic excitability that the effect may become sufficiently strong to trigger an "antidromic" action potential in those same afferent fibers.

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[1] Barron, D. H., *J. Neurophysiol.*, **3**, 403 (1940).
[2] Barron, D. H., and B. H. C. Matthews, *J. Physiol.*, **85**, 73 (1935).
[3] *Ibid.*, **92**, 276 (1938).
[4] *Ibid.*, **94**, 26P (1938–39).
[5] Brooks, C., McC., K. Koizumi, and J. L. Malcolm, *J. Neurophysiol.*, **18**, 206 (1955).
[6] *Ibid.*, **19**, 61 (1956).
[7] Eccles, J. C., *The Physiology of Synapses* (Berlin: Springer, 1964).
[8] Eccles, J. C., in *Progress in Brain Research*, ed. J. C. Eccles and J. P. Schadé (Amsterdam: Elsevier, 1964), vol. 12, p. 65.
[9] Frank, K., *Abstract, Intern. Physiol. Congr., 19th*, **7**, 362 (1953).
[10] Gotch, F., and V. Horsley, *Phil. Trans., Roy. Soc. London, Ser. B*, **182**, 267 (1891).
[11] Habgood, J. S., *J. Physiol.*, **121**, 264 (1953).
[12] Hagbarth, K. E., and D. I. B. Kerr, *J. Neurophysiol.*, **17**, 295 (1954).
[13] Hughes, J., and H. S. Gasser, *Am. J. Physiol.*, **108**, 295 (1934).
[14] Hursh, J. B., *J. Neurophysiol.*, **3**, 166 (1940).
[15] Lloyd, D. P. C., and A. K. McIntyre, *J. Gen. Physiol.*, **32**, 409 (1949).
[16] Pollock, L. J., and L. E. Davis, *Arch. Neurol. Psychiat.*, **10**, 391 (1923).
[17] Renshaw, B., *J. Neurophysiol.*, **3**, 373 (1940).
[18] Skoglund, C. R., and B. Uvnäs, *Acta Physiol. Scand.*, **6**, 149 (1943).
[19] Toennies, J. F., *J. Neurophysiol.*, **1**, 378 (1938).
[20] Tregear, R. T., *J. Physiol.*, **142**, 343 (1958).
[21] Wall, P. D., *J. Physiol.*, **142**, 1 (1958).