FURTHER STUDIES ON THE FUNCTIONAL PROPERTIES OF SPINAL AXONS IN VIVO*

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Beyond their recovery cycle of excitability (17) other functional characteristics of spinal axons can be conveniently examined in vivo. The studies presented here are intended to furnish additional standards against which to compare the behavior of excised spinal axons (16). The first section considers briefly auxiliary evidence for the existence of a conspicuous L fraction (14) of membrane properties in spinal tracts. The second section provides the results of an examination of their rhythmic state.

In their most general sense the findings demonstrate that central axons behave essentially like peripheral nerve. However, certain noteworthy differences suggest that central axons occupy an extreme position in the spectrum of properties encountered in neural tissues.

RESULTS

1. Evidence for an L Fraction in Central Axons in Vivo

Classical studies have distinguished two general groups of membrane properties in peripheral axons (4-6, 10-12, 14). One, stable to environmental variation and related predominantly to those parameters defining the spike potential, Lorente de Nó has termed "Q" (14). The other, labile and normally reflected more closely in both the resting level of excitability and afterpotentials, he has labelled "L." It is a characteristic of tissues supporting a large phase of post-spike supernormality, as do central axons (17), that great enhancement of resting excitability intervenes before depression when the value of the associated labile fraction is diminished under the influence of most depolarizing agents.

To test these relations in spinal tracts three representative variables (oxygen, potassium, and citrate) were selected as being practical for study in vivo from among those agents known to affect the L fraction of peripheral nerve.

Fig. 1 presents an experiment typical of eight showing the excitability...
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changes produced by anoxia of ventral column axons in the intact spinal cord. The cat was a spinal preparation, curarized to minimize movements during hypoxia and ventilated on a closed system with CO₂ absorption. General experimental conditions were similar to those already described (17). The upper curve plots the amplitude of response to supramaximal stimuli recorded 3 cm. from the point of initiation at the cord ventrum. The lower curve plots the magnitude of response to stimuli of constant current strength set initially to elicit a volley one-half of the maximal value. Since the stimulating electrodes were oriented longitudinally along the cord, excitability of low threshold parent fibers was being tested (18, 17).

At 27 minutes the oxygen in the breathing system was replaced by nitrogen. Within 8 minutes the height of the submaximal response began to increase even though maximal volley size fell. It was not investigated whether the decrease in maximal volley height initially represented a fall in the amplitude of the individual axon spikes, resulted from a decrease in number of fibers transmitting, or was only an apparent decrement due to increased temporal dispersion. It is important to note only that the concurrent increase in submaximal volley height signifies that more fibers were responding to the sub-

![Figure 1](image-url)

**Fig. 1.** Anoxic hyperexcitability of ventral column *in situ*. Description in text.
maximal stimulus and consequently that excitability was rising. Excitability continued to rise for at least 6 minutes before conduction block became severe. Results identical with these have been obtained in thoracic dorsal columns as well.

When solutions containing an excess of potassium chloride or sodium citrate are applied topically to the cord dorsum at the testing cathode, under conditions similar to those described elsewhere (17), enhancement of resting excitability occurs just as it does with hypoxia. Typical results are presented in Fig. 2 and are described in conjunction with the observations in the next section.

The finding that the action of all three variables results in a phase of hyperexcitability is, within the framework established by Lorente de Nó (14), consistent with the existence of post-spike supernormality in central axons, as well as their supernormality when under the influence of externally applied catelectrotonus; e.g., DCV (17). Therefore, a well defined L fraction may be said to exist in myelinated fibers of central tracts.

2. The Rhythmic State in Central Axons

Variations in the concentration of a wide variety of materials normally present in the environment of peripheral nerve or spinal root incite them to fire autonomously (2, 8-12, 14). In addition, the literature contains observations suggesting that the intensity and facility with which such firing occurs in a given tissue may be related to the degree of development of its positive after-potential. Thus, frog sensory roots and mammalian motor nerves develop better autonomous firing and have a larger positive after-potential than do frog motor roots and mammalian sensory nerves, respectively (12, 8, cf. 3 and 15).1

Since a careful search in dorsal columns failed to yield any sign of a positive after-potential (17) they were investigated to determine whether their rhythmic state was in any way unique. Of the several outstanding candidate manipulations—increase of potassium ion concentration, decrease of calcium ion, or CO₂ removal—the first can best be accomplished in required degree and selectivity in vivo. The results show that central axons are unusually reluctant to enter the rhythmic state although the difference from peripheral nerve in this respect is not absolute.

Potentials were recorded from dorsal roots (L₇ or S₁) cut as far distally as possible shortly before topical application of test solutions to the cord dorsum or dorsal root. Cord and roots were covered with mineral oil equilibrated with 5 per cent CO₂, 95 per cent O₂ as suggested by Lloyd (13). On the ipsilateral cord dorsum, 2 to 3

1 But, note Adrian's observation to the contrary on rhythmicity induced by injury of mammalian motor and sensory nerves (1).
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cm. rostral to the root entry zone, a pair of stimulating electrodes was arranged with the cathode proximal to the root. At a frequency of one per second a small testing volley was initiated at the cord by a current pulse of constant strength and recorded at the root. Under these conditions Krebs's solution (7) containing varying concentrations of KCl was applied either to the rootlet (1 cm. or less from the root-cord junction but several centimeters from the cut end) or to the cord dorsum at the stimulating cathode (well above the region where contamination of the rootlet might occur). Two measurements were then taken. (1) The oscilloscope was monitored continuously for evidence of autonomous firing and (2) changes in excitability occurring at the cord dorsum were determined by noting the variation in the size of the testing volley appearing at the dorsal root. Thus, even in the absence of firing it could be determined whether or not an effect had been produced on tract axons by the applied solutions. The experiments were performed in nine nembutalized cats with identical results.

A typical experiment is presented in Fig. 2. Excitability change is plotted in arbitrary units on the ordinate as the variation in height of the testing volley. Representative oscillograms displaying the level of random activity recorded at the root are given at the times when they were obtained during the experiment. Immediately following the control period, a drop of Krebs's solution of normal composition was placed on the cord dorsum at the testing cathode to provide the necessary additional control for the altered extracellular conductance appearing at the stimulating electrodes with application of the test solutions. The resultant shunting produced transient ineffectiveness of the stimulus current which returned to the initial level in a few minutes. Krebs's solution containing three times (20 mM) the normal concentration of KCl was then applied. It can be observed that a process was introduced which competed with the effect of shunting so as to hasten the recovery of the testing volley size. The process finally resulted in a small amount of absolute hyperexcitability. Hyperexcitability was even more pronounced when 60 mM KCl in Krebs' was applied. The beginning of the phenomenon was also apparent with the addition of a pure solution of 100 mM KCl but was rapidly obscured by a second period of transient depression. This depression cannot be the result of shunting but must be ascribed to the fact that enough KCl had penetrated the pial barrier to produce subnormal excitability.

It will be observed that at no time in the excitability cycle do the oscillograms show any sign of autonomous firing, nor have they in other preparations in which the concentration range between 20 and 100 mM KCl was carefully explored over the length of lumbar and thoracic dorsal columns for the purpose of taking the tissue to maximal hyperexcitability and then to depression. These

1 Usually no attempt was made to preserve isotonicity of the applied solutions, hence their conductivity increased with the concentration of KCl. The only effect of this variable would be to enhance the degree of shunting with successive increments in KCl concentration.
findings have been more fully investigated in vitro (16) where it has been found that central tracts do not enter the rhythmic state following the action of a wide variety of agents which incite firing in peripheral nerve and roots.

However, this difference between central and peripheral axons is not absolute since autonomous firing can be evoked in the former by at least one agent; namely, sodium citrate. Fortunately, this makes it possible to determine the effectiveness with which firing would be recorded at a dorsal root 2 to 3 cm. or more away from its origin in the columns. In Fig. 2 (at 25 minutes) after recovery from the effects of 100 mM KCl, 70 mM sodium citrate was applied to the cord dorsum at the testing cathode. Hyperexcitability of large degree en-
sued and was accompanied by marked firing in the related oscillogram. This serves to validate the experimental method. Since the effect can appear within several seconds it probably results from activity in columns rather than in dorsal horn collaterals of the afferent cell.

If one now examines the axonal tissue distal to the root-cord junction, the response to applied KCl is found to differ strikingly. Dorsal roots, even within a few millimeters of the root-cord junction, behave like peripheral nerve with respect to the development of autonomous firing under the influence of KCl.

Fig. 3. Autonomous activity resulting from K⁺ applied to dorsal root (1 and 2) and lack thereof upon K⁺ application to dorsal columns (3–7) which, however, are activated by addition of citrate (8). Description in text.

These experiments suggest that important changes occur in the membrane properties of the primary afferent neuron as it enters the central nervous system. Central myelinated axons in situ, unlike their peripheral counterparts,
show absolute resistance to the development of autonomous firing when acted upon by varying concentrations of potassium ion. Accordingly, support is given the empirical correlation made in peripheral nerve and now extended to central axons between the degree of autonomous firing and the prominence of the positive after-potential. Until adequate formulation of mechanism is possible there is profit in describing the monotonic post-spike recovery of membrane potential in spinal tracts (17) as an extreme overdamped member of a family of recovery processes in neural tissues which are usually oscillatory. The reluctance of tracts to enter the rhythmic state is apparently another consequence of the highly damped nature of the system. It may be noted that such unusual stability in central axons might tend to prevent them from entering into reverberant linkage with the intramedullary currents they help initiate and to which they are in turn exposed.

![Image of periodic oscillations](image.png)

**Fig. 4.** Periodic oscillations (390 c. p. s.) recorded from a dorsal root but induced by a stimulus to the dorsal columns following application to the latter of sodium citrate. Time = 1000 c. p. s.
It is also practicable to study in situ the fundamental frequency of the rhythmic state in central tracts. When under the influence of citrate, the firing is synchronized by a stimulus to the dorsal columns there are recorded at the dorsal root heavily damped periodic oscillations like those found in peripheral nerve. However, the frequency of the tract oscillations, demonstrated in Fig. 4, is unusually high (340 to 400 c.p.s.) compared to that in mammalian peripheral nerve (200 to 300 c.p.s.) (16) (chapter XV, Fig. 64 of reference 14). Because similar frequencies are also found in spinal tracts in vitro (16) it is reasonable to allocate their origin to tract fibers as such rather than to activity arising in their presynaptic collaterals. However, it is not yet certain where the transition to the higher frequency oscillation occurs along the primary afferent cell in its approach to the central nervous system.

SUMMARY

Mammalian spinal tracts in situ demonstrate a phase of marked hyperexcitability during hypoxia or on the application of an excess of potassium or citrate ion. This is in keeping with the fact that they also show post-spike supernormality as well as hyperexcitability under cathodal polarization (17). Behavior of this kind indicates that central axons carry a well developed L fraction of membrane properties.

The rhythmic state in central axons in situ, unlike peripheral nerve or spinal root, is not induced by the action of excess potassium ion. This appears to be related to the absence of a positive after-potential in dorsal columns (17). However, sodium citrate can elicit autonomous firing in central axons. When synchronized by an applied stimulus the resulting periodic oscillations have a fundamental frequency (340 to 400 c.p.s.) which is significantly greater than that of peripheral nerve.

BIBLIOGRAPHY


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