glandins E_1 and E_2, thought to inhibit adenyl cyclase activity (*14*), reduces the slowing of spontaneous discharge obtained by application of NE from another barrel of the same micropipette.

Our studies of ultrastructure show nerve terminals in the cerebellar cortex containing large granular vesicles, characteristic of the nerve endings in the brain, which contain monoamine (*15*). Moreover, autoradiography of cerebellar cortex after subdural injection of H^3-NE reveals uptake and binding of this monoamine in nerve terminals adjacent to dendrites of Purkinje cells.

These data on ultrastructure, together with our findings on NE sensitivity of Purkinje cells and previous reports of NE-containing axons and high turnover rates of NE in the cerebellar cortex, suggest that this monoamine should be given serious consideration as a possible neurotransmitter in the cerebellum.

We propose that the action of NE on Purkinje cells may be specifically mediated by the formation of cyclic-AMP, although other possibilities cannot be eliminated. The lengthy time course of the effect of NE relative to that of cyclic-AMP, the similarity of response to these two substances, and the effects of theophylline and prostaglandins E_1 and E_2 all support such a stepwise mechanism. Synaptic inhibitions of long duration, reflecting underlying metabolic effects, have been described for vertebrate and molluscan ganglia (*16*). We suggest that, in the mammalian cerebellum, the NE released by the postulated adrenergic terminals affects Purkinje cells by transynaptic activation of a metabolic system.

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C-Fiber Responses in the Ventrolateral Column of the Cat Spinal Cord

Abstract. *Saphenous nerve C-fiber volleys generate, in the ventrolateral column of the cat spinal cord, a conducted postsynaptic response revealed by averaging the integrated temporally dispersed axonal discharges. This finding is compatible with the hypothesis that the feline ventrolateral column includes a major pathway related to nociception.*

Central reflex and sensory effects of unmyelinated C-fiber afferent volleys are well known, but summated central tract potentials evoked by C volleys have never been observed, even with closely spaced repetitive pulse stimulation. Their identification is hindered by temporal dispersion in nerve, polysynaptic relay, and central tracts where recordable amplitude from the fine fibers is small at best. Furthermore, the strength of the applied stimulus unavoidably engenders multifiring in myelinated A fibers and initiates a postsynaptic disturbance preceding C-evoked activity. Methods of differential block of myelinated fibers have been successfully applied to confine the input to C fibers, and spike activity responses have been described in the ventral thalamus, caudal medulla, and dorsolateral and propriospinal tracts of the cat spinal cord (*1, 2*).

In a study of directly recorded ventrolateral column (VLC) discharges evoked by myelinated afferents, we failed to record responses that could be related to C volleys. However, depression of responses evoked by A afferents after conditioning volleys in C fibers alone suggested that this column does relay C input (*3*). Attempts to reduce temporal dispersion by stimulating C fibers in dorsal roots, or averaging the poststimulatory activity, or both, were also unsuccessful. The postsynaptic firing generated by C volleys is revealed by averaging the integrated evoked activity.

In nine cats, decerebrated under ether anesthesia, the saphenous nerve was dissected, cut distally, and placed on an array of stimulating, polarizing, and recording electrodes (Fig. 1D). After laminectomy and opening of the dura the animals were paralyzed with gallamine triethiodide and artificially ventilated; small doses of the drug were supplemented intravenously each 20 to 30 minutes. Contralateral VLC responses were recorded monopolarly through a stainless steel electrode. electrolytically sharpened (*4*) and insulated to within 30 to 70 μm of the tip (resistance 60 to 100 kilohm); a similar reference electrode was inserted into nearby muscle (Fig. 1, D and E). Trains of ten stimuli, 100 per second, at appropriate strength for A-delta and C fibers were delivered to the nerve

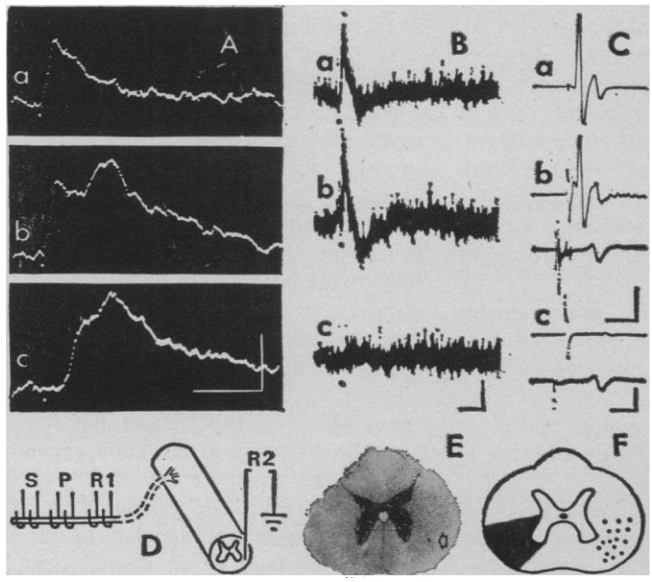

Fig. 1. (A) Integrated ventrolateral column discharge at low thoracic level. Dots indicate moment of stimulus application. (B) Conventional single-sweep recording of VLC response. (C) The monitored afferent nerve volley. (a) Myelinated beta and delta (stimulus strength ten times the threshold for beta axons). (b) Mixed A myelinated and C unmyelinated (stimulus strength 200 times the threshold for beta axons). (c) Unmyelinated C input; stimulus strength as in (b). Nerve volley is displayed in (Cb) and (Cc) at different speed and gain to show beta and delta (upper beam) and C (lower beam) inflections. (D) Disposition of stimulating (*S*), polarizing (*P*), and recording (*R1* and *R2*) electrodes. (E) Recording locus. (F) (Right side) Location of 16 recording points of integrated discharge at low thoracic level; (left side) hatched area of conventionally recorded compound response to myelinated A afferents. Calibration: (Ac) for integrated discharge: time base 500 msec; voltage calibration is obtained by integrating and averaging ten trains of ten square waves of 1 msec duration, 50 μv, 100 per second; (Bc) for conventional recording: 100 μv and 100 msec; (Cc) 1 mv and 2 msec for (Ca) and upper beam of (Cb) and (Cc), 200 μv and 20 msec for lower beam of (Cb) and (Cc).

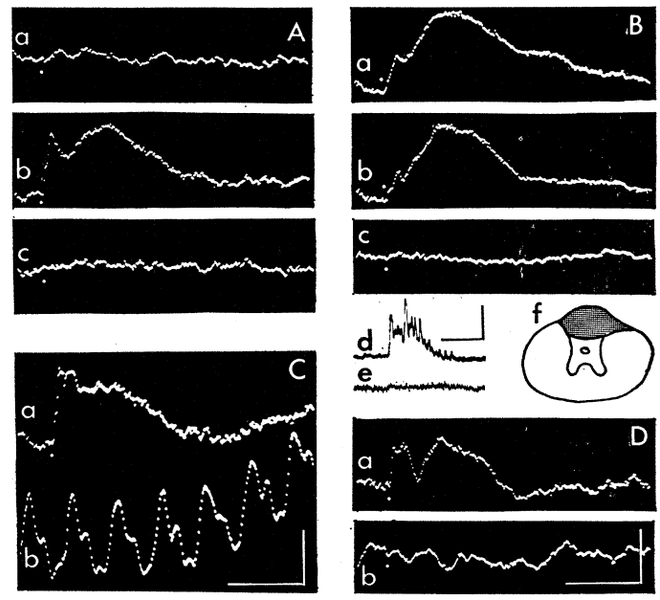

Fig. 2. (A) (a) Integrated paraspinal muscle recording, leads 20 mm apart. (b) Integrated ventrolateral column discharge at low thoracic level after mixed A and C volley. (c) Same as (b) after cutting of the dorsal roots. (B) (a) Integrated VLC discharge at midthoracic level after mixed A and C volley. (b) Same as (a) after section of the dorsal columns. (c) Same as (a) after section of the whole cord. (d) Dorsal column response after single peripheral stimulus at strength for A myelinated fibers. (e) Same as (d) after section of the dorsal columns. (f) Diagram of histological dorsal column lesion. (C) (a) Integrated VLC discharge at low thoracic level after mixed A and C volley. (b) Simultaneous averaged recording of the changes in blood pressure. The lack of synchronization of pulse waves results in an apparently rapid pulse rate. (D) (a) Integrated VLC discharge at the cervical level after mixed A and C volley. (b) Same as (a) after section of the thoracic cord. Calibration: (Bd) for (Bd) and (Be), 10 msec and 500 μv; (Cb) for blood pressure recording: 500 msec and 50 mm-Hg; (Db) for integrated responses: same as in Fig. 1.

in the nonpolarized state and during anodal polarization, which ensures differential block of myelinated fibers (*5*); afferent volleys were monitored diphasically (Fig. 1, C and D). Ventrolateral column activity was led after amplification to an electromyograph integrator with a low-frequency filter providing 50 percent attenuation at 3 cycle/sec in the preamplifier portion, and a 0.2-second time constant in the integrator portion, and then to a LINC computer for averaging responses evoked by ten successive stimulus trains at 10- to 20-second intervals. Nonintegrated responses to each stimulus train (*3*) could be monitored in parallel on a conventional oscilloscope. In some animals the dorsal column potential after single peripheral or antidromic dorsal column stimulation was recorded monopolarly. Electrolyzed recording loci were located in the fixed tissue by the Prussian blue reaction. Blood pressure was monitored by a polygraph through a carotid cannula and transducer, and reactions to stimulation were also averaged on the LINC computer.

The activity generated in the VLC at a low thoracic level by successive stimulus trains at A-delta strength appeared after integration and averaging as a monophasic deflection (A wave), reaching its peak in about 50 to 70 msec and lasting from 200 to 300 msec (Fig. 1, Aa and Ca). Precise measurement of initial latency was not possible on this prolonged (2.02 seconds) time base. Direct simultaneous recording on a single sweep of the oscilloscope showed the compound positive-negative postsynaptic response to myelinated afferents (*3*, *6*) superimposed by a rich spike activity. This response started 5 msec after the first pulse of each stimulus train, reached its positive peak 50 to 60 msec later, and lasted about 200 msec (Fig. 1Ba). We relate the A wave to integrated postsynaptic axonal discharges triggered by A fibers; its prolonged duration with respect to the directly recorded response is most probably due to the time constant of the integrating device and to the contribution by poorly synchronized late discharges.

Raising the nerve stimulus from A to C strength (Fig. 1Cb) somewhat augmented the amplitude of directly recorded response (Fig. 1Bb) because of multifiring in myelinated fibers, without adding any late component except further increased thickness of base line activity. Yet the integrated record showed a second wave of activity (C

wave) riding on the descending limb of the A wave, reaching its peak 450 to 600 msec after the stimulus and lasting up to 1 or 2 seconds (Fig. 1Ab).

During polarization block of myelinated fibers (Fig. 1Cc) the A wave disappeared altogether from the integrated record, and the C wave arose from a flat base line a little more than 150 msec after the stimulus (Fig. 1Ac). Direct single-sweep recording still showed increased base line activity and an irregular initial wavelet presumably related to unblocked A-delta axons (Fig. 1Bc). Anodal block cannot eliminate a residual activity in some 5 percent of fast axons without decrement of slower components (*5*). The latency of the C wave corresponded rather well to values expected from C-fiber input latency at the dorsal root entry zone (150 msec in the experiment of Fig. 1 over a conduction distance of 21 cm), plus synaptic delay and conduction time in the postsynaptic path. Since A and C afferents converge at least in part on the same ascending neurons (*3*), this value should not be more than 5 msec. An unknown delay in the intraspinal presynaptic arbor can be neglected in view of the short intraspinal course of peripheral unmyelinated fibers (*7*).

Neither nerve polarization without nerve stimulation nor trains of stimuli of A-delta strength during polarization block of A fibers produced significant deflection of the integrated activity. These observations exclude the possibility that the late component of integrated record derives from unblocked beta or delta axons. We therefore infer that the C wave represents integrated spike discharges in ascending neurons evoked by C afferents, since C fiber activation was the condition sufficient and necessary for its appearance.

The discrepancy between conventional and integrated record with respect to A and C responses deserves comment. Conventional single-sweep recording (Fig. 1B) can detect only the compound mass discharge generated by synchronous myelinated input. Dispersed unitary discharges evoked by C volleys become lost in background activity (*2, 3*); because of the temporal dispersion of brief unit potentials evoked by C fibers, simple averaging techniques are not adequate. Voltage integration is suitable to collect the scattered axonal discharges that are more abundant and prolonged after stimulation of C fibers while the elevation of the base line in the A wave is filtered out by the recording system. Averaging of integrated activity further improves the quality of the records.

The integrated response was recorded from a ventrolateral sector of the cord corresponding to the zone of conventionally recorded compound response to A afferents (Fig. 1F) (*3*). Movements of the electrode tip inside that area were somewhat less critical for the integrated than for conventional recording.

Although accurate measurements were hindered by the prolonged time base adopted, the conduction velocity within the cord was of the same order of magnitude for A and C waves, in accordance with the common locus of the ascending projections of A and C input. We know that the directly recorded response to myelinated afferents traverses the VLC at 14 to 30 m/sec (*3*).

The C wave was produced in all the animals tested, although in a few cases when the differential efficacy of the block was suboptimal (*5*), the A wave could not be completely suppressed or the C wave was reduced.

Because of the great possibility of spurious results in averaging techniques, the following controls were made to rule out an artifactual origin of the A and C waves.

1) Stimulus artifacts were excluded since the response outlasted the duration of the stimulus and was abolished both by barbiturates and by section of the dorsal roots.

2) Muscle potentials were avoided by recording during pharmacologically induced immobility with the result that no significant activity was recorded from electrodes in the muscle (Fig. 2Aa), no matter how spaced and positioned. Conversely, bipolar electrodes 1 to 2 mm apart in the VLC gave results similar to monopolar recording, although as could be predicted, the A and C waves were somewhat reduced in amplitude.

3) Field effects of conducted nerve and root potentials need not be considered, since cutting the lumbar dorsal roots at the entry zone abolished the VLC response to A, C, and mixed volleys (Fig. 2, Ab and Ac). Conducted potentials at the distal stump of the root were not affected by the section.

4) Neither presynaptic intraspinal dorsal root potentials nor segmental relayed postsynaptic intraspinal potentials (*8*) could be responsible, since the potential was equally well recorded at low thoracic and high cervical levels. In addition, the cervical response was abolished by spinal cord section at the midthoracic level (Fig. 2D).

5) Field effects of dorsal column potentials were a negligible source of contamination since section of these tracts did not interfere with VLC response recorded a few centimeters craniad (Fig. 2, Ba and Bb). The wave could be abolished by subsequent enlargement of the lesion to the ventral half of the cord (Fig. 2Bc). The completeness of the dorsal column section was confirmed by the disappearance of the local dromic and antidromic responses, and was checked on the fixed tissue (Fig. 2, Bd, Be, and Bf).

6) Blood pressure elevation regularly followed repetitive stimulation of C fibers. However, the course of the C wave was unrelated to that of blood pressure changes which started 1 to 2 seconds after the stimulus train, when the average response was already completely developed (Fig. 2C), and attained their peak 6 to 8 seconds later.

Ventrolateral column tracts subserve transmission to higher levels of the central nervous system of myelinated as well as nonmyelinated input, both of which are potently associated with nociception. This finding is in line with the view that VLC includes, in the feline as well as in primates, a major pain pathway (*3*), although transmission of impulses to be recognized as painful is certainly not its only function (*9*).

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