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Ventral root projections of myelinated dorsal root ganglion cells in the cat

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Since the original formulations by Bell[2] and Magendie[21], there have been successive attempts to demonstrate exceptions to division of spinal roots into ventral, outgoing motor and dorsal, incoming sensory pathways[30]. Data suggesting the presence of primary afferent fibers in mammalian ventral roots have been persistent in the anatomic[7,8,33,35], electrophysiologic[6,9,15,16,26,27], and clinical[12,32,36] literature. While converse suggestions of spinofugal activity in the dorsal roots[1,13] have been either discredited[31] or relegated to rare anomalies[5,28], recent evidence for unmyelinated afferents in the ventral root has been more compelling[6–8], prompting the present systematic search for myelinated afferents.

Anatomical studies of ventral root degeneration patterns and physiological studies of teased filament activity are complicated by the well established presence of small numbers of unipolar cell bodies (presumably ganglion cells) scattered throughout the ventral roots[25,29,35,37]. In the present study, extracellular microelectrode records were obtained from presumed cell bodies in the dorsal root ganglion (DRG) and the projections of these DRG cells were determined by orthodromic and antidromic electrical activation from both dorsal and ventral roots as well as from peripheral nerve.

A total of 185 L7 DRG units were recorded in 11 male and female adult cats. L4–S1 laminectomies were performed under deep pentobarbital anesthesia in a stereotaxic frame. The animals were paralyzed with gallamine during unit recording and artificially ventilated via tracheotomy. EKG and rectal temperature were continuously monitored and the temperature of the mineral oil-filled spinal pool was automatically maintained at 36–38 °C. Pairs of platinum hook electrodes with shielded leads were used to stimulate and record evoked potentials from intradural spinal roots elevated in the oil. The L7 DRG was exposed *in situ* with its dural sheath intact. The intact sciatic nerve just distal to the hamstring muscular branch was fitted with a longitudinally slit Silastic cuff containing a pair of platinum foil electrodes 1 cm apart.

Single unit recordings of DRG cell activity were obtained from closely spaced pairs of glass insulated platinum–iridium[38] or Parylene insulated tungsten[20] microelectrodes using differential amplification and filtering to cancel the large intragangli-

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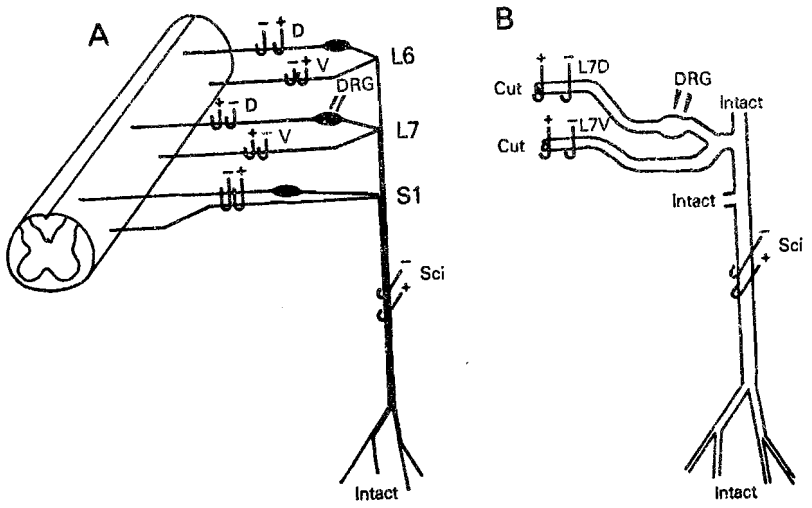

Fig. 1. Probe configurations employed. A: 6 bipolar hook electrodes on intact sciatic (Sci) nerve and indicated spinal roots, used for stimulating (polarities indicated) and recording evoked potentials in the first 6 preparations. B: 3 bipolar hook electrodes placed on intact sciatic nerve and proximally cut L7 dorsal and ventral roots, used as before in the last 5 preparations.

onic evoked potentials produced by synchronous electrical driving. An attempt was made to classify the physiologic modality of each unit as cutaneous or proprioceptive based on response to manual manipulation of the skin, muscles, and joints of the lower leg. Thermal, noxious, and vibratory stimuli were not employed and fibers responding primarily to such modalities are probably among the 'unknown' category. This category also includes units lost during testing and, possibly, spindle afferents made relatively unresponsive by the motor paralysis. Most units were recognized on the basis of spontaneous activity or response to manipulation of the leg during micromanipulator advancement of the microelectrodes. To improve representation of high sensory threshold and slowly conducting units, some penetrations were made while applying supramaximal electrical stimulation to the sciatic nerve.

Two different spinal root probe configurations were employed, as shown in Fig. 1. Six cats had 5 pairs of platinum hook electrodes placed on elevated but uncut segments of the indicated roots (Fig. 1A). A total of 47 units were examined for projections to the dorsal and ventral roots of the adjacent L6 and S1 segments as well as to the L7 level. No DRG cell projections of what has been termed the 'recurrent collateral' type to adjacent roots were found (in agreement with the most recent appraisal of their scarcity[28]). The subsequent preparations were designed to optimize stimulus isolation by stimulating only the distal stumps of cut L7 dorsal and ventral roots (Fig. 1B). In all cases there was at least 14 mm of free root in oil between the DRG and the nearest stimulating hook. The methods and findings regarding conduction velocities are discussed in previous paper[19].

Electrical stimulation (10 pulses/sec) was applied in succession to the sciatic nerve and available spinal root electrode pairs to identify all intact processes of each

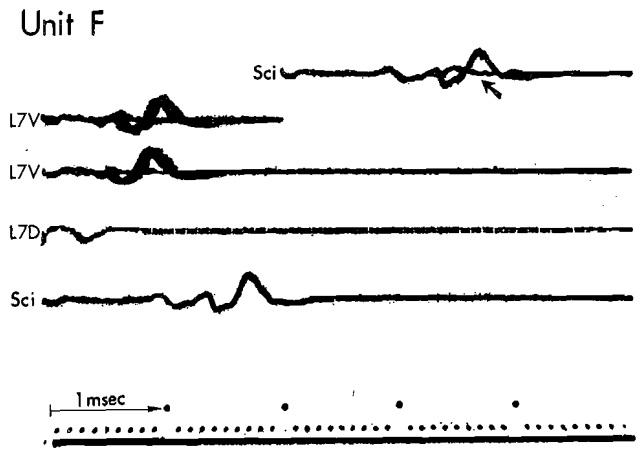

Fig. 2. Multiple sweep microelectrode recording showing response of the DRG unit to sciatic stimuli (trace Sci, 114 mm from cathode, 2.5 V, 1.3 msec latency), no response to L7 dorsal root stimuli (trace L7D), and all-or-none responses to threshold L7 ventral root stimuli (traces L7V, 20 mm from cathode, 15 μA, 0.6 msec latency). The top trace displacement indicates a repeat sciatic nerve stimulus which fails to propagate to the DRG soma (arrow) on those sweeps when the preceding L7V stimulus is effective. Traces synchronized to start of 0.1 msec stimulus pulse.

isolated cell. All apparent projections were confirmed by collision loss of unit response as the interval between the appropriate stimuli was reduced to a critical level. Electrically isolated bipolar stimulators (Devices 2533) were connected to the electrode pairs via a matrix board and shielded, twisted pair leads (12 pF maximum cross talk). Monophasic, direct-coupled stimuli (0–1 mA constant current) were always applied with the cathode as in Fig. 1A and B, with pulse durations of 0.1 or 0.3 msec.

A number of tests were routinely made to rule out the possibility of stimulus current spread. At the beginning and at frequent intervals during each experiment (particularly after each ventral root afferent described below was found), the threshold was determined for electrical stimulation in each hook electrode pair to produce a minimal evoked potential in each of the other pairs. Thresholds varied from 5 to 100 μA depending on root caliber and spacing between the electrodes of each pair, and the hooks and oil bath were revised if thresholds or degree of isolation changed. In preparations with uncut roots (Fig. 1A), stimuli 10 times the level required for evoking a maximal potential in the sciatic nerve gave no discernible volleys in adjacent roots. In cut root preparations (Fig. 1B), stimulation of the ventral or dorsal root at up to 100 times the maximal response level failed to evoke discernible volleys in the other root. The relative thresholds for stimulating the processes of each DRG unit tended to be roughly inversely correlated with the conduction velocity and in no case exceeded 500 μA.

Of the 185 L7 DRG units isolated, 129 had electrically identified and collision confirmed proximal and distal processes. Of these, 124 were classical DRG cells with one process in the sciatic nerve and one in the associated L7 dorsal root; their conduction velocities (10–120 m/sec) are discussed elsewhere[19]. The remaining five (3.9%)

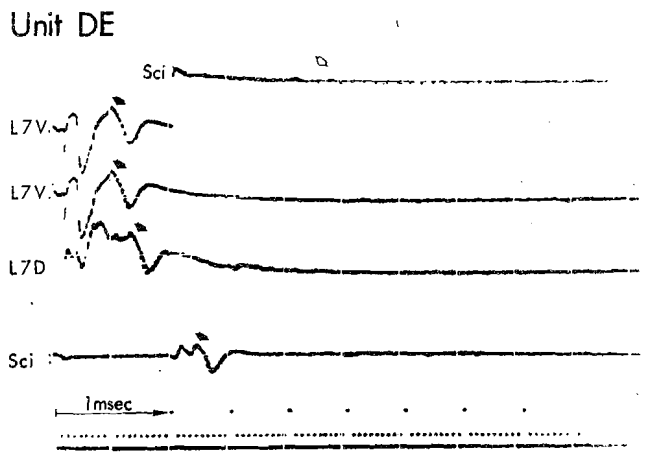

Fig. 3. Multiple sweep record as in Fig. 2, showing response of the DE unit (solid arrows) to sciatic stimuli (lower trace Sci, 91 mm from cathode, 0.15 V, 1.2 msec latency), L7 dorsal root stimulation (trace L7D, 18 mm from cathode, 350 μA, 0.65 msec latency), and L7 ventral root stimulation (traces L7V, 14 mm from cathode, 360 μA, 0.45 msec latency.) The displacement of the top trace (at Sci) indicates the same sciatic nerve stimulus as in the bottom trace, this time colliding with a preceding action potential produced by L7V stimulation and therefore failing to propagate to the DRG soma (open arrow).

had ventral root projections as described below. No more than one atypical unit was found in any given preparation. Fig. 4 summarizes the DRG units by modality and peripheral conduction velocity, indicating fairly good representation of the normal myelinated afferent population[4,18] above 10 m/sec except for some under-representation of both cutaneous and proprioceptive fibers in the 10–30 m/sec range.

Unit F: the 22nd of 38 units from the 11th preparation (adult male) prepared as in Fig. 1B (cut roots). The cell responded phasically to extension of the toes with no cutaneous receptive field. The conduction velocity from sciatic nerve stimulation was 81 m/sec and the unit was proximally driven only by L7 ventral root stimuli with a conduction velocity of 33 m/sec. The action potentials and collision trials are shown in Fig. 2.

Unit A: the 5th of 9 units isolated from the third cat (adult female) prepared as in Fig. 1A (intact roots) was physiologically activated by moving a single guard hair on the lateral aspect of the sole of the foot. The orthodromic conduction velocity from sciatic nerve stimulation was 81 m/sec; the unit was antidromically activated only via the L7 ventral root where the velocity of conduction to the DRG was 54 m/sec. It was possible to shift the stimulating probes while holding the unit, changing the ventral root current threshold somewhat, but it was never possible to drive the unit with supramaximal stimuli to the L7 dorsal root or adjacent segment dorsal or ventral roots.

Unit B: the 8th of 12 units isolated from the fourth cat (adult male prepared as in Fig. 1A) responded phasically to ankle dorsiflexion and tapping over the calf muscles. Distal conduction velocity was 74 m/sec and the unit was excitable proximally by

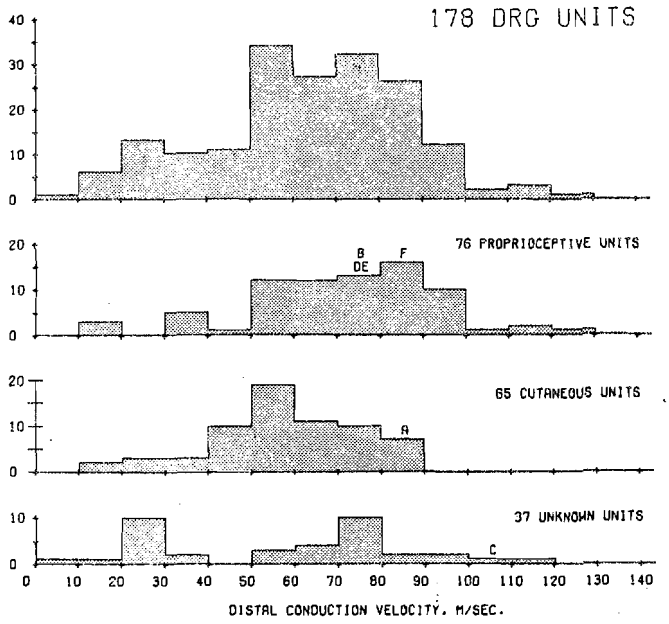

Fig. 4. Histograms of numbers of units recorded *versus* conduction velocity of the distal (sciatic nerve) process, grouped by somesthetic modality. The shaded areas include only the units with classical (L7D only) or unidentified proximal projections; the ventral root afferents are identified by letters over the appropriate columns (see text).

electrical stimulation of the L7 ventral root only, where the conduction velocity was 20 m/sec.

Unit DE: the 21st unit of 28 studied in the ninth cat (adult male prepared as in Fig. 1B) had a tonic discharge when the ankle was dorsiflexed, with further phasic increase upon rapid dorsiflexion of the ankle or extension at the knee. The unit had a 76 m/sec conduction velocity from the sciatic nerve cathode and was also clearly driven by pulses to both ventral and dorsal roots at comparable currents (see Fig. 3). The dorsal root conduction velocity was 30 m/sec and the ventral 28 m/sec. Cross-collision experiments were successful in all three possible combinations of projection pairs and with both orderings of dorsal and ventral root paired stimuli, confirming that all unit action potentials came from the same cell. The unit was held while the mineral oil was sucked away around the roots and ganglion. After confirming the physical separation of the stimulating probes and roots in air, the stimulation tests were unchanged. Fig. 3 shows the DRG cell unit potential being evoked by sciatic, dorsal root, and ventral root stimulation, the top trace showing collision extinction of the sciatic response when preceded by a ventral root response.

Unit C: the 3rd unit studied out of four in the sixth preparation (adult male prepared as in Fig. 1A and used subsequently for C-fiber volley experiments) was not physiologically excitable with any of the maneuvers employed. The sciatic nerve conduction velocity was 104 m/sec. The unit was electrically excitable with a 10 μA pulse (0.3 msec) to the L7 ventral root where the conduction velocity was 28 m/sec. The

unit also appeared to be driven with 153 μA threshold from the L7 dorsal root, a threshold well within the range of other units and still an order of magnitude below levels tested for current spread. The latency would have represented 34 m/sec conduction in the dorsal root, but the unit was lost before this could be confirmed by cross-collision between dorsal and ventral root stimuli.

The data presented here suggest that a considerably smaller percentage of myelinated afferents project to spinal cord via ventral root than seems to occur among unmyelinated DRG cells[7,8]. However, such ventral root myelinated afferents can arise from cell bodies in the ganglion proper and transmit apparently typical somesthetic information to terminations as yet unknown. Whether they constitute a consistent population or vary widely in number among spinal levels, specimens, or species (as do ventral root unipolar somata[25]), remains to be seen.

While constituting at most a small percentage of the total afferent input, recent anatomical and physiological evidence for sprouting of primary afferents in partially deafferented spinal cord[17,22,24] support the notion that ventral root afferents may play a significant role in the recovery of function noted following dorsal rhizotomy[3,23,34]. The apparent ability of DRG cells to have more than one proximal process may also further complicate anatomic analyses by fiber counts and size spectra[10,11,14].

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