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# Long-range projections of Aδ primary afferents in the Lissauer tract of the rat

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**Abstract**

Electrical microstimulation has been used to activate fine myelinated primary afferents running within the Lissauer tract. Stimulation of the tract at the L2/L3 border produced antidromic volleys which were recorded on the dorsal roots of more caudal spinal segments. Antidromic volleys were present in all cases for roots as far caudal as the S2 segment (L3, $n = 12$; L4, $n = 6$; L5, $n = 6$; L6, $n = 9$; S1, $n = 3$; S2, $n = 6$; observations in a total of 15 rats). These fibres were collaterals of primary afferents with conduction velocities in the dorsal root of up to $17.3 \pm 2.3\ m\ s^{-1}$ (mean ± S.D., $n = 6$; range 14–20 $m\ s^{-1}$). Conduction velocities within the Lissauer tract were slower; the fastest contributing fibres had conduction velocities of $9.2 \pm 2.2\ m\ s^{-1}$ (range 6–12 $m\ s^{-1}$). Lesions of the Lissauer tract caudal to the stimulation site abolished the volleys on roots lying caudal to the lesion. Most previous works have suggested that primary afferents project in the Lissauer tract for only one or two spinal segments. The present study shows that some fibres project rostrally for up to seven spinal segments (L2–S2).


*Keywords:* Primary afferents; Lissauer's tract; Spinal cord; Pain; Propriospinal; Dorsal horn

The Lissauer tract is a distinct region of axons that lies above the spinal dorsal horn between the dorsal and lateral funiculi [16]. Functionally, the Lissauer tract has been implicated in the regulation of spinal receptive field size [11,25] and the gating of transmission through the spinal dorsal horn [20]. While the tract was originally believed to consist entirely of primary afferents, it is now also known to contain propriospinal axons [2–8,20]. Modern estimates suggest that approximately two-thirds of the axons in Lissauer's tract in the rat are of primary afferent origin and that 80% of these are unmyelinated [4]. Myelinated fibres in the tract are of fine calibre, typically less than 5 μm in diameter (range 1–8 μm) [6] The majority of anatomical studies have suggested that most of these afferents project rostrocaudally in the tract for only one or two spinal segments [3,4,6,8,12,19,20], although a few have shown more extensive projections, at least for some afferents [10,18,21]. Electrophysiological studies have also shown long-ranging projections for some Aδ-fibres in the sural nerve of the cat [23]. Thus, antidromic compound action potentials with conduction velocities in the Aδ-fibre range were found to be evoked by stimulation of the Lissauer tract up to five spinal segments rostral to the root-entry zone of the sural nerve fibres [23]. In the present study, electrophysiological methods were used to demonstrate the presence of fine myelinated primary afferents projecting seven segments rostral to their parent dorsal root in the rat.

Experiments were performed on adult male Sprague–Dawley rats anaesthetized with urethane ($1.25\ g\ kg^{-1}$ i.p., supplemented if required). Experimental procedures conformed with and were licensed under UK legislation (Scientific Procedures Act, 1986). The trachea, carotid artery and jugular vein were cannulated and the rat was mounted in a stereotaxic frame providing support via ear bars and pelvic clamps. Rectal temperature was monitored and used to regulate a homeothermic blanket. The electrocardiogram was recorded via percutaneous electrodes in the right and left forelimbs.

A laminectomy was made to expose the spinal cord from thoracic level to the cauda equina. The cord was transected at mid-thoracic level. Skin flaps and muscle around the exposure were raised and tied to form a pool, which was filled with warm paraffin to protect the exposed cord. Dorsal roots were cut and mounted across Ag/AgCl wires to record dorsal root potentials and root volleys. Stimuli to the Lissauer tract were delivered via a tungsten-in-glass microelectrode from an isolated constant current source and were monitored by record-

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ing the voltage-drop across a resistor placed in the current path. Pulse sequences were generated using Pulser software [13]. All recordings were digitized and averaged using a PC and interface card (micro1401 MkII; Cambridge Electronic Design, UK) with Signal or Spike2 for Windows software. For recording, gallamine triethiodide (20 mg i.v.) was administered to achieve neuromuscular blockade and the rat was artificially ventilated. Expired $CO_2$ was monitored, and ventilation volume adjusted, to maintain an end-tidal $CO_2$ concentration of 3–4%.

This study has taken advantage of the position of the Lissauer tract at the L2/L3 spinal level, where it occupies a thin strip exposed at the spinal cord surface. As shown in earlier studies [14,24,26], it is possible to stimulate the Lissauer tract selectively at this level, i.e. without effective current spread to activate fast-conducting, high-calibre primary afferents of low electrical threshold in the neighbouring dorsal roots or dorsal columns. In these circumstances, Lissauer tract stimulation evokes a dorsal root potential (DRP) that has a characteristically long latency-to-onset [1,14,24–26] and this can be used to localize the tract. The purpose of this study was to examine whether, with these stimuli, there was evidence of activation of slower-conducting primary afferents running within the Lissauer tract and, if so, which dorsal roots were the parents of those afferents.

DRPs evoked by Lissauer tract stimulation were recorded together with the antidromic compound action potentials evoked on neighbouring roots as shown in Fig. 1A. A pair of electrodes was placed on the L2 root to record the DRP with the most proximal electrode 1 mm or less from the root entry zone. More caudal roots were also lifted onto a pair of electrodes to record the antidromic volleys. These electrodes were positioned as far distal as possible to reduce interference from the stimulus artefact and with an inter-electrode distance of 10–15 mm. The dorsal root was crushed just proximal to the most distal electrode to allow a monophasic action potential to be recorded.

Preliminary experiments showed that substantial antidromic volleys were present on dorsal roots L3, L4 and L5 with stimulation of the Lissauer tract of 10 μA or less at the L2/L3 border. More caudal roots were examined in subsequent experiments and antidromic volleys were present in all cases for roots as far caudal as the S2 segment (L3, $n = 12$; L4, $n = 6$; L5, $n = 6$; L6, $n = 9$; S1, $n = 3$; S2, $n = 6$; observations in a total of 15 rats). Volleys on the more caudal roots were invariably smaller than those observed on roots closer to the stimulus. In one experiment, the volley was recorded from the first coccygeal root. A small volley was present but only with stimuli of 20 μA. Subsequent observations were restricted to the dorsal roots of the L3–S2 segments.

For the traces illustrated in Fig. 1B–E, stimuli of increasing intensity (2–50 μA as indicated) were delivered to the Lissauer tract at the L2/L3 segmental boundary. Delayed-onset DRPs were apparent on the L2 root with stimuli up to 10 μA (Fig. 1B) and these were associated with potentials on the L3, L6 and S2 roots that had a form and latency consistent with antidromic volleys conducted at 2–20 m $s^{-1}$ (Fig. 1C–E). These potentials increased in size with increasing stimulus intensity from 2 to 10 μA. With 50 μA stimuli, the DRP was markedly larger and of much shorter latency-to-onset. This change in the DRP was

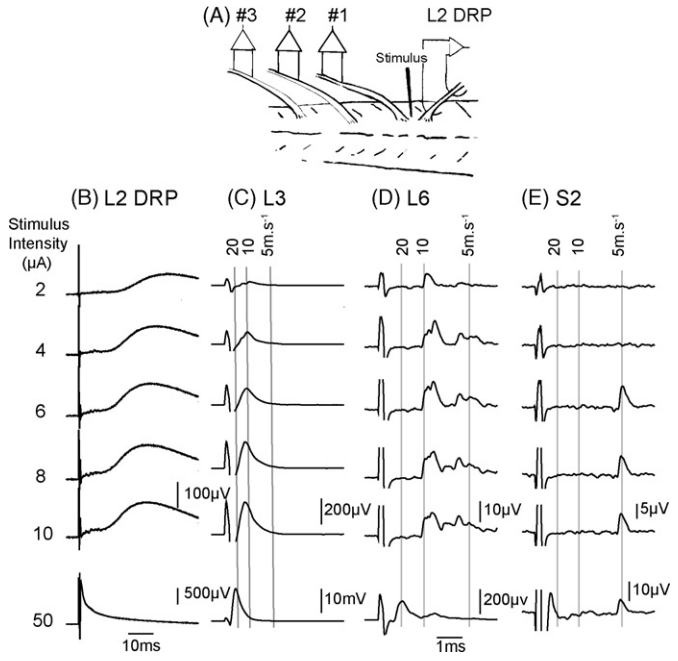


Fig. 1. Root volleys evoked by Lissauer tract stimulation. (A) Recording arrangement. Stimuli were applied to the Lissauer tract while recording the L2 DRP together with antidromic volleys on more caudal roots. (B–E) Averaged traces with increasing intensities of stimulation from 2 to 50 μA as indicated to the left. All stimuli were 200 μs pulses delivered at 1 Hz. In (B), the DRPs recorded from the L2 root are shown while (C–E) show the antidromic volleys recorded simultaneously from the L3, L6 and S2 dorsal roots. Note the change in timebase between (B) and the traces of (C–E). Voltage calibrations shown for 10 μA stimuli apply for all traces with stimuli of 2–10 μA. Negativity upwards in this and all other figures. Vertical grey lines indicate the estimated conduction velocities calculated over the full distance between the stimulus sites and the most proximal recording electrode on the root which in this case was 10, 19 and 18 mm for L3, L6 and S2 roots, respectively.

accompanied by the appearance of a larger and faster conducted volley on the L3 root (note the changes in scale in Fig. 1 with 50 μA stimuli). This indicated spread of current to low-threshold primary afferents in the nearby dorsal roots or dorsal columns with 50 μA stimuli. An early volley was also apparent on the L2 DRP trace at this intensity of stimulation.

It remains to demonstrate that the potentials in Fig. 1C–E are antidromic volleys evoked by direct activation of primary afferents by Lissauer tract stimulation. Plainly, as the roots were cut, the potentials must arise centrally. They might, however, be synaptically evoked as Lissauer tract stimulation evokes primary afferent depolarization and this could give rise to antidromic volleys in the roots analogous to the dorsal root reflexes evoked by dorsal root or cutaneous nerve stimulation (reviewed in [27]). Two observations indicate that the volleys are the result of direct activation of primary afferents. First, their latency is shorter than that of the DRP; therefore, they precede the onset of the evoked primary afferent depolarization (Fig. 1B–E). Secondly, the potentials always reliably followed pairs of stimuli delivered at up to 500 Hz in frequency ($n = 12$ roots in four rats). Examples of this are shown in Fig. 2 for the L3, L6 and S2 roots. These data indicate direct rather than synaptic activation.

To show that the activated afferents ran through the Lissauer tract, it was lesioned caudal to the stimulus site as shown in