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A horizontal slice preparation for examining the functional connectivity of dorsal column fibres in mouse spinal cord

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ABSTRACT

In spinal cord injury (SCI) research, axon regeneration across spinal lesions is most often assessed using anatomical methods. It would be extremely advantageous, however, to examine the *functional* synaptic connectivity of regenerating fibres, using high-resolution electrophysiological methods. We have therefore developed a mouse horizontal spinal cord slice preparation that permits detailed analysis of evoked dorsal column (DCol) synaptic inputs on spinal neurons, using whole-cell patch clamp electrophysiology. This preparation allows us to characterise postsynaptic currents and potentials in response to electrical stimulation of DCol fibres, along with the intrinsic properties of spinal neurons. In addition, we demonstrate that low magnification calcium imaging can be used effectively to survey the spread of excitation from DCol stimulation in horizontal slices. This preparation is a potentially valuable tool for SCI research where confirmation of regenerated, functional synapses across a spinal lesion is critical.

1. Introduction

In the rodent spinal cord, the dorsal columns (DCols) contain longitudinally orientated ascending and descending fibres that are often deliberately damaged in various spinal cord injury (SCI) models. Typically, axons within the DCols, such as those in the corticospinal tract (CST), are then examined for signs of regenerative growth, primarily using *anatomical* methods (Bareyre et al., 2005; Carmel et al., 2010; Goldshmit et al., 2004; Iseda et al., 2004; Liu et al., 2010). Importantly, intracellular recording techniques such as sharp electrode and whole-cell patch clamp electrophysiology are now increasingly used in experimental SCI research as they can be used to measure intrinsic neuronal properties and function, thus providing valuable data on SCI processes/recovery (Beaumont et al., 2008; Dougherty and Hochman, 2008; Li et al., 2007; MacFarlane and Sontheimer, 1997; Rank et al., 2011; Taccola et al., 2008). Crucially, these techniques can also be used to provide unequivocal evidence for the existence of functionally viable synapses that have regenerated *across* a spinal lesion (Fenrich and Rose, 2009). Although this information is essential if functional recovery from SCI is to be attributed to regenerated axons, such experiments are technically difficult and rarely attempted (Fenrich and Rose, 2009).

We have therefore developed a mouse horizontal spinal slice preparation that preserves longitudinal fibres, allowing investigation of stimulated DCol inputs onto spinal neurons using the whole-cell patch clamp technique. Importantly, this technique permits high-resolution, low noise current- and voltage-clamp analysis, enabling detailed investigation of the type and efficacy of synaptic inputs on recorded neurons. Our data show DCol inputs can be activated to evoke synaptic responses in spinal neurons over extended rostro-caudal distances in horizontal spinal cord slices. In addition, we demonstrate the use of calcium imaging to monitor the spread of excitation evoked by DCol stimulation. In short, this preparation offers an ideal approach to study DCol connectivity and function *across* a spinal lesion.

2. Materials and methods

2.1. Horizontal slice preparation

All experimental procedures were approved by the University of Newcastle Animal Care and Ethics Committee. Mice (C57Bl/6, both sexes; see Sections 2.3 and 2.6 for ages) were housed in an animal care facility under a 12-h light/dark cycle and had continuous access to food and water. Mice were anaesthetised with ketamine

Abbreviations: ACSF, artificial cerebrospinal fluid; AP, action potential; CNS, central nervous system; CST, corticospinal tract; DCols, dorsal columns; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; FCIP, fluorescent calcium indicator protein; IPSC, inhibitory postsynaptic current; IPSP, inhibitory postsynaptic potential; ROI, region of interest; S-ACSF, sucrose substituted artificial cerebrospinal fluid; SCI, spinal cord injury.

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(100 mg/kg, i.p.) and decapitated. The entire vertebral column, ribs, and surrounding soft tissue were isolated and submerged in ice-cold, oxygenated, sucrose substituted, artificial cerebrospinal fluid (S-ACSF; containing (in mM): 250 sucrose, 25 $NaHCO_3$, 10 glucose, 2.5 KCl, 1 NaH_2PO_4, 1 $MgCl_2$, and 2.5 $CaCl_2$; pH 7.3). Using a ventral approach, the spinal cord (segments T9 – S2) was dissected free and glued ventral side down to a cutting stage using cyanoacrylate glue (Loctite 454; Loctite, Caringbah, Australia). To ensure the dorsal surface of the spinal cord was parallel to the plane of the blade, extra glue was applied underneath the thoracic and upper lumbar segments to compensate for the narrow diameter compared to the mid-lumbar region. The stage and tissue were placed in a bath containing ice-cold, oxygenated S-ACSF, and horizontal slices (250 μm thick) were cut, commencing at the rostral end of the cord, using a vibrating microtome (HM 650V; Microm; Walldorf, Germany) (Fig. 1A).

Generally, two or three horizontal slices from the dorsal spinal cord were obtained. Because of the rapid taper of the sacral spinal cord, our slices did not extend far beyond the lumbar region, and thus consisted primarily of caudal thoracic and lumbar spinal segments. The first and most superficial slice contained the DCols and superficial dorsal horns (laminae I–II). The second slice contained a wide band of the DCols, flanked by dorsal horn grey matter, and the white matter of the lateral funiculus. The third slice contained a thin band of DCols, deep dorsal horn/intermediate zone grey matter, and the white matter of the lateral funiculus (Fig. 1A, right panel). Slice orientation was determined by examining the width and taper of each slice. The rostral (thoracic) end was not as wide as the lumbar segments and the caudal (sacral) end generally tapered to a point. The spinal cord segments that maintained the most consistency with respect to DCol width and depth, and were therefore examined in most detail, were L2 to L5. After cutting, slices were immediately transferred to a humidified storage chamber containing oxygenated ACSF (118 mM NaCl replacing sucrose in S-ACSF) and allowed to equilibrate for 1 h at room temperature (22–24 °C) before recording commenced.

2.2. Stimulation of the DCols in horizontal slices

A bipolar stimulating electrode, fabricated from insulated tungsten microelectrodes (100 μm shank diameter, 80 μm tip separation, 1–2 MΩ impedance; FHC, ME, USA), was positioned using a micro-manipulator (LBM-7; Scientifica, Bedford, UK) so that the tips were positioned in the DCols, slightly below the tissue surface, at the rostral end of the slice (Fig. 1B, left panel). In some experiments, the stimulating electrode was placed in the grey

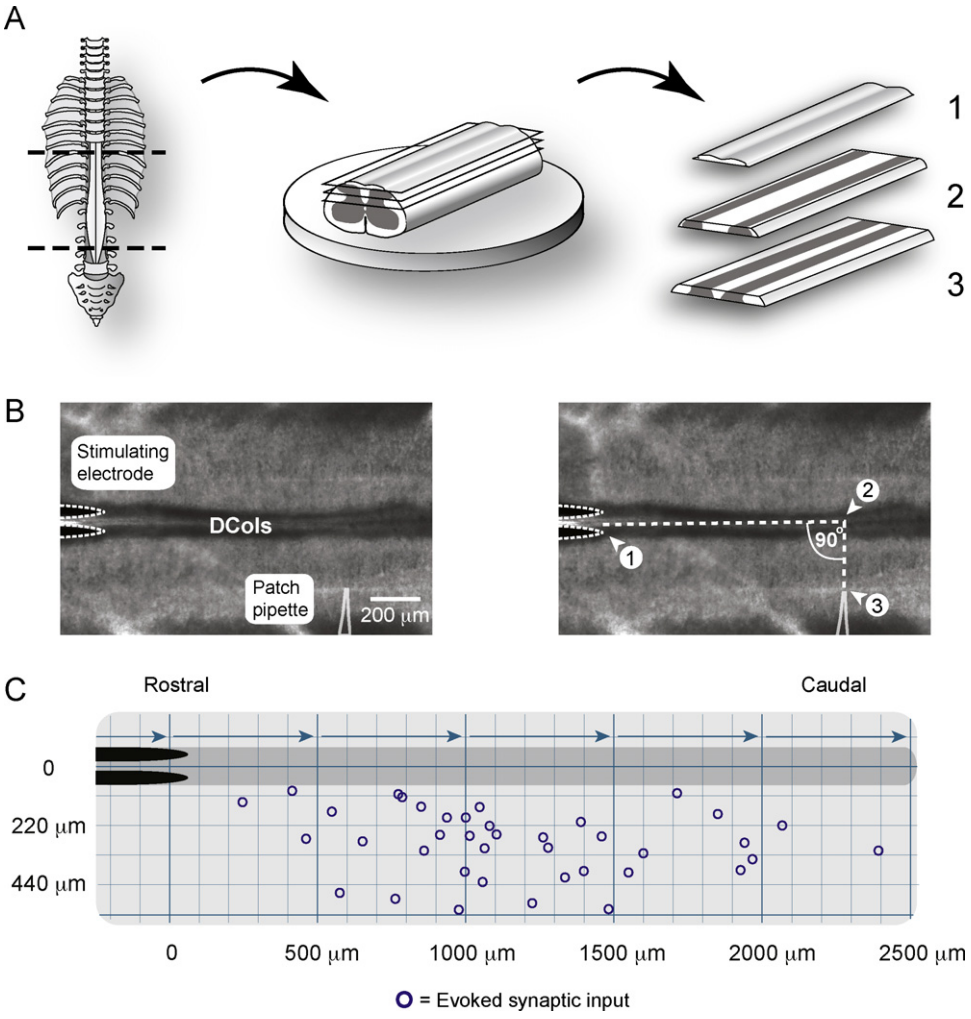

Fig. 1. Slice preparation and recording configuration. (A) The thoraco-sacral spinal cord (T9 – S2: dashed lines) was excised and glued to a cutting stage (ventral side down). Two to three slices (250 μm thick) containing the DCols were cut in the horizontal plane. See Section 2.1 for more detail. (B) A bipolar stimulating electrode was positioned in the DCols and recordings were made in the grey matter caudal to the stimulation site (left panel). The rostrocaudal distance between the stimulating electrode and recording location was measured along the length of the DCols (i.e., 1–2, right panel). The mediolateral position of the patch pipette was measured perpendicular to the DCols (i.e., 2–3, right panel). (C) Location of recorded neurons relative to the stimulating electrode.