A horizontal spinal cord slice preparation for studying descending synaptic inputs to neurons in the mouse spinal cord

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Since the introduction and widespread use of *in vitro* spinal cord slice preparations, studies of evoked synaptic transmission in spinal neurons have concentrated on inputs from two sources; those from primary afferents and local circuit neurons. This focus is due largely to practical considerations. For example, peripheral inputs can be readily activated by stimulation of dorsal roots that often remain attached to spinal cord slices. Similarly, local inputs can be studied within a slice by low intensity focal stimulation of visualized neurons or even recording from pairs of neurons. The other major source of synaptic input to the spinal cord, from more distant propriospinal, brainstem, midbrain and even cortical regions, has been overlooked in slice work because of the difficulty of maintaining the integrity of long descending axons in a spinal cord slice. A preparation that enables study of descending synaptic connections is now needed as recent studies suggest that it is possible for regenerating axons to regrow across a spinal cord lesion (Goldshmit *et al.*, 2004). So far these studies have only presented anatomical and behavioural evidence for successful axon regrowth across spinal cord lesions. It is not known if sprouting or regenerating axons make functional synaptic connections with neurons above or below the lesion site. Accordingly, we have taken advantage of the small size of the mouse spinal cord to develop a horizontal spinal cord slice preparation that permits investigation of synaptic transmission at connections between descending axons and spinal neurons. Mice (C57Bl/6; both sexes, aged 19 - 41 days) were anaesthetised with Ketamine (100 mg/kg, i.p.) and decapitated. A length of spinal cord spaning from mid-thoracic (~ T8) to mid-lumbar (~ L4) segments was removed and glued ventral side down onto a cutting stage. The stage was oriented in a cutting chamber to allow cutting of horizontal slices (300 μm thick). A substantial length of cord containing midline fibre tracts (dorsal columns) could be retained in at least two slices. Patch clamp recordings (KCH_3SO_4 internal) were made in neurons in the dorsal grey matter. These recordings were most likely made in the superficial and deep dorsal horn regions in the first and second slice, respectively. Synaptic responses were evoked in dorsal horn neurones by stimulating the dorsal columns with a bipolar electrode (200 μm tip separation) at various distances rostral to the recording site. Responses were evoked in 26 of 32 recordings with success highly dependent on the distance between stimulating and recording site (0 to 500 μm = 75%; 500 to 1000 μm = 94%; 1000 to 1500 μm = 86%; and 1500 to 2000 μm = 33%). In voltage-clamp (holding potential -60 mV), three different types of response that exhibited constant latency and low failure rate after stimulation at 1.2 × threshold were distinguished: single component monosynaptic (15/26); dual component monosynaptic (8/26); and multi component polysynaptic (3/26). Subsequent current clamp recordings in a subset of neurones (13/26) revealed that some evoked responses contained an inhibitory component (5/13). Some neurones (13/25) receiving evoked synaptic inputs were also categorized according to their discharge patterns during depolarising current step injection (800 ms, 20 pA steps): Tonic firing (5/13); initial bursting (6/13); and delayed firing (2/13). In summary, these data indicate that a variety of descending synaptic inputs, which travel in the dorsal columns, can be preserved in horizontal slices of the mouse spinal cord. These descending inputs can be excitatory or inhibitory and innervate a heterogeneous population of target neurones in the superficial and deep dorsal horn. This information shows that horizontal spinal cord slices in the mouse may in future provide a model to assess the functional relevance of anatomical regrowth across spinal cord lesions.

Goldshmit Y, Galea MP, Wise G, Bartlett PF & Turnley AM. (2004) *Journal of Neuroscience,* **24:** 10064-73.