A preparation for studying axon regeneration and descending synaptic connections after spinal cord injury in mice

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Recent evidence suggests manipulation of molecular pathways and exercise can improve function after spinal cord injury (SCI), presumably by plasticity associated with axon regeneration and sprouting (Goldshmit *et al.*, 2004). Surprisingly, we know little about the synaptic connections regenerating or sprouting axons make to "bridge" a spinal cord lesion.

Purpose: To develop a horizontal spinal cord slice preparation for electrophysiological examination of synaptic connections between descending axons and spinal neurons.

Methods: Mice (C57Bl/6, > P19-41) were anaesthetised (Ketamine 100 mg/kg i.p.) and decapitated. Horizontal slices (300 μm thick) containing T8-L4 spinal segments were cut and whole-cell recordings were obtained from visualized neurons in the intermediate zone (KCH_3SO_4 internal, at 23°C). Evoked synaptic responses were obtained by stimulating the dorsal columns at various distances rostral to the recording site. In mice, the dorsal columns contain corticospinal and propriospinal axons.

Results: Synaptic responses were evoked in 26 of 32 recordings. The separation between stimulating and recording sites ranged from 0.3-1.9 mm. In voltage-clamp, three different types of responses were observed: single component monosynaptic (15/26); dual component monosynaptic (8/26); and multi component polysynaptic (3/26). Subsequent current-clamp recordings showed some responses contained an inhibitory component (5/13). A range of action potential discharge patterns was also observed in neurons that demonstrated evoked synaptic responses: Tonic firing (5/13); Initial bursting (6/13); and Delayed firing (2/13).

Conclusions: The *in vitro* horizontal slice preparation could be used for future study of descending synaptic connections to spinal neurons in both normal mice and in those demonstrating functional recovery after SCI.

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