Preparation of rodent lumbar spinal cord slices for electrophysiological studies of pain circuits.

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Introduction

The dorsal horn of the spinal cord is the major receiving zone for primary afferent axons that transmit information from sensory receptors in the skin, viscera, joints, and muscles of the trunk and limbs to the central nervous system. The superficial dorsal horn (SDH) modulates nociceptive input is universally accepted. SDH contains neurons selectively responsive to noxious and/or thermal stimuli. This has led to numerous studies that have investigated the mechanisms underlying the phenomena, with a view to identifying novel targets for the treatment of pathological pain states. A major goal has been to define the synaptic circuits involving the various neuronal components of the dorsal horn. Several groups paved the way to study individual spinal neuronal populations and identified the afferent fiber pathways to selectively stimulate and record pain circuits in transverse or para-sagittal in-vitro spinal cord slices. These recordings can also be performed in pain models, such as after nerve injury or inflammation as a result of tissue injury, and the ensuing behavioral transformation can be observed in cellular correlates in the *ex vivo* slices.

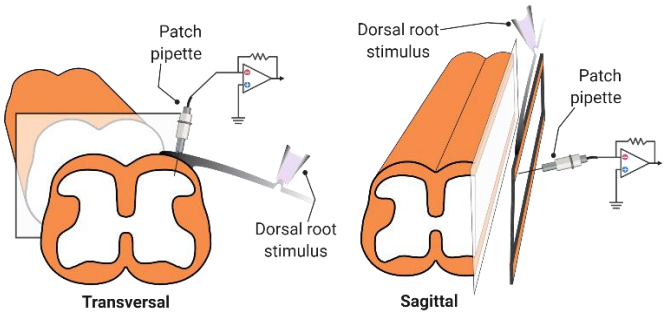
Procedures

Typically, rodents (mice or rats) either wild type or those expressing fluorescent markers are used in the preparation of spinal cord slices. Animals are anesthetized with approved protocols and perfused transcardially with 10 ml of ice-cold oxygenated, sucrose-containing artificial cerebrospinal fluid. The lumbar spinal cord is rapidly isolated by laminectomy, placed in oxygenated, ice-cold, sucrose-aCSF, cleaned of dura mater. The nerve roots are either cut close to the cord or spared (~10 mm) depending on the experiment conducted. The spinal cord is immersed in low-melting-point agarose and transverse or parasagittal slices (300–450 µm) are cut in ice-cold, sucrose-aCSF using a vibrating microtome (**7000smz-2**; Campden Instruments, Lafayette, IN, USA). All slices are incubated for 15 min at room temperature in NMDG containing recovery solution (Li and Baccei 2016). The slices are then transferred to normal aCSF used for electrophysiological recording (Griggs *et al.*, 2019; Taylor *et al.*, 2019; Sinha *et al*., 2021).

Patch-clamp recordings: A single parasagittal spinal cord slice is transferred to a fixed stage mounted under an upright microscope, where it is continuously superfused with oxygenated aCSF. Recordings from neurons are obtained under direct visualization using differential interference contrast (DIC) optics or with epifluorescent microscopy for fluorescent labelled cell bodies. Recording pipettes (3-6 MΩ) containing appropriate intracellular recording media. Patch-clamp recordings in either current- and voltage-clamp modes are performed on SDH neurons using an Axon Instruments Multiclamp 700B amplifier (Molecular Devices). Signals are low-pass filtered at 4-20

kHz, amplified 1-20 fold, sampled at 5-10 kHz and analyzed offline using pClamp software (Molecular Devices). Experimental data were recorded approximately 5-10 min after establishing whole-cell configuration. All recordings are performed at room temperature on neurons selected from the selected SDH region. In current clamp mode we looked at firing patterns in response to a series of 7-9 depolarizing current injections in 20 pA steps lasting 1 second. In voltage clamp mode we record voltage-activated currents. At present, the lab is focused on identifying fast activating and fast inactivating A-type currents that the excitatory SDH neurons readily exhibit. Other neurons possibly also exhibit slow hyperpolarization-activated inward currents and T-type calcium currents .

Dorsal root stimulation (DRS): DRS experiments are carried in slices prepared with approximately 4 mm long dorsal root entering the dorsal horn L3-L4 segments. Electrical stimulation of the attached dorsal root is performed using a glass suction electrode to evoke EPSCs. We used stimuli of 0.1 ms pulse width at variable frequencies (0.03 to 50 Hz) and intensities (10 to 600 µA), which putatively recruits Aβ- (10 – 25 µA), Aδ- (30 – 70 µA) and C- (100 to 600 µA) fibers.



Schematic of transverse or para-sagittal spinal cord slice preparation used for electrophysiological recordings. Created with BioRender.

Specific examples

We have used slices prepared in this manner to study the acute inflammation induced sensitization of nociceptive neurons that triggers the accumulation of calcium permeable (CP) APMA receptors in the dorsal horn of the spinal cord. Using the current-voltage (I-V) relationship we evaluated the inward rectification, a key characteristic of CP-AMPARs, via C-fiber stimulus-evoked AMPAR-mediated EPSCs in lamina II neurons. We found that the intraplantar injection of complete Freund's adjuvant (CFA) induced an inward rectification at 3 d that persisted to 21 d after injury (Taylor *et al*. 2019).

In another example we used the slice preparation combined with calcium imaging to study whether spinal MG signals through TRPA1, AC1, PKA, and Epac1/2 to cause PDN in type 2 diabetes. We specifically showed in calcium imaging experiments that MG, which was greater in db/db mice as compared to BKS controls (Griggs *et al*. 2019).

Our recent study involved an adult ex vivo spinal cord slice preparation from Y1eGFP reporter mice, wherein we characterized the firing patterns of lamina II neurons. We identified distinct heterogenous Y1 receptor expressing population based on detailed firing patterns analysis. By electrical stimulation at a frequency and intensity sufficient to recruit C- and/or Aδ-fibres, both of which are essential for the transmission of noxious somatosensation, we identified the type of peripheral termination on Y1eGFP neurons. We also studied the neuropeptide Y inhibition of responsiveness of Y1eGFP neurons to primary afferent input, which might be mediated by activation of GIRK channels. We also showed that different type of voltage-activated current closely associated with a specific firing pattern. The correlation between firing pattern and ionic conductance will facilitate the functional classification of SDH neurons that is required to understand the neuronal pain circuitry (Sinha *et al*. 2021).

Our future studies are designed to determine the cellular correlates in the ex vivo slices to behavioral studies of persistent pain models.

References

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