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Research report

Mechanosensory afferent input and neuronal firing properties in rodent spinal laminae III–V: re-examination of relationships with analysis of responses to static and time-varying stimuli

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Abstract

Relationships between neuronal firing pattern and mechanosensory input in the deep dorsal horn were investigated using whole-cell recordings from isolated hamster spinal cord with innervation from an attached skin patch. Neurons that fired repetitively to depolarizing current (tonic cells) responded to both moving and static stimulation of their cutaneous receptive fields, and discharged continuously for the duration of stimulus application. Neurons responding to depolarizing current with transient, rapidly adapting firing (phasic cells) were significantly more responsive to stimulus movement than to static skin contact. Phasic cells typically issued a brief discharge at the onset or termination of a stimulus; their responses during static skin contact were weaker than tonic cells. Tonic cells were activated during both ramp and steady-state skin indentations, whereas phasic cells responded with their strongest excitation to displacement velocities exceeding 8 μm/ms. Mechanosensory input to phasic cells originated primarily from low threshold receptors, whereas tonic cells demonstrated a mixture of inputs from both low and high threshold sources. A third class of neurons responded to depolarizing current with a pronounced firing delay and displayed a sensitivity to cutaneous stimuli that was similar to tonic cells except they showed a modest decrease in firing as skin indentation velocity increased. The results suggest a correlation between functional properties of mechanoreceptive afferent fibers and intrinsic discharge properties of laminae III–V neurons that may significantly influence integration of cutaneous mechanosensory information at the first spinal relay.

Theme: Sensory systems
Topic: Spinal cord

Keywords: Whole-cell recordings; In vitro; Deep dorsal horn; Interneurons; Skin indentation

1. Introduction

The spinal dorsal horn plays an important role in integration and transmission of diverse sensory information from the body surface and internal organs (see Refs. [7,13,15,19,32,47,56] for reviews). Dorsal horn neurons receive convergent input from multiple sensory channels [3,12,17,20,21,34,54,55,59] and differ in their intrinsic membrane and discharge properties [18,22,28,33,36,37,39,40,43,52], indicating that integration of somatosensory information at the first spinal relay is mediated by complex interactions between physiologically diverse elements.

In their provocative studies over twenty years ago, Tapper et al. [8,50] proposed that primary afferent fiber systems innervating hairy skin are distributed non-randomly onto dorsal horn neurons, and suggested that some selection process directs these projections onto their postsynaptic targets. This proposition is important because it implies that integration of cutaneous sensory information begins by segregating afferent input into functional circuits according to channel modality.

Functional diversity among somatic afferents and the possibility of non-random distribution of these inputs raises

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the question of whether afferent signals are matched to intrinsic properties of the postsynaptic neurons. The few studies investigating such a relationship have reached mixed conclusions. Lopez-Garcia and King [33] reported that rat dorsal horn neurons activated by both low and high threshold mechanical stimuli displayed tonic firing to pulses of depolarizing current, whereas those neurons selectively responsive to noxious stimuli often fired with a rapidly adapting (i.e., 'phasic') discharge. Their conclusion that a relationship exists between a neuron's biophysical properties and its responses to cutaneous afferent stimulation, however, went unsupported by Jiang et al.'s [28] subsequent study that examined somatic and visceral sensory inputs to deep dorsal horn neurons.

The object of the present study was to re-examine relationships between neuronal intrinsic properties and sensory afferent function in the spinal dorsal horn and help resolve this question. The work was undertaken in light of recent, more detailed observations of dorsal horn neuronal intrinsic properties [18,22,37,43]. Given a recent finding that dorsal horn neurons with phasic discharge patterns exhibit differential responsiveness to static and time-varying membrane depolarizations [43], it seemed natural to ask whether these cells also respond differentially to static and dynamic components of a sensory stimulus delivered to their receptive fields, possibly contributing to feature extraction processes underlying movement and transient detection.

The study focused on Rexed's laminae III–V, the so-called deep dorsal horn, a major termination site for hair, tactile, and muscle afferents [7], and a source of reflex [23] and ascending sensory [56] pathways. Experiments were conducted on isolated preparations of spinal cord innervated by sensory afferents serving an excised patch of hairy skin [42,45,46], allowing comparisons between neuronal intrinsic properties and functional characteristics of sensory input. The observations offer fresh evidence of a relationship between neuronal discharge properties and mechanosensory afferent input. The results are consistent with the idea that connections between cutaneous afferent fibers and spinal neurons form synaptic networks that are related to the functional properties of afferent end organs.

2. Materials and methods

2.1. Tissue preparation

All procedures involving animals conformed to standards set by the National Institutes of Health (*Guide for the Care and Use of Laboratory Animals*, publication 865-23) and were approved by the All-University Committee on Animal Use and Care of Michigan State University.

Procedures for preparing isolated spinal cord with partially intact afferent input have been published in detail [42,45]. Briefly, the vertebral column from thorax to sacrum and a patch of shaved flank skin (~20 × 25 mm) with intervening peripheral innervation were removed from urethane-anesthetized (1.5 mg/g, i.p.), 3- to 4-week-old (40−55 g) male Syrian hamsters and placed in an ice-cold (5–8 °C) dissection solution [2], containing (mM) 216 sucrose, 2.5 KCl, 0.25 $CaCl_2$, 10 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose (pH 7.35−7.45, 290−310 mOsm/l) gassed with 95% O_2/5% CO_2. The cord was hemisected and peripheral nerves innervating the skin flap, including dorsal roots, dorsal root ganglia, and cutaneous nerve branches, were freed from bone and muscle.

Dissected tissues were transferred to a twin-compartment chamber (25–27 °C) where the cord was submerged in a well with oxygenated artificial cerebrospinal fluid containing (in mM) 125 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose (pH 7.35−7.45; 290−310 mOsm/l) flowing at 8 ml/min. A nylon grid supported the skin patch over an adjacent well through which flowed an oxygenated synthetic interstitial fluid (3 ml/min) modified from Bretag [5], containing (in mM) 108 NaCl, 3.5 KCl, 1.5 $CaCl_2$, 0.7 $MgSO_4$, 1.7 NaH_2PO_4, 26 $NaHCO_3$, 10 Na gluconate, 5.6 glucose and 7.6 sucrose (pH 7.35–7.45; 290−290 mOsm/l), keeping the underside continually moist. The intervening nerves were placed in a conjoining groove and covered by a mixture of mineral oil and petroleum jelly. A dorsal root and cutaneous branch were placed in contact with bipolar platinum-iridium electrodes (5 mm separation) for purposes of activating volleys in sensory afferents.

2.2. Electrophysiological recording

Electrical activity from neurons in laminae (L) III–V was recorded using the 'blind' cell-attached, tight-seal configuration [4]. Patch clamp pipettes were fabricated from borosilicate glass (N-51A; Drummond Scientific, Broomall, PA, USA) and filled with internal solution containing (mM) 130 potassium gluconate, 5 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 11 EGTA, 10 HEPES, 2 ATP (equine, magnesium salt) and 0.1 GTP (lithium salt), (pH 7.3, 280−285 mOsm/l) (DC resistances 5−7 MΩ). Biocytin (2%; free base, Sigma Chemical Co., St. Louis, MO, USA) was added to provide morphological information about the recorded cells and verify recording location [43].

Recording pipettes were advanced into the cord (15–20° from vertical) at the junction between LX and the dorsal columns, about 0.2 to 0.5 mm rostral to the entry zone of the dorsal root-cutaneous nerve under study. After establishing a high resistance seal (1–2.5 GΩ), cell-attached recording configuration was achieved by rupturing the cell membrane with gentle mouth suction applied via polyethylene tubing attached to the pipette holder. Signals (0−5 kHz bandwidth) were amplified by Axoclamp 2 or Axopatch 1D amplifiers (Axon Instruments, Foster City, CA, USA), passed through a digital data recorder and stored on magnetic tape or computer-analyzed on-line using a