

Short communication

## Temporal plasticity of dorsal horn somatosensory neurons after acute and chronic spinal cord hemisection in rat

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### Abstract

Unilateral T13 hemisection of the rat spinal cord produces a model of chronic spinal cord injury (SCI) that is characterized by bilateral hyperexcitability of lumbar dorsal horn neurons, and behavioral signs of central pain. While we have demonstrated that responsiveness of multireceptive (MR) dorsal horn neurons is dramatically increased at 28 days after injury, the effects of acute hemisection are unknown and predicted to be different than observed chronically. In the present study, the consequences of T13 hemisection are examined acutely at 45 min in MR neurons both ipsilateral and contralateral to the site of injury, and compared to the same class of cells at 28 days after injury ( $n=20$  cells total per group: 2–3 cells/site of the cord from  $n=5$  animals). Acutely, ipsilateral to the hemisection, both spontaneous and evoked activity of MR neurons were significantly increased, whereas contralaterally, only evoked activity was significantly increased. In animals 28 days after hemisection, spontaneous activity of MR neurons was comparable to intact levels ipsilaterally, and cells exhibited hyperexcitability to evoked stimuli bilaterally. Expansion of cutaneous receptive fields was observed only in hindpaws ipsilateral to the lesion, acutely. These results demonstrate dynamic plasticity in properties of dorsal horn somatosensory neurons after SCI.

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In previous studies, we have demonstrated that following unilateral T13 spinal cord hemisection injury (SCI), animals develop behaviors consistent with chronic central pain (allodynia and hyperalgesia) [5]. Long-term changes in the electrophysiologic properties of lumbar dorsal horn neurons are also evident after injury [9]. These include the occurrence of irregular spontaneous interspike intervals, shifting of cell phenotypes to favor populations that respond to noxious stimuli, and bilateral hyperexcitability to both innocuous and noxious peripheral stimuli. Surprisingly, however, in these chronically hemisected animals,

the frequency of ongoing spontaneous background activity of dorsal horn neurons, and the sizes of cutaneous receptive field areas, remain equivalent to naive animals.

Changes in firing properties of dorsal horn neurons likely result from the interruption, or interference with, tonic descending inhibitory controls. Early work using an acute cold block paradigm supports this contention; cold block applied rostral to the recording site causes an immediate increase in spontaneous activity as well as evoked responsiveness of dorsal horn neurons [3,12,15]. Similarly, acute rostral hemisection causes immediate increases in background and evoked activity in neurons on the side ipsilateral to the injury in cats [1]. We have shown that chronic hemisection SCI dramatically reduces lumbar levels of tissue 5-HT, and that intrathecally-delivered 5-HT

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agents can modify pain-related behaviors [10]. Additionally, 5-HT topically applied to the surface of the cord modulates abnormal electrophysiologic properties of lumbar dorsal horn neurons [11]. Similarly, loss of tissue 5-HT [7], neuronal hyperexcitability [9], and pain-related behaviors [8] can be reversed by engraftment of 5-HT-secreting cell transplants.

Because of discrepancies between predicted and observed changes in spontaneous discharge rates and in cutaneous receptive field sizes, in consideration of bilateral neuronal hyperexcitability and nociception observed after chronic hemisection, the current study was undertaken to examine the effects of acute unilateral T13 hemisection on properties of multi-receptive wide dynamic range (MR) neurons in the lumbar enlargement both ipsilateral and contralateral to the injury site. We hypothesize that if disparate electrophysiologic changes are observed in dorsal horn neurons of acute versus chronically injured animals, form of functional plasticity is occurring after SCI.

In one group, male Sprague–Dawley rats ( $n=10$ ), 150–175 g were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and the T13 spinal cord segment was unilaterally hemisected using a #11 scalpel blade, without damage to the posterior vessel or its branches. Postoperative treatments included saline (2.0 cc s.c.) and penicillin-G (0.35 ml/kg i.m.). From between 28 and 35 days postoperatively, animals exhibiting robust mechanical allodynia and thermal hyperalgesia ('chronic';  $n=5$  animals; cf. Ref. [8]) were selected for electrophysiological experiments. Extracellular single unit recordings ( $n=20$  neurons total were analyzed from 2–3 neurons/side of the cord/animal) were obtained. In a second group of animals ( $n=5$ ), unit recordings were obtained immediately before ('intact';  $n=20$  neurons total, 2–3 neurons/side of the cord/animal), and 45 min immediately after unilateral T13 hemisection ('acute';  $n=20$  neurons total, 2–3 neurons/side of the cord/animal).

For electrophysiological recording, rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and supplemented (5 mg/kg per h) through a jugular vein catheter. Animals were fixed in a stereotaxic frame and the spinal cord was exposed from T12 to L6 and covered with warm mineral oil. MR neurons were classified by their graded responses to brush, press and pinch stimuli (delivered first). Units were isolated in the L3–L5 segments medial to the dorsal root entry zone up to a depth of 1000  $\mu\text{m}$ . Recordings were made with insulated tungsten (12 M $\Omega$ ) microelectrodes (A-M Systems, Carlsborg, WA, USA). Once a cell was identified (by its background activity and evoked response), spontaneous activity was measured followed by cutaneous receptive field delineation and tracing on a template of the hindpaw. Mechanical stimuli were applied to the lateral and ventral surfaces of the hindpaw: (1) brushing the skin with a cotton-tipped applicator; (2) pressure, by attaching a large arterial clip with a weak grip to a fold of the skin (144 g/mm<sup>2</sup>); and

(3) pinching, by applying a small arterial clip with a strong grip to a fold of skin (583 g/mm<sup>2</sup>). Next, various strength von Frey filaments (3.84, 9.96 and 204.1 mN) and 47 °C thermal stimuli consisting of a heated steel probe (surface area 1 cm<sup>2</sup>) were applied. Stimuli were each applied serially for 20 s (enough time to permit stimulus accommodation), separated by 20 s of baseline activity. In experiments recording activity before and acutely after hemisection, it was ensured that isolated units remained intact and held for the duration of each experiment using Spike 2 template matching routines. Electrical signals were amplified and fed into a window discriminator, displayed on analog and digital storage oscilloscopes, processed by a data collection system (CED 1401+; Cambridge Instruments, Cambridge, UK; 486 PC, Dell, Austin, TX, USA), and stored on a computer. Records were analyzed off-line with Spike 2 software (v3.13, Cambridge Electronic Design, Cambridge, UK). Data were compiled as mean  $\pm$  S.D. and separated into intact, ipsilateral (same side of the animal as the lesion) and contralateral (opposite/intact side of the animal to the lesion) groups for both acute and chronic recordings.

Statistical tests were evaluated at the alpha level significance of 0.05, by two-tailed analyses using parametric tests (SigmaStat v1.0). Significance of the effects of hemisection was determined using the paired Student's *t*-test for comparisons between control and acute recordings in the same cells or two-sample Student's *t*-test for comparisons between control and chronic recordings in different cells.

Sampled MR neurons were isolated in both superficial and deep dorsal horn laminae I–V (50–1000  $\mu\text{m}$ ) ipsilateral and contralateral to the hemisection. Bilaterally, the mediolateral distribution of recorded units extended from the dorsal median septum to the edge of the dorsolateral funiculus, and the units were concentrated medially in the spinal cord at L3–L5, a region receiving a major input from the plantar (lateral–ventral) aspect of the hindpaw. In naive intact animals, MR response profiles were such that cells responded to all types of innocuous and noxious peripheral stimulation, and in a graded manner to increasing intensity von Frey filaments.

Representative (typical) peristimulus time histograms (spikes/1 s bin) are shown in Fig. 1A for neurons sampled from intact and acutely hemisected animals (+45 min), and from chronically hemisected (+28 days) animals. Records are shown for dorsal horn neurons both ipsilateral and contralateral to the lesion side. In the histograms showing intact and +45 min evoked rates, records shown are from the same neuron both before and after hemisection (arrow). In both acute (95% of all sampled units ipsilaterally, 65% contralaterally) and chronic animals (100% of all sampled units on both ipsilateral and contralateral sides), hyperexcitability occurred to all peripheral stimuli bilaterally, however spontaneous background activity increased on the side ipsilateral to the lesion only in acutely hemisected animals (85% of all sampled units).



These functional alterations of dorsal horn neurons are most likely conferred by permanent changes in descending controls, particularly serotonergic [10] that exert a critical inhibitory influence on dorsal horn neurons [16]. Indeed, 5-HT receptor agonists readily decrease bilateral hyperexcitability after hemisection [11]. The puzzling observations of contralateral effects may be explained in part by bilateral features of 5-HT denervation, described by Skagerberg and Bjorklund [14], showing that descending serotonergic fibers originate from the raphe magnus (dorsal, intermediate, and ventral serotonergic pathways) innervate not only the ipsilateral dorsal horn but also the contralateral dorsal horn at the same segmental level of termination by way of fibers that cross in lamina X. The relative proportion of fibers coursing to the contralateral side may reflect the proportion of contralateral neurons that exhibited changes after injury. The reduction in spontaneous activity and reduction in receptive field in MR dorsal horn neurons from chronic hemisected animals may reflect the partial return of this 5-HT innervation [10] or by other descending or dorsal horn-intrinsic systems.

Our results are in general accordance with those reported by others regarding onset and offset of increased spontaneous activity [1], but are in disagreement with data demonstrating that permanent changes in receptive field size begin at 10–15 days postoperatively in cats following spinal cord hemisection [2]. One possible reason for the discrepancy could be the differing nature of the lesion: Brenowitz carried out a unilateral hemisection, but intentionally spared the dorsal columns (DC). Since DC stimulation can produce inhibition of dorsal horn neurons [13], it could be the case that in our model, interruption of a DC-mediated feedback circuit could confer additional expansion of peripheral somatosensory fields. Additionally, the dorsal funiculi contains descending medullary fibers that synapse on dorsal horn neurons [4] that may also facilitate receptive field expansion through interruption of further descending inhibition. Others have shown similar results with at- and above-level receptive field expansion in rodent SCI models [6]. It may also be the case that the type (pentobarbital vs. methoxyflurane-oxygen for example) and depth of anesthesia could affect spontaneous activity and thus confound results from laboratory to laboratory.

In summary, the electrophysiological evidence presented here demonstrates an important dynamic temporal plasticity in properties of centrally-deafferented dorsal horn neurons. The partial return towards normal function may be related to the partial territorial reinnervation of 5-HT [10]; which contributes to chronic central pain that develops and persists secondary to SCI.

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