A role for uninjured afferents in neuropathic pain

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Abstract: Diseases and injuries to the nervous system can lead to a devastating chronic pain condition called neuropathic pain. We review changes that occur in the peripheral nervous system that may play a role in this disease. Common animal models for neuropathic pain involve an injury to one or more peripheral nerves. Following such an injury, the nerve fibers that have been injured exhibit many abnormal properties including the development of spontaneous neural activity as well as a change in the expression of certain genes in their cell body. Recent data indicate that adjacent, uninjured nerve fibers also exhibit significant changes. These changes are thought to be driven by injury-induced alterations in the milieu surrounding the uninjured nerve and nerve terminals. Thus, alteration in neural signaling in both injured and uninjured neurons play a role in the development of neuropathic pain after peripheral nerve injury.

Key words: nerve injury; hyperalgesia; unmyelinated afferents; myelinated afferents; nociceptors; neuropathy

Neuropathic pain is a chronic pain that develops as a result of pathology in the nervous system. This pathology can be due to diseases secondarily affecting the nervous system such as diabetes, shingles, or AIDS, or can be due to trauma of the nerve. Patients with neuropathic pain following nerve injury often complain of ongoing burning pain as well as pain to light touch. This hyperalgesia can exist well outside of the innervation zone for the injured nerve.

What accounts for this neuropathic pain? In this review, we will describe some of the peripheral factors that we think play a role in signaling this neuropathic pain.

1 Spinal nerve ligation (SNL) model of neuropathic pain

Much of what we know about neuropathic pain comes from experiments in animal models. A number of different animal models have been developed. Most of these models are based on producing an injury to some part of the peripheral nerve. Many of our recent studies have used the SNL model developed by Chung[1]. In this model, one or more of the lumbar spinal nerves are ligated and transected. This model has the advantage that the effects on injured and uninjured afferents can be easily investigated.

Each of the animal models of neuropathic pain leads to signs of abnormal pain behavior. Most studies investigate alterations in evoked pain behavior (i.e., withdrawal responses to stimuli applied to the paw). For example, a decreased threshold to mechanical stimuli or a decreased latency to heat stimuli applied to the paw are common observations. Enhanced responsiveness to cooling stimuli is also reported. Unfortunately, good behavioral assays for ongoing pain are lacking.

2 Ectopic activity develops in injured afferents

So what changes occur in the nervous system after these traumatic nerve injuries? A common finding is that the injured afferents develop abnormal spontaneous activity. For injuries to distal nerves, spontaneous activity and ectopic sensitivity to heat and mechanical stimuli develops in C and A fiber afferents[2,3]. Following an SNL, spontaneous activity initially develops at the site of injury but eventually progresses to the dorsal root ganglion (DRG). Surprisingly, this spontaneous activity in injured afferents after SNL develops primarily in large myelinated fibers[4,5].

How is it that this spontaneous activity leads to the development of neuropathic pain? One model to explain neuropathic pain is what we call the injured afferents hypothesis. A simplified schematic of this model is illustrated in Fig. 1. After an injury to the L5 spinal nerve, the nerve distal to the injury site degenerates. The spontaneous activity that...
originates at the injury site and the DRG propagates to the spinal cord where it leads to the development of sensitization in dorsal horn pain signaling neurons. Touching the skin with von Frey probes leads to activation of low-threshold mechanoreceptors. Before injury, this neural activity leads to the perception of touch. The sensitization that occurs in the spinal cord as a result of the injury leads to an enhanced synaptic efficacy between low-threshold afferents and pain signaling neurons. Now, von Frey stimulation leads to activation of these neurons which accounts for the hyperalgesia.

There are a number of reservations about this injured afferent hypothesis. Several authors have demonstrated that most of the ongoing spontaneous activity in injured fibers following an SNL originates from large myelinated afferents and that many of these afferents may have originally innervated muscle\(^6,7\). However, ongoing activity in muscle afferents is physiological and does not produce pain, at least in normal animals. Another reason to question the role of spontaneous activity in large myelinated fibers comes from experiments investigating mechanisms of secondary hyperalgesia. It is thought that activity in C-fiber afferents is needed to produce central sensitization and to produce secondary hyperalgesia\(^8\). However, after an SNL, spontaneous activity does not appear to develop in the injured C-fiber afferents.

One way to examine the role of spontaneous activity from the injured root in the development of neuropathic pain is to cut the dorsal roots. Cutting the dorsal root would disconnect the source of spontaneous activity from the spinal cord. Thus, it should eliminate the central sensitization and lead to a reversal of the neuropathic behavior. The results of this experiment are controversial: in some papers, cutting the dorsal roots eliminated the neuropathic behavior\(^9\), whereas in others the neuropathic behavior did not decrease\(^10,11\). Regardless, it appears that ectopic activity from injured afferents is not a necessary requirement for the development of neuropathic pain behavior since a dorsal root ganglionectomy (where the injured afferents are removed) and a ventral rhizotomy (where the afferents are not directly injured) lead to neuropathic behavior\(^12,13\).

### 3 The uninjured afferents hypothesis

An alternative model to explain neuropathic pain is what we call the uninjured afferents hypothesis. A simplified schematic of this hypothesis is shown in Fig. 2. Cutting of the L5 spinal nerve leads to Wallerian degeneration of the axons distal to the cut and partial denervation of the tissue. Wallerian degeneration involves invasion of the nerves by macrophages and the release of cytokines and chemokines. Therefore, the adjacent, uninjured fibers are embedded in a pool of reaction products associated with degeneration. These reaction products could lead to a change in the properties of the uninjured axons in the L4 spinal nerve. In addition, the properties of the surviving nerve fibers in the partially denervated tissue could be altered for example due to the excess of growth factors.

One possibility is that the reaction products associated with Wallerian degeneration lead to spontaneous activity in adjacent, uninjured C-fiber afferents. We know, for
example, that exogenous administration of TNFα along the nerve leads to activity in nociceptive afferents. Spontaneous activity in uninjured C fibers could lead to the development of central sensitization. Now mechanical stimulation of the peripheral low-threshold mechanoreceptors would lead to pain. Another possibility is that the nociceptors in the partially denervated skin are sensitized to natural stimuli. We know, for example, that mRNA for NGF is upregulated in denervated skin, and that NGF leads to a sensitization of nociceptors. The sensitization after nerve injury could lead to an enhanced response of the uninjured nociceptors to mechanical stimuli which could account for the mechanical hyperalgesia.

We now have evidence that spontaneous activity develops in uninjured C-fibers of the L4 spinal nerve after an L5 lesion. In these experiments, we ligated and cut the L5 spinal nerve. At various times after this lesion, we recorded from the L4 spinal nerve (Fig. 3A). Therefore, we recorded from afferents that were not directly injured but were adjacent to those undergoing Wallerian degeneration. An electrode was placed on the sciatic nerve to identify and count the C fibers at the recording electrode. What we found was that the C fibers developed low grade spontaneous activity. Figure 3B is an example of one C fiber that exhibited 3 action potentials over the 5 min recording period. The fiber also responded to pinching of its receptive field and was classified as a nociceptor (Fig. 3C).

For the control animals, less than 10% of the C fibers exhibited spontaneous activity. However, within one day of the lesion almost half of the C fibers had developed spontaneous activity (Fig. 4). So although the rate of discharge was low, a large proportion of the C fibers exhibited spontaneous activity. The incidence of spontaneous

Fig. 2. Schematic diagram of uninjured afferents hypothesis for neuropathic pain.

Fig. 3. Spontaneous activity develops in uninjured C-fiber afferents in the L4 spinal nerve after an L5 spinal nerve injury. A: Action potential activity was recorded (R) from single C fibers in the L4 spinal nerve of the rat. A distal stimulating electrode (S1) on the sciatic nerve was used to identify and count the number of C fibers at the recording electrode. B: Example of low grade spontaneous activity in a C fiber. C: This fiber responded to pinching of the receptive field and was classified as a nociceptor. D: Electrical stimulation at S1 revealed that this fiber had a conduction velocity (CV) of 0.5 m/s. E: The electrical and mechanically evoked action potentials had the same shape as the spontaneous action potential. F: This fiber had a punctate receptive field (RF) on the leg. (Reproduced with permission from reference 17).
activity was still high one week after the lesion. The spontaneous activity appears to originate from the receptive field, since injection of 10 $\mu$L of lidocaine into the receptive field eliminated the spontaneous activity.

We wondered whether low rates of activity in C fibers are sufficient to produce central sensitization and mechanical hyperalgesia\cite{18}. To test this, we electrically stimulated the heel of normal rats with 120 pulses over a period of 10 min. We then tested their response to a 150 mN von Frey probe applied to the middle of the foot. In a blinded fashion, we electrically stimulated at an intensity to activate A-fibers or C-fibers. After electrical stimulation at C-fiber strength, the incidence of paw withdrawal increased to about 80% at 10 min after stimulation. In contrast, no increase was seen following A fiber stimulation. This provides evidence that low frequency activity in C fibers is able to produce mechanical hyperalgesia in rats.

We have recently determined that the conductive properties of uninjured C fibers also change after a L5 spinal nerve injury\cite{19}. We investigated the activity-dependent slowing of conduction that is characteristic of C fibers. In an in vitro preparation, the tibial nerve was electrically stimulated with 120 pulses over 2 min, and the action potential latency of single C fibers in the L4 spinal nerve of the rat was recorded. In normal animals, the action potential latency increases with repeated electrical stimulation. In the lesioned animals, this activity-dependent slowing was significantly enhanced. These results demonstrate that the conduction properties of uninjured C fibers in the L4 spinal nerve are altered following an L5 spinal nerve injury, and that the expressions of ion channels involved in action potential conduction are changed. Previously, it has been reported that the TTX-resistant sodium channel NaV1.8 is upregulated in peripheral axons of uninjured afferents\cite{20}.

There is evidence that uninjured C fiber nociceptors develop enhanced responsiveness to natural stimuli. For example, several authors have shown that uninjured nociceptors developed a responsiveness to catecholamines\cite{21,22}. In addition, Shim et al.\cite{23} has reported that uninjured C fiber nociceptors are sensitized to mechanical and heat stimuli.

In addition to these physiological changes, molecules related to nociception are upregulated in the L4 DRG neurons after an L5 spinal nerve ligation. For example, there is an increase in the expression of the neuropeptide CGRP, the transduction channels TRPV1 and TRPA1, and the growth factor BDNF\cite{24-28}. Upregulation of TRPV1 and TRPA1 may account for the hyperalgesia to heat and cold stimuli observed in spinal nerve lesioned animals.

In conclusion, the neural mechanisms of neuropathic pain are still incompletely understood. Although injured afferents certainly play a role, recent evidence indicates that the properties of uninjured afferents are greatly altered. Since the uninjured afferents survive in the peripheral tissues, they are potential targets for local therapies.

REFERENCES

7. Proske U, Iggo A, Luff AR. Mechanical sensitivity of regenerat-
ing myelinated skin and muscle afferents in the cat. Exp Brain Res 1995; 104: 89-98.