# Central Neuronal Changes After Nerve Injury: Neuroplastic Influences of Injury and Aging

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*Peripheral nerve injury produces a hyperexcitability of primary afferents and neurons in the spinal cord that is considered important in the development of nerve injury–induced pain. The authors recently developed a nerve injury model in the trigeminal region of the rat to study the neuronal mechanism of neuropathic pain in the trigeminal system. The escape thresholds to mechanical stimulation applied to the whisker pad area were significantly lower in rats with an inferior alveolar nerve (IAN) transection than those evoked from the contralateral, sham-operated whisker pad. Also, background activity and mechanically evoked responses in infraorbital nerve (ION) afferents and hyperpolarization-activated current (Ih) in trigeminal ganglion ION neurons were increased following IAN transection. Background activity and mechanically evoked responses of wide dynamic range (WDR) neurons in trigeminal subnucleus caudalis on the ipsilateral side relative to the transection were also significantly increased after the operation. A large number of cells expressed c-Fos-like immunoreactivity in the caudal medulla and upper cervical spinal cord following non-noxious mechanical stimulation of the faces of rats with IAN transection. The effect of aging on spinal dorsal horn neurons and the involvement of nerve injury in producing abnormal pain sensation in rats with advancing age were also studied. The incidence of licking behavior in response to noxious radiant heat stimulation of the hind paw was lower in the aged rats than in younger adults, but paw withdrawal latency was shorter and the activities of spinal dorsal horn neurons were higher in the aged rats. Furthermore, the descending inhibitory systems were impaired in the aged rats. These observations suggest that the changes in neuronal activity in the aged rats likely corresponded to the changes observed in the rat model of peripheral nerve injury.* J OROFAC PAIN 2004;18:293–298

**Key words:** aging, dorsal horn, medulla, neuropathic pain, trigeminal nerve

It has been reported that peripheral nerve injury produces an extensive increase in primary afferent activity.[1–3] Bennett and Xie developed a neuropathic pain model in which the sciatic nerve is chronically constricted with chromic gut (chronic constriction nerve injury, CCI).[4] The induction of spontaneous firing and increased evoked responses are typical phenomena in spinal nociceptive neurons following CCI.[1–5] In the trigeminal system, inferior alveolar nerve (IAN) transection also causes a high-frequency background activity in the injured nerve, and this hyperexcitability of the injured IAN is considered to be involved in nerve injury-induced allodynia in the trigeminal region.[6] The increase in nerve activity can also affect intact uninjured primary afferent

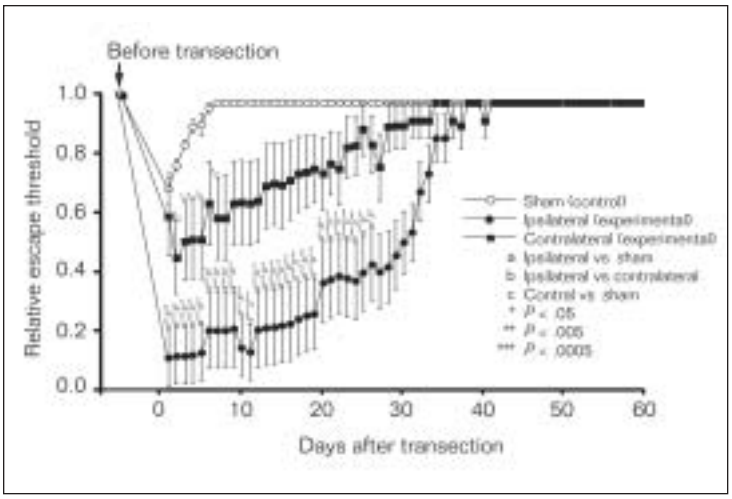


**Fig 1** Changes in the mechanical threshold intensity for eliciting escape behavior following IAN transection. The changes in threshold intensity were plotted as relative values, where escape threshold values were compared before transection and at different periods after IAN transection. A relative threshold of 1.0 was defined as the absence of a response to the highest stimulus intensity (40 g). Sham = sham-operated rats; contralateral = changes of escape threshold when mechanical stimulation was applied to the contralateral side relative to the IAN transection; ipsilateral = changes of escape threshold when the mechanical stimulation was applied to the ipsilateral side relative to the IAN transection (From Iwata et al).[15] Used with permission.

neurons.[7] This hyperexcitability of primary afferent neurons also contributes to the changes in protein synthesis in primary afferent ganglion neurons, such as nerve growth factor (NGF) and brain-derived nerve growth factor (BDNF).[8] The abnormal increase of peripheral nerve activity is thought to be conveyed to the central nervous system (CNS), resulting in an increase in the excitability of the second-order neurons and the development of nerve injury–induced pain. This, however, has not been studied in detail in the trigeminal brainstem nuclei.

Previous studies of age-related changes in morphology and conduction velocity of unmyelinated fibers and neuropeptides in spinal ganglion cells[9–12] suggest that aging also produces some changes in the peripheral nociceptive system. In addition, aging may be associated with reduced pain sensitivity in humans.[13] Iwata et al[14] reported that formalin stimulation of the face induces a larger number of c-Fos-positive neurons in the trigeminal spinal subnucleus caudalis (Vc) in the aged rats compared with adult rats. These data suggest that the central mechanism in nociception may change with advancing age. However, little is known about the response properties of neurons in nociceptive pathways in the aged CNS.

In order to evaluate the differences and similarities in the physiological properties of nociceptive neurons in nerve-injured rats and aged rats, a series of experiments were carried out to analyze the CNS neuronal activity in trigeminal nerve–injured rats and in aged rats. For these purposes, a series of experiments using a trigeminal nerve–injured model with transection of the IAN was developed.[15] Aged rats (more than 29 months old) were used in another series of experiments.[16] Both series involved behavioral observations, single neuron recording, and c-Fos labeling.

## Caudalis Neuronal Activity Following IAN Injury

It is known that IAN injury causes an extensive increase in activity in the uninjured infraorbital nerve (ION) as well as in injured afferents.[15] A rat model of trigeminal neuropathic pain in the ION region following IAN injury was developed. The effects of IAN transection on escape behavior evoked by mechanical stimulation applied to the whisker pad area innervated by the ION as well as on Vc neuronal activity in response to noxious and non-noxious stimulation of the face were analyzed.[15]

The escape thresholds were significantly lower than those evoked from the contralateral and sham-operated whisker pad from 1 day post-surgery until 28 days after the transection. They returned to the preoperative level by 40 days after transection (Fig 1). Wide dynamic range (WDR), nociceptive-specific (NS), and low-threshold mechanosensitive (LTM) neurons (n = 540) were recorded from the Vc of untreated naive rats, from the Vc of sham-operated rats with a skin incision, and from the ipsilateral and contralateral Vc of rats with an IAN transection. The response properties of NS neurons were not significantly affected by IAN transection. On the other hand, the background activity of WDR neurons on the side ipsilateral to the transection was significantly increased at 2 to 14 days after the operation compared to that of the naive rats. Innocuous and noxious mechanical stimuli-evoked responses of LTM and WDR neurons were significantly enhanced at

2 to 14 days after the IAN transection. The mean area of the receptive fields of WDR neurons was also significantly larger in the ipsilateral Vc at 2 to 7 days after transection than in naive rats. The changes in background and mechanically evoked activity generally appeared similar to those reported for "central sensitization,"[3,17] but modulation of noxious and non-noxious thermal responses of WDR and NS neurons following IAN transection was not observed.

The expression of c-Fos in the medulla and upper cervical spinal cord of rats with IAN transection was also studied. Fos protein–like immunoreactive (Fos protein-LI) cells were observed in the transition zone between trigeminal spinal subnuclei Vc and interpolaris (Vi/Vc) and in the transition zone between C1 and C2 (C1/C2 zone) after non-noxious mechanical stimulation of the whisker pad area in the rats with IAN transection. Fos protein-LI cells were found bilaterally in the Vi/Vc zone and ipsilaterally in the C1/C2 zone. The largest number of Fos protein-LI cells was observed in the Vi/Vc zone, both ipsilaterally and contralaterally. However, no significant change in Fos expression was observed in these 2 regions following thermal stimulation of the face. The number of Fos protein-LI cells increased after application of 1 g, 4 g, and 16 g stimuli as compared to rats without mechanical stimulation.

The present findings suggest that the increase in neuronal activity of WDR Vc neurons in the Vi/Vc and C1/C2 zones following IAN transection may play an important role in the development of the mechanical allodynia in a region adjacent to the area innervated by an injured nerve.

## ION Primary Afferent Neuronal Activity Following IAN Injury

The IAN transection model was also used for an analysis of ION primary afferent activity (see details in Tsuboi et al[18]). Single fiber recordings from the ION and patch clamp recordings from trigeminal ganglion (TRG) ION neurons were performed in rats with IAN transection to clarify the involvement of primary afferent neurons in abnormal sensations in the trigeminal region adjacent to an injured nerve.

The activity of 68 single ION fibers was recorded at 3 days after IAN transection. Evoked activities in C-fibers were not affected by IAN transection, whereas those in A-fibers were significantly affected by IAN transection; for example, increases in spontaneous activity and mechanically evoked responses were seen.

Since the A-fiber responses were significantly affected by IAN transection, patch clamp recording was performed in middle- to large-diameter TRG neurons. The $I_K$ (sustained) and $I_A$ (transient) currents were significantly smaller in TRG neurons of rats with IAN transection as compared to those of naive rats, while the hyperpolarization-activated current ($I_h$) was significantly larger. Furthermore, current injection into TRG neurons induced high-frequency spike discharges in the rats with IAN transection.

These data are supported by findings of alterations in uninjured afferents following injury of adjacent spinal afferents.[7,8] Furthermore, the data suggest that changes in potassium ($K^+$) current and $I_h$ observed after IAN transection in the uninjured TRG neurons reflect an increase in the excitability of TRG neurons innervated by the ION. This increase appears to result in the development of mechanical allodynia in the area adjacent to the injured neurons innervated by the IAN.[18]

## The Effect of Aging on Spinal Dorsal Horn Neurons

The aged rats exhibited a significantly shorter paw withdrawal latency to noxious thermal stimulation of the hind paw compared to young rats. On the other hand, paw licking was less frequent in aged animals compared with young ones.[16] The responses of single dorsal horn nociceptive neurons to 50°C and 54°C heating of the skin in the aged animals were significantly greater than those of young animals. The mean receptive field sizes of WDR and NS neurons in the aged animals were significantly larger for the high-threshold area but were smaller for the low-threshold area of WDR neurons compared to those of young animals. Background activity of WDR and NS neurons was significantly higher in aged animals than in young animals. High-frequency afterdischarges following noxious stimulation were also more common in the aged rats.

It is possible that changes in descending modulatory pathways contributed to the increased activity of dorsal horn neurons in aged rats. To assess this possibility, a reversible spinal block was produced by application of a local anesthetic onto the dorsal surface of the thoracic cord at a level (T1) that was rostral to the spinal dorsal horn recording site. In the young rats, neuronal responses to heat stimulation were significantly increased during the spinal block, as illustrated in Fig 2. These results indicate that the net effect of the descending system is inhibitory in young rats. The spinal block did not

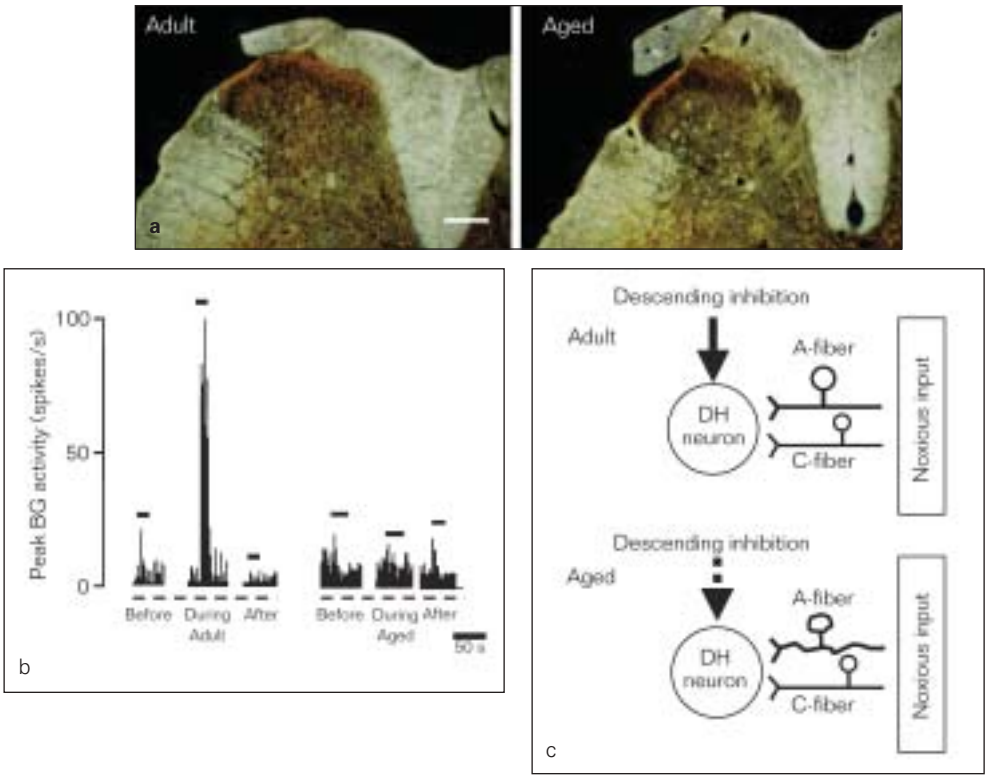


**Fig 2** *(a)* The 5-HT immunohistochemistry of the spinal dorsal horn in aged (32-month-old) and young adult (9-month-old) rats. Dense labeling with 5-HT antibody was observed in the dorsal horn of the younger adult rats, but such labeling was sparse in aged rats. Bar = 200 μm. *(b)* Responses of dorsal horn neurons after spinal block. Before = response of dorsal horn neuron during application of a 48°C stimulus before spinal block; during = response during application of a 48°C stimulus during spinal block; after = 5 min after heat stimulus application. BG = background activity. *(c)* Schematic illustration of the possible neural mechanisms (ie, reduced net descending inhibition) underlying the change in the dorsal horn in the aged rats. DH = dorsal horn.

have these effects in aged rats. Thus, there appears to be a reduced net descending inhibition (Fig 2c), or an increased descending facilitation, in aged rats. Background activity was significantly increased in both aged and young animals during the spinal block. Afterdischarges following noxious stimulation were significantly increased during the spinal block in young rats.

Since these descending pathways act in part through the release of 5-hydroxy tryptamine (5-HT) and dopamine, further comparisons were made between the immunohistochemical distribution of 5-HT and dopamine β-hydroxylase (DBH), an essential enzyme for norepinephrine synthesis, in the spinal cord of the aged and young rats. A large number of 5-HT and DBH-immunoreactive (ir) fibers were observed in both superficial laminae (laminae I and II) and deep laminae (laminae III and IV) of the dorsal horn of the young animals. Such fibers were much sparser in aged animals (Fig 2a). The total area occupied by 5-HT-ir fibers was significantly smaller in the aged animals than in the young rats. The areas occupied by DBH-ir fibers were significantly smaller in laminae I and II of the aged rats than in the comparable laminae of the young rats.

## Concluding Remarks

ION activity was significantly increased after IAN transection, which may account for the associated increase in Vc activity. As noted and as illustrated in Fig 3, the activity of the large-diameter myelinated nerve fibers in the ION was increased following IAN transection. The I*h* is an important current induced in the uninjured TRG ION neurons following IAN transection. Thus, IAN transection enhanced the ability for spike potentials to be generated in the uninjured afferent neurons. It has been suggested in

an analysis of extraterritorial hyperalgesia using the CCI model that cross-talk may occur between injured and uninjured afferents, with an injury-induced barrage of action potentials in injured afferents, causing activation of adjacent uninjured afferents.[7] The sprouting of primary afferent terminals in the CNS has been considered an alternative mechanism for the injury-related increase in the neuronal activity of second-order neurons.[19] Experiments were conducted using cholera toxin subunit B tracer in order to determine whether IAN injury causes central sprouting in Vc (data not shown), but no sprouting of the injured IAN was apparent in Vc.

It has been reported that primary afferent C-fibers are not affected by age, whereas A-fibers are impaired with advancing age.[10–12] The authors of the present study have demonstrated that aging also causes an extensive increment of neuronal activity in the spinal dorsal horn. Furthermore, descending modulatory systems were significantly impaired in the aged rats. A loss of descending inhibitory effects in aged animals would upset the dynamic balance in central nociceptive pathways after injury and lead to a relative absence of descending inhibition and greater enhancement of spinal dorsal horn excitability, as demonstrated in the present study (see Fig 2c). An imbalance of these modulatory systems may also be a mechanism contributing to both acute and chronic pain conditions, especially those involving deep tissues such as muscle and viscera.

It is possible that peripheral nerve injury had occurred in the aged rats. Such injury might be analogous to the nerve-damage model, which also is associated with an increase in neuronal activity. On the other hand, the mechanisms underlying the central changes may be different between the IAN-transected rats and aged rats. For example, a decrease in GABAergic inhibitory interneurons may occur following peripheral nerve injury,[20] and this could also contribute to the increase in second-order neuronal activity following IAN injury.

Finally, the authors have demonstrated many physiologic and morphologic changes in rats with a peripheral nerve injury and in aged rats. The damage of the peripheral nerve and the associated changes in nociceptive pathways could be involved in the abnormal pain sensations that may be seen in elderly people[13] and in peripheral nerve-injured patients.[3,6]

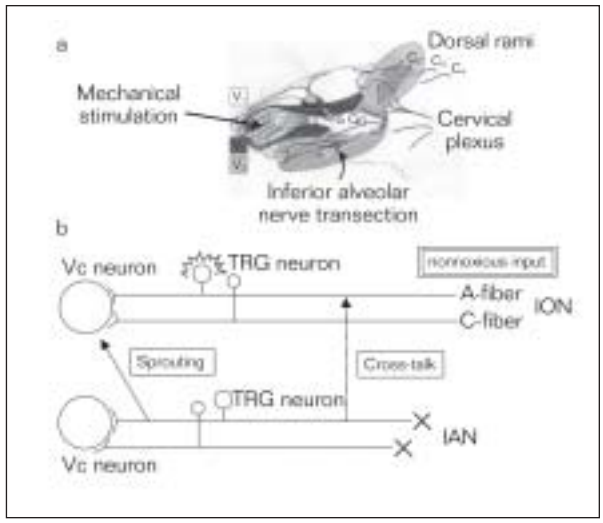


**Fig 3** *(a)* Schematic illustration of the IAN transection model and *(b)* possible mechanisms underlying the abnormal activity of the primary and second-order neurons.

## Acknowledgments

This study was supported in part by research grants from the Sato and Uemura Funds of Nihon University School of Dentistry, Japan; a grant for promotion of multidisciplinary research projects from the Ministry of Education, Science and Culture, Sports, Science, and Technology, Japan; and a grant-in-aid for scientific research (14571761) from the Ministry of Education, Science and Culture, Sports, Science, and Technology, Japan.

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