The expression of deafferentation dysesthesias reduced by dorsal root entry zone lesions in the rat

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Extensive longitudinal lesions of the dorsal root entry zone (DREZ) are effective in relieving some chronic deafferentation pain in humans. A deafferentation syndrome follows C5-T2 dorsal root ganglionectomies in rats. The syndrome consists of biting and scratching the completely and partially denervated limb areas, respectively.

This study examines the effect of DREZ lesions on the deafferentation syndrome in the rat. Of 37 rats, 24 underwent C5-T2 ganglionectomies only, five received C4-T3 micromechanical DREZ lesions only, and eight underwent ganglionectomies plus simultaneous DREZ lesions. The animals were observed for 45 days postoperatively. Histological analysis of the spinal cord lesions was performed.

All rats with ganglionectomies alone exhibited the deafferentation syndrome; however, no rats with DREZ lesions alone showed this feature. Only 25% of rats with combined ganglionectomies and DREZ lesions exhibited the deafferentation syndrome in the first 30 days, whereas 80% of the animals with ganglionectomies only did so. Although 75% of the animals with combined lesions eventually bit the insensitive forepaw, this behavior was significantly attenuated: the day of onset was delayed and the extent of self-mutilation was reduced. Postmortem histological examination of the DREZ lesions indicated a close association between the completeness of the dorsal horn destruction and the reduction or prevention of self-mutilation.

These data support the validity of the animal model and also the hypothesis stating that the deafferentation syndrome results from abnormal spontaneous neural activity in the dorsal horn. Moreover, the variability of the histological findings in these experiments stresses the importance of making contiguous and complete dorsal horn lesions in human DREZ surgery.

KEY WORDS  •  ganglionectomy  •  deafferentation syndrome  •  dorsal root entry zone  •  pain  •  rat

CHRONIC deafferentation pain can result from loss of primary afferent input to the central nervous system. Patients with these disorders present one of the most challenging pain management problems confronting health-care professionals. Until relatively recently, there has been little to offer these patients. Medical treatment is largely limited to antidepressant, sedative, or anticonvulsant medication, or psychological counseling. Prior to the dorsal root entry zone (DREZ) operation, surgical therapy often employed other neurodestructive, but eventually ineffective procedures.

In 1976, Nashold, et al., reported an operation performed on a patient with brachial plexus avulsion and deafferentation pain. The procedure presumably destroyed the dorsal horn (laminae I to V of Rexed) of the spinal cord over the several segments of nerve root avulsion. The hypothesis behind this operation was that when neurons in the dorsal horn, normally involved in the signaling of pain, are deprived of their usual afferent input, they function in a pathological manner. The rostral transmission of abnormal neuronal activity (altered firing pattern) is experienced by the patient as pain. Thus, destroying these pathophysiological neurons relieves the discomfort. Since their initial successful DREZ procedure, Nashold and colleagues, as well as several other groups, have obtained good results with the use of the DREZ operation for brachial plexus avulsion pain and for a variety of other deafferentation pain syndromes.

Several models of chronic deafferentation pain have been developed in animals in an attempt to better understand this type of pain in man. In these animal models, the sensory input is experimentally decreased or abolished, either by peripheral nerve transection, dorsal root avulsions, rhizotomies, or ganglionectomies. The deafferentation syndrome produced in rats by all of these procedures consists of scratching the partially
DREZ lesions for deafferentation dysesthesias

### TABLE 1
Grading scale for self-inflicted wounds*

<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>removal of one or more nails</td>
</tr>
<tr>
<td>1–5</td>
<td>removal of each distal half digit</td>
</tr>
<tr>
<td>1–5</td>
<td>removal of each proximal half digit</td>
</tr>
<tr>
<td>1</td>
<td>removal of hair anywhere on limb or shoulder</td>
</tr>
<tr>
<td>2</td>
<td>damage to skin anywhere other than digits</td>
</tr>
<tr>
<td>2</td>
<td>muscle exposed</td>
</tr>
<tr>
<td>3</td>
<td>muscle damage</td>
</tr>
</tbody>
</table>

* A maximum of 19 points is possible.

innervated and biting the anesthetic portion of the limb, sometimes to the point of amputation (autotomy). Human cases of similar behavior associated with brachial plexus avulsion have been recently described and successfully treated by DREZ surgery. We and others believe these behaviors to be objective evidence of spontaneous dysesthesias. Ganglionectomies may serve as an experimental model to study certain aspects of chronic dysesthesias and/or pain caused by deafferentation in man. Ganglionectomies do not induce traumatic injury to the spinal cord (as do root avulsions), and they eliminate a possible role for ventral root or sympathetic afferents as might exist with dorsal rhizotomies or peripheral nerve injury. Painful dysesthesias in man have been described following ganglionectomies.

This study examines the effect of DREZ lesions on the deafferentation syndrome following ganglionectomies in the rat. It thereby assesses the validity of this animal model and also tests the hypothesis that the deafferentation syndrome results from abnormal spontaneous activity in the dorsal horn.

**Materials and Methods**

**Laboratory Preparation**

A total of 37 Sprague-Dawley rats underwent either right C5–T2 dorsal root ganglionectomies only (24 rats), right C4–T3 micromechanical DREZ lesions only (five rats), or combined right dorsal root ganglionectomies plus simultaneous right DREZ lesions (eight rats). The animals were anesthetized with intraperitoneal ketamine hydrochloride (90 mg/kg) prior to surgery. This dose was sufficient to produce profound anesthesia within minutes, which was maintained with supplemental doses (20 mg/kg).

Under aseptic conditions, right hemilaminectomies were performed from C-4 to T-3 utilizing a dissecting microscope. Microinstruments were used to remove the ganglia (C5–T2) extradurally. In the animals undergoing DREZ surgery, the dura was opened over the DREZ and subpial contiguous dorsal horn lesions (C4–T3) were made longitudinally using a microneedle with a 1-mm angled tip. This mechanical technique was employed because no sufficiently small radiofrequency electrode was available. The dorsal root entry zone provided a visible landmark. All rats were housed individually. The animals were examined daily postoperatively to assess areas of peripheral insensitivity. Sensory examination tested responsiveness to light touch and pinprick.

**Neurological Evaluation**

Through daily observations, the onset of biting was noted, and the self-inflicted wounds were assessed using a grading scale that assigned a numerical value to the extent of biting (Table 1). The grading scale ranged from 0 to 19, with 19 representing the most extensive biting. We have used this grading scale in various other publications. In this experiment, the rats were observed for 45 days after surgery. The onset of biting was recorded and a deafferentation syndrome score was assigned at Days 30 and 45 postoperatively.

**Animal Care**

The animals were observed by the Duke University Institutional Animal Care and Use Committee after the deafferentation procedure and were found to meet the National Institutes of Health guidelines for care of experimental animals. The rats exhibited normal sleep-wake cycles and gained weight. There was no piloerection, pupillary dilation, or any other evidence of discomfort except for the biting behavior. Animals were given antibiotic medications to prevent wound infections and, in fact, no infections occurred. The rats were sacrificed immediately upon reaching an autotomy score of 19 or after postoperative Day 45.

**Histological Examination**

The spinal cords were removed and placed in buffered 10% formalin and embedded in paraffin; 10-μm sections were taken at 200-μm intervals throughout the deafferented and/or lesioned region. Sections were stained with cresyl violet and Luxol fast blue. The slides were then examined microscopically and the lesions were reconstructed by camera lucida. Lesions included areas of neuron cell body loss, dense gliosis, and demyelination.

**Statistical Analyses**

The behavioral data were analyzed using Statworks software on a Macintosh II computer and significance levels were determined by reference to published statistical tables. Chi-squared, Mann-Whitney U, t-test, analysis of variance (ANOVA), Kolmogorov-Smirnov, and Kruskal-Wallis testing were employed as appropriate.

**Results**

All rats with ganglionectomies only exhibited the deafferentation syndrome by 45 days, whereas none of the rats with DREZ lesions only did so. Although 75% of the rats receiving combined ganglionectomies and DREZ lesions eventually bit the forepaw, this behavior was much reduced compared to the control group. Table 2 shows that the onset of biting behavior was significantly delayed in the animals that underwent ganglionectomies plus simultaneous DREZ lesions.
TABLE 2
Onset and degree of biting behavior in rat groups*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Rats</th>
<th>Biting Onset (days)</th>
<th>Day 30</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>ganglionectomies only</td>
<td>24</td>
<td>15.7 ± 2.8</td>
<td>4.75 ± 1.1</td>
<td>8.08 ± 1.2</td>
</tr>
<tr>
<td>ganglionectomies &amp; DREZ lesions</td>
<td>8</td>
<td>29.8 ± 3.8†</td>
<td>0.25 ± 0.16†</td>
<td>2.5 ± 9.4†</td>
</tr>
<tr>
<td>DREZ lesions only</td>
<td>5</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Means are expressed ± error of the means. For a definition of the biting score see Table 1. DREZ = dorsal root entry zone; NA = not applicable.
† Statistically significant, see text.

(29.8 ± 3.8 days) as compared to rats undergoing ganglionectomies only (15.7 ± 2.8 days). The onset of self-mutilation was indexed by the number of days between the spinal cord surgery and the first occurrence of biting. Since this was deemed to be interval data, one-way ANOVA was performed on the day of onset. Data from the groups showed the results to be significantly different (p = 0.002). Based on this finding, t-tests were performed on pairs of group means. The group with ganglionectomies and the group with combined ganglionectomies plus DREZ lesions differed at p = 0.027 (only six rats in the combined-lesion group bit).

The extent of biting was also significantly decreased in the animals undergoing ganglionectomies plus simultaneous DREZ lesions, both on Day 30 and Day 45 postoperatively. On Day 30, the animals undergoing the combined procedure had a deafferentation syndrome score of 0.25 ± 0.16 points compared with 4.75 ± 1.1 points for the rats with ganglionectomies only. On Day 45, this significant difference persisted (2.5 ± 9.4 points vs. 8.08 ± 1.2 points). Since the extent of biting was deemed to be ordinal data, the Kruskal-Wallis ANOVA was used to examine the degree of biting for the various groups on postoperative Days 30 and 45. Data from the three groups were found to be significantly different with respect to extent of biting on both postoperative Days 30 and 45 (p = 0.001). Based on this result, the Kolmogorov-Smirnov test was performed on different pairs of deafferentation syndrome score means. The scores for the groups receiving DREZ lesions only or undergoing the combined lesions were significantly different from that for the group with ganglionectomies only (p < 0.05).

By Day 30, 19 (79%) of the 24 rats with ganglionectomies only had bitten as compared to only two (25%) of the eight in the combined-lesion group. On Day 45, 100% of the animals with ganglionectomies only had bitten while only six (75%) of the eight in the combined-lesion group had done so. These differences were significant (p < 0.05, chi-squared test) at both Days 30 and 45 postoperatively.

Self-mutilation in the group with ganglionectomies only typically occurred within the C5-T2 dermatomes. Four of the six animals that bit in the combined-lesion group (scores of 8, 4, 3, and 3 points, Table 1) preferentially mutilated the C8-T2 dermatomes. There was histological evidence of marked sparing of the dorsal horn at the C8–T3 spinal segments in these four animals. The other two animals that bit in this combined-lesion group exhibited only minimal self-mutilation, removing only one nail each.

In addition, the two combined-lesion animals with no biting and the two that bit only one nail had spinal lesions that virtually involved the entire dorsal horn from at least C-5 to T-2 (Fig. 1 left). However, the combined-lesion animal with the highest biting score showed substantial sparing of some dorsal horn areas between C-5 and T-2 (Fig. 1 right). There was no histological evidence of spinal cord damage in the animals with ganglionectomies only, but the dorsal column was atrophied on the deafferented side.

![Fig. 1. Spinal cord sections at C-5 of two rats that underwent combined right dorsal root ganglionectomies and simultaneous right dorsal root entry zone lesions. Left: This rat exhibited minimal biting behavior. A complete lesion of the right dorsal horn is seen at this level. Right: This rat exhibited the most severe biting behavior. There is sparing of the right dorsal horn at this level.](image-url)
DREZ lesions for deafferentation dysesthesias

The deafferented limb was not truly paralyzed in any of the animals although hypotonic paresis was present. Animals in all three groups exhibited peripheral sensory loss in the distal forelimb postoperatively. The peripheral sensory loss in the forelimb in the two rats in the combined-procedure group that never self-mutilated was not noticeably different from the loss that was apparent in the rats receiving ganglionectomies only.

Discussion

The data presented here suggest that extensive lesions of the dorsal horn, made simultaneously with cervicothoracic ganglionectomies, reduce the expression of the deafferentation syndrome in the rat, as measured by reduced incidence, delayed onset, and decreased extent of self-mutilation.

Our results support the premise that self-mutilation reflects spontaneous deafferentation dysesthesias which have their pathophysiological cause in the dorsal horn.5 Self-mutilation is not simply a response to sensory loss, since adding the DREZ lesions does not diminish the degree of sensory loss, although it significantly alleviates the deafferentation syndrome. Moreover, because the affected limb is not paralyzed, the deafferentation syndrome is not due to limb immobility.29 Similar conclusions were reached earlier by Basbaum1 concerning the effects of spinal lesions on the deafferentation syndrome of dorsal rhizomatoses.

The mechanism of deafferentation pain and self-mutilation is not well understood. A prevailing view is that extensive deafferentation of dorsal horn nociceptive transmission neurons produces hyperactivity and hypersensitivity of the neurons.10,22 Some studies have found abnormally increased spontaneous discharge or altered somatotopic organization in dorsal horn neurons after deafferenting lesions.11,12,16,22 Another hypothesis is that, after deafferentation, the dorsal horn nociceptive neurons are released from strong central inhibition. Some experiments seem to indicate that descending inhibition is diminished after dorsal horn deafferentation.8,24,38 Finally, it is possible that deafferentation leads to a fundamental change in dorsal horn neurochemistry. There is evidence for a denervation supersensitivity to substance P.15,40

Our experimental results also emphasize the importance of making contiguous and complete dorsal horn lesions in human DREZ surgery. Friedman, et al.6 found that patients with brachial plexus avulsion who were treated with a relatively small number of large radiofrequency lesions had a lower incidence of long-term pain relief than patients treated with a greater number of smaller contiguous radiofrequency lesions.

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