

Injury Prior to Neurectomy Alters the Pattern of Autotomy in Rats

Behavioral Evidence of Central Neural Plasticity

Joel Katz, Ph.D.,* Anthony L. Vaccarino, Ph.D.,† Terence J. Coderre, Ph.D.,‡ Ronald Melzack, Ph.D.§

A common property of phantom limb pain is that a preamputation lesion continues to be felt in the same location of the phantom limb after amputation. A model of the phantom limb in the rat is provided by sectioning the sciatic and saphenous nerves. This procedure leads to self-mutilation of the denervated hindpaw, a behavior known as autotomy. There is strong evidence that autotomy is a response to painful or dysesthetic sensations referred to the anesthetic limb. The present study examined the hypothesis that the site of autotomy behavior can be altered by an injury given prior to denervation. Experiment 1 evaluated the effects of a thermal injury applied (under sodium pentobarbital anesthesia) to the medial or lateral hindpaw and digits before or after sciatic and saphenous nerve transections in order to determine whether autotomy is directed specifically to a previously injured site. The results revealed that autotomy onset occurred in the injured region of the paw in a significantly greater proportion of rats, compared to an uninjured control group, if the thermal injury had been induced *before* denervation: 100% of the rats with medial paw injury induced prior to neurectomy initiated autotomy in the medial digits, and 55.6% of rats with lateral paw injury initiated autotomy in the lateral digits. Rats injured after autotomy showed no such preference (medial, 33.3% and lateral, 37.5%) relative to the uninjured controls (medial, 33.3% and lateral, 17%). These results suggest that central cells, sensitized by the thermal injury, contribute to enhanced autotomy in the absence of further inputs from the injured paw. However, rats injured after neurectomy displayed enhanced autotomy compared to uninjured controls, suggesting that peripheral, injury-related factors may also contribute

to the autotomy among injured animals. In order to minimize the contribution of peripheral factors produced by cutaneous injury, experiment 2 examined the effects, on autotomy behavior, of noxious electrical or mechanical stimulation of the sciatic nerve (under sodium pentobarbital anesthesia) prior to neurectomy. Electrical stimulation of the sciatic nerve prior to denervation altered the pattern of self-mutilation compared to control rats. These experiments suggest that the alteration in the site of autotomy onset by a prior injury depends in part on a sensory memory mechanism in the central nervous system that is sustained in the absence of further inputs from the injured region and that long outlasts the duration of noxious stimulation. The results imply that a preoperative anesthetic block of the relevant peripheral nerves and/or spinal cord cells involved in nociceptive transmission would decrease the intensity of post-operative pain and reduce the incidence of chronic pain syndromes such as phantom limb pain. (Key words: Autotomy. Deafferentation. Memory. Pain: phantom limb. Plasticity. Sensitization. Spinal cord.)

A GROWING BODY of clinical and laboratory data shows that injury produces a prolonged change in central nervous system (CNS) function that influences responses to subsequent somatosensory inputs. The data strongly suggest that this injury-induced "neuroplasticity" may contribute to the experience of pain long after the offending stimulus has been removed or the injury has healed. Models have been developed to examine, in rats, the long-term central physiologic and behavioral effects of brief noxious inputs.¹⁻⁵ High-intensity electrical stimulation of afferent nerve fibers or injury to nociceptors in skin has been found to induce neural and behavioral changes that persist even when inputs from the injured region are later blocked by local anesthesia^{1,6} or interrupted by nerve section³⁻⁵ or dorsal rhizotomy.⁷ Simply cutting afferent nerve fibers in the absence of a prior noxious conditioning stimulus produces a long-term facilitation in spinal cord cells that persists in the absence of sustaining inputs from the transected nerve after the central cut end has been immersed in a local anesthetic solution.⁸ Moreover, bathing peripheral nerves in a local anesthetic prior to nerve transection reduces the incidence of behaviors indicative of pain in the weeks after the neurectomies.⁹ These animal studies indicate that brief noxious inputs are capable of producing changes in the excitability of spinal cord cells and in behaviors indicative of pain, long after the offending stimulus has been removed.

Perhaps the most striking clinical evidence of injury-induced central neuroplasticity in humans comes from studies of amputees who report phantom limb pain, which

* Assistant Professor, Department of Behavioural Science, University of Toronto; MRC Fellow, Department of Psychology, The Toronto Hospital, Toronto General Division.

† NSERC Fellow, Department of Psychology, University of California, Los Angeles.

‡ Director, Pain Mechanisms Laboratory, Clinical Research Institute of Montreal; Chercheur Adjoint, Department of Medicine, University of Montreal; Associate Member, Department of Psychology, McGill University.

§ Professor, Department of Psychology, McGill University.

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Address reprint requests to Dr. Katz: Department of Psychology, The Toronto Hospital, Toronto General Division, 200 Elizabeth Street, CW2-306, Toronto, Ontario, Canada, M5G 2C4.

resembles a pain experienced in the limb before amputation.¹⁰⁻¹² For example, amputees may report the sensation of a painful preamputation ulcer on the phantom foot or the burning pain of gangrene that was present at the time of amputation. These somatosensory pain "memories" are not merely cognitive recollections but are direct experiences of pain that are referred to the phantom limb in the same location and with the same qualities of sensation as the past pain.¹⁰ A recent review¹⁰ indicates that between 12.5 and 79% of amputees report similar pains before and after amputation. Although more than 50 types of therapy are used to treat phantom limb pain, only 7% of patients receive a significant degree of pain relief.¹³⁻¹⁵ Furthermore, the pain persists so that 60% of amputees have phantom limb pain as long as 7 yr after amputation.¹⁶ The protracted course of phantom limb pain indicates that it is affected by long-lasting changes in CNS function.

A rodent model of deafferentation pain has been developed in which peripheral neurectomy or dorsal rhizotomy is followed by self-mutilation behavior termed "autotomy."¹⁷ The animal bites and scratches the distal portions of the insensitive paw to the point of amputation. There is strong evidence¹⁸ that autotomy is a response to painful or dysesthetic sensations referred to the denervated limb and represents a behavioral model of phantom limb pain or anesthesia dolorosa.¹⁹ This model has since been used to explore the effects of a prior injury on the subsequent development of pain referred to the anesthetic limb. Several studies have shown that chemical^{7,20} or thermal³⁻⁵ injury of the paw prior to deafferentation increases the severity of autotomy or leads to a shift in the site of self-mutilation. Since all sensory input from the injured paw is eliminated as a consequence of deafferentation, the enhanced autotomy has been attributed to increased pain due to the sensitization of central cells by the earlier injury, thus reflecting a change in central neural function that long outlasts the duration of injury.

However, peripheral factors also may contribute to the enhanced autotomy observed in these animal studies. Tissue damage, inflammation, and the local release or production of toxic chemicals (histamine, bradykinin, prostaglandins, or substance P) at the site of injury may provide olfactory, visual, or gustatory cues directing animals to lick, scratch, or bite at the injured and anesthetic site. Only two studies have controlled for this possibility, by injuring the paw after denervation.^{3,7} In one of these studies,³ injury of the paw before, but not after, neurectomy enhanced autotomy behavior. In the other study,⁷ both pre- and postdenervation injuries enhanced autotomy behavior. That injury of a limb after deafferentation also increases autotomy in some instances suggests that peripheral factors may also contribute to the enhanced autotomy among injured animals.⁷

The present study examined the effects of two types of injury on the pattern and severity of autotomy in an attempt to model the descriptions of human amputees who report the persistence of a preamputation pain referred to a specific location of the phantom limb. Experiment 1 evaluated the effects of a thermal injury applied to the medial or lateral hindpaw and digits either before or after sciatic and saphenous nerve transections, thus examining the possibility that autotomy may be directed specifically to a previously injured site. Experiment 2 examined the effects of noxious electrical or mechanical stimulation applied directly to the sciatic nerve prior to neurectomy, thereby minimizing the contribution of peripheral factors since the procedure does not produce a cutaneous injury.

Materials and Methods

SUBJECTS AND HOUSING

Experiments were approved by the McGill University Animal Care Committee. The subjects were 91 male hooded Long-Evans rats weighing 300–500 g at the time of surgery. The rats were housed individually, had access to food and water at all times, and were maintained on a 12-h light cycle.

NERVE SURGERY

Peripheral neurectomy was performed under sodium pentobarbital (Nembutal, Abbott) anesthesia (65 mg/kg) administered by intraperitoneal injection. Anesthesia with this dose of pentobarbital lasted approximately 2 h. The sciatic and saphenous nerves of the hindpaw were dissected free at midhigh level, ligated, and cut distal to the ligation. Approximately 5 mm of the distal end of the nerve was removed to prevent reinnervation. The wound was then sutured and treated with a topical antibiotic (Furacin) to prevent infection. Rats were placed on a heating pad and warmed with a heat lamp until recovery.

AUTOTOMY SCORING

Starting the day after surgery, the rats were examined and scored daily for signs of autotomy using a scale developed by Wall *et al.*¹⁷ A score of 1 point was assigned for the removal of one or more nails, an additional point for injury or removal of each distal half-digit, another point for each proximal half-digit, and a point each for the distal and proximal halves of the hindpaw (maximum autotomy score = 13). The degree (autotomy score) and the site(s) of self-mutilation were recorded each day beginning the day after surgery. To minimize stress and discomfort, rats were killed (by an overdose of sodium pentobarbital) 4 days after the day of autotomy onset (*i.e.*,

on day 5) or when a score of 11 or greater was reached. Rats that did not exhibit autotomy were killed after 56 days.

EXPERIMENT 1

Treatment

Forty-one rats were randomly assigned to one of five treatment groups. While anesthetized with sodium pentobarbital (65 mg/kg), two groups of rats received a thermal injury of selected regions of the hindpaw 30 min before sciatic and saphenous nerve transections. Either the medial two digits (D1 and D2) and medial half of the hindpaw ($n = 7$) or the lateral two digits (D4 and D5) and lateral half of the hindpaw ($n = 9$) were immersed in 55° C water for 25 s. To control for the peripheral effects of the injury, two additional groups of rats received the same treatment of either the medial ($n = 7$) or lateral ($n = 9$) digits and hindpaw immediately after denervation (fig. 1). A fifth group of rats ($n = 9$), whose treatment involved only sciatic and saphenous nerve transections, served as uninjured controls.

To ensure that the thermal injury was produced at a water temperature of 55° C, water was heated to 60° C in a 10-l thermal bath, and for each rat, 0.5 l was transferred to a beaker where the temperature was monitored until it had dropped by 5° C, at which point the preselected regions of the paw were carefully immersed. Non-selected digits and hindpaw regions were covered with masking tape as a precaution against accidental immersion

(which never occurred). Thermal injury of this sort produces a reddening and inflammation of the skin but no signs of blister, tissue necrosis, or scarring.

Site of Autotomy Onset

Three categories (medial, lateral, and other) were identified for the purpose of scoring the hindpaw region(s) at which each rat first exhibited signs of autotomy (site of autotomy onset). Rats that initiated autotomy within 1) D1, D2, or the medial half of the hindpaw were classified as "medial," 2) D4, D5, or the lateral half of the hindpaw were classified as lateral, and 3) D3 or any combination of two categories (e.g., medial and lateral) were classified as "other" (fig. 1).

EXPERIMENT 2

Treatment

Fifty rats were randomly assigned to one of two treatment groups or two control groups. While under sodium pentobarbital (65 mg/kg), rats received either electrical or mechanical stimulation of the sciatic nerve before the sciatic and saphenous nerves were sectioned. In the electrical-stimulation group ($n = 15$), the exposed nerve was placed on electrodes and stimulated at C-fiber strength^{6,8} (5 mA, 5 ms, 2 Hz) for 30 s (Grass S-88 stimulator, oscilloscope, and PSIU-6 isolation unit). In the mechanical stimulation group ($n = 10$), the exposed nerve was lifted and gently tugged with the stimulating electrodes for 30

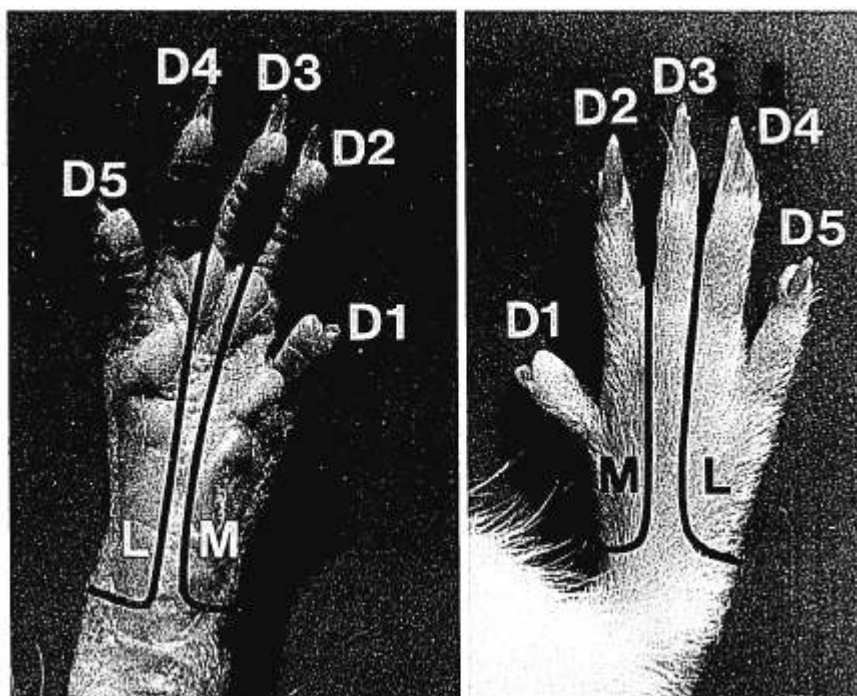


FIG. 1. Plantar (left) and dorsal (right) views of the right hindpaw of a rat showing the regions that were immersed in 55° C water for 25 s to produce a thermal injury. D1–D5 = digit 1 through digit 5; M and L = to medial and lateral aspects of the hindpaw, respectively. Rats received a thermal injury of the medial two digits (D1 and D2) and hindpaw or the lateral two digits (D4 and D5) and hindpaw before or after sciatic and saphenous nerve transections. See text for details of the system of scoring the degree and site of autotomy onset.

s, but current was not delivered. One control group (n = 15) had the sciatic and saphenous nerves transected after careful placement of the sciatic nerve on the stimulating electrodes, which were neither activated nor moved. A segment of the exposed sciatic nerve had been locally anesthetized prior to electrode placement in this control group in order to minimize input from mechanical stimulation associated with electrode placement. Local nerve anesthesia was produced by wrapping cotton pledgets soaked in 0.5% lidocaine around the nerve for 10 min prior to electrode placement. Nerve sections were performed proximal to the site of anesthesia. A second control group (n = 10), whose treatment involved only sciatic and saphenous nerve transections, served as uninjured controls. Animals were examined daily for signs of autotomy.

DATA ANALYSIS

The site of autotomy onset was analyzed by a chi-squared (χ^2) test of significance for two-way tables to determine whether the groups differed significantly in the observed frequency of rats that initiated autotomy in various regions of the paw. The severity of the self-mutilative behavior on day 5 after autotomy onset was assessed by a χ^2 test of significance for two-way tables comparing the frequency of rats in each group having a score of less than 3 with the frequency having a score of 3 or more. Autotomy onset latencies were submitted to a one-way, independent samples analysis of variance comparing the groups. This was followed by multiple comparisons (Newman-Keuls) if the group main effect reached statistical significance. χ^2 tests with 1 degree of freedom were evaluated using Yates's²¹ continuity correction. $P < 0.05$ was considered significant for all statistical tests.

Results

EXPERIMENT 1

The frequency of rats with autotomy scores above criterion (≥ 3) on day 5 is shown in table 1 for animals injured either before or after neurectomy (combined medial and lateral groups) and for uninjured controls. The

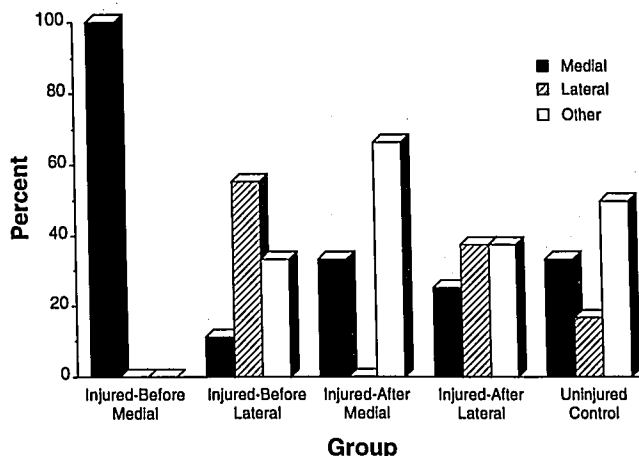


FIG. 2. Site of autotomy onset for the uninjured control group and for rats with thermal injuries of the medial or lateral hindpaw (and digits) before or after sciatic and saphenous nerve transections. Chi-squared test for two-way tables demonstrated a significant ($P < 0.002$) difference among the five groups.

presence of an injury, whether induced before or after neurectomy, led to a significant increase in the number of rats showing high autotomy scores when compared to uninjured controls ($\chi^2(2) = 7.79, P < 0.02$).

The site of autotomy onset differed significantly depending upon whether the injury was induced before or after nerve section ($\chi^2(8) = 19.2, P < 0.002$). Figure 2 shows that 100% of the rats with medial paw injury induced prior to neurectomy initiated autotomy in the medial digits and that 55.6% of rats with lateral paw injury initiated autotomy in the lateral digits. Rats injured after neurectomy showed no such preference (medial, 33.3%; lateral, 37.5%) relative to uninjured controls (medial, 33.3%; lateral, 17%).

The mean number of days to autotomy onset was calculated for rats injured either before or after neurectomy (combined medial and lateral groups) and for uninjured controls (table 1). The analysis of variance revealed a significant main effect ($F(2,38) = 3.59, P < 0.04$) for the group factor, and subsequent *post hoc* multiple comparisons indicated that the latency to autotomy onset was significantly earlier both for rats injured before neurectomy

TABLE 1. Number of Days to Autotomy Onset and Percentage of Rats with Autotomy Scores above Criterion (≥ 3) on Day 5 for Animals Who Received a Thermal Injury of the Hindpaw before or after Sciatic and Saphenous Nerve Transections and for Uninjured Controls

	Treatment Group			P
	Injured Before Denervation (n = 16)	Injured After Denervation (n = 16)	Uninjured Control (n = 9)	
Autotomy onset (days)	9.3 ± 3.5*	13.9 ± 4.73*	29.9 ± 8.34	0.05
Day 5 autotomy score ≥ 3 (%)	81.3*	81.3*	33.3	0.02

Data for days to autotomy onset are mean ± SEM.

* Differs significantly from uninjured control group.

TABLE 2. Number of Days to Autotomy Onset and Percentage of Rats in Experiment 2 with Autotomy Scores above Criterion (≥ 3) on Day 5

	Treatment Group				P
	Local Anesthesia (n = 15)	Control (n = 10)	Mechanical Stimulation (n = 10)	Electrical Stimulation (n = 15)	
Autotomy onset (days)	25.2 \pm 4.4	35.3 \pm 7.3	21.2 \pm 6.6	17.4 \pm 4.0	NS
Day 5 autotomy score ≥ 3 (%)	20	40	80	66.7	0.01

Data for days to autotomy onset are mean \pm SEM.

NS = not significant ($P > 0.05$).

($P < 0.05$) and for rats injured after neurectomy ($P < 0.05$) when compared to the uninjured control rats.

EXPERIMENT 2

Table 2 shows that the groups differed significantly in the number of rats with autotomy scores above criterion (≥ 3) on day 5 ($\chi^2(3) = 11.7$, $P < 0.01$). A significantly greater proportion of rats in the experimental conditions (the groups receiving either electrical or mechanical stimulation) displayed higher autotomy scores when compared to the two uninjured control conditions ($\chi^2(1) = 9.68$, $P < 0.001$).

Figure 3 shows the site of autotomy onset for rats in the two experimental and two control groups. The site of autotomy onset was significantly altered among animals that received electrical stimulation of the sciatic nerve prior to neurectomy ($\chi^2(6) = 19.1$, $P < 0.004$). Only rats that were given electrical stimulation initiated autotomy at sites on the lateral aspect of the leg above the cutaneous distribution of the sciatic nerve (compared to the me-

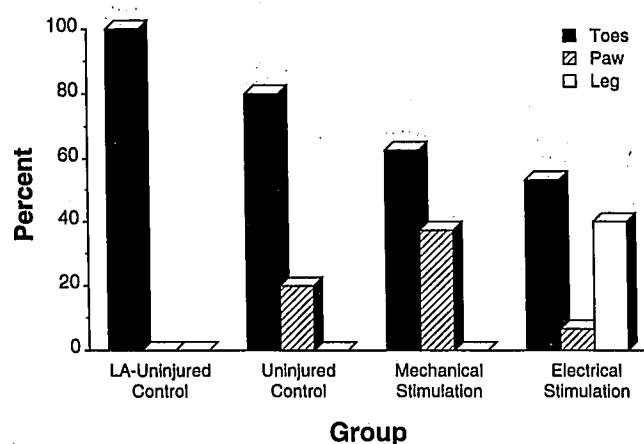


FIG. 3. Site of autotomy onset for control rats and rats that received electrical or mechanical stimulation of the sciatic nerve prior to nerve transections. Chi-squared test for two-way tables demonstrated a significant ($P < 0.004$) difference among the four groups. LA-Uninjured Control = rats that received local anesthetic applied to the sciatic nerve before electrode placement and nerve section.

chanical-stimulation group ($\chi^2(2) = 6.13$, $P < 0.05$) and to the combined control groups ($\chi^2(2) = 9.0$, $P < 0.01$). The latency to autotomy onset (table 2) was not significantly different for the four groups ($F(3, 46) = 1.92$, $P > 0.05$).

Discussion

The results demonstrate that thermal injury of selected regions of the hindpaw or noxious stimulation of the sciatic nerve prior to sciatic and saphenous nerve transections alters the usual pattern of self-mutilation seen in control rats. These results provide behavioral evidence consistent with recent demonstrations that brief noxious inputs are able to produce changes in spinal cord cells that long outlast the duration of the stimulus.^{3,6} The enhanced autotomy is also consistent with the findings of other studies using the same model, in which the injury was induced by thermal or chemical means prior to deafferentation.^{3-5,7,18} Furthermore, the change in the site of autotomy onset parallels the descriptions of human amputees who report the persistence of a preamputation pain referred to the same location of the phantom limb after amputation.¹⁰

These experiments strongly suggest that prior injury or noxious stimulation is capable of producing central changes that influence autotomy behavior after the sciatic and saphenous nerves are sectioned. Since the nerve sections produce a deafferentation of the originally injured region, the central effects of the injury are sustained in the absence of further inputs from the injured region. A contribution of peripheral factors is not ruled out, however, since a thermal injury given after nerve section is capable of increasing the severity of autotomy despite its inability to direct autotomy specifically to the injured site, as seen in rats injured before the neurectomy.

The shift in the site of autotomy in rats that received a thermal injury of the hindpaw (fig. 2) or high-intensity electrical stimulation of the sciatic nerve (fig. 3) prior to neurectomy can be explained, in part, by a memory mechanism in which dorsal root ganglion cells or CNS

cells, sensitized by the noxious stimuli, contribute to enhanced autotomy in the absence of subsequent input from the site of injury. Evidence of this central mechanism was provided, in experiment 1, by the pronounced preference among medially injured rats to initiate autotomy only in the injured region of the paw if the injury was induced *before* denervation (fig. 2). In addition, the incidence of autotomy onset at lateral sites among rats injured in the lateral hindpaw before nerve transections is more than twice that of control rats that did not receive a thermal injury. On the other hand, the pattern of autotomy among rats injured after neurectomy (whether in the medial or lateral region) was not different from that of the uninjured control rats. The absence of autotomy onset in the lateral region of the paw among rats injured in the medial region after neurectomy can be explained by the relatively higher percentage of autotomy in the "other" category, which included rats exhibiting autotomy at both medial *and* lateral sites.

The significantly earlier autotomy-onset latencies and greater number of higher autotomy scores among rats injured before denervation may also be a reflection of hyperactivity or hypersensitivity of central cells, but similar results from rats injured after denervation suggest that a peripheral mechanism also may be involved.⁷ A central mechanism in which spinal cells become sensitized due to the intense afferent barrage produced by the thermal injury is ruled out in the "injured-after" groups by the fact that the injury barrage did not reach the CNS. Thus, the results suggest that some injury-related peripheral cues direct the rat (injured before or after nerve transections) to attack the denervated region earlier, and to a greater degree than controls. It may be that visual, olfactory, or gustatory information that signals the presence of a cutaneous injury provides a sufficient condition for the enhanced autotomy (so that the self-mutilative behavior need not reflect pain or dysesthesia). However, that areas adjacent to the injured region also are attacked suggests that cues from the injury site are not the only peripheral factor involved. Another possibility is that chemical substances released from the site of injury enter the bloodstream and excite regenerating fibers in the neuroma that project to central cells subserving the denervated hindpaw, or that such substances directly stimulate dorsal root ganglion or central cells that are already sensitized by inputs associated with the neurectomy.

Since experiment 2 was designed to minimize the influence of peripheral cutaneous factors, the results strongly support the hypothesis that high-intensity electrical stimulation of the sciatic nerve sensitizes central cells representing the territory innervated by the stimulated nerve. We suggest that after sciatic and saphenous nerve transections, these cells retain a hyperactive or hypersensitive state, resulting in activity that is referred to regions

of the anesthetic limb subserved by the sciatic nerve, and producing a pattern and extent of autotomy behavior uncharacteristic of control rats. That the pattern of autotomy is changed so that rats will attack areas not even innervated by the sciatic nerve (*i.e.*, the leg) suggests that the electrical stimulation has led to significant changes in the function of CNS cells adjacent to those supplied by the sciatic nerve.

Direct stimulation of peripheral nerves²² or dorsal roots²³ results in both orthodromic and antidromic effects, the latter producing vasodilatation and plasma extravasation in the innervated skin regions subserved by the nerves or roots. It is possible, therefore, that the autotomy displayed by rats in experiment 2 was directed to the cutaneous receptive fields of afferent fibers because of antidromic release of certain substances. However, autotomy onset occurred, on the average, more than 2.5 weeks after sciatic and saphenous transections, making it unlikely that chemicals released from the nerve endings at the time of stimulation were present at the time of autotomy onset. In addition, the finding in the electrical-stimulation group that autotomy spread to areas that are not even innervated by the sciatic nerve suggests that the noxious electrical stimulus triggers CNS changes within neurons adjacent to those innervated by the sciatic nerve. Taken together, these considerations suggest that the altered site of autotomy onset in the electrical-stimulation group is not due to an antidromically induced release of substances that cue rats to scratch or bite at specific locations of the denervated paw.

The enhanced autotomy observed in rats that received mechanical stimulation of the sciatic nerve implies that part of the effect of electrical stimulation applied directly to the sciatic nerve may be due to the mechanical forces exerted on the nerve and surrounding tissue in combination with the injury barrage produced by the neurectomy itself.^{8,9,24} The enhanced autotomy produced by 30 seconds of mechanical stimulation paralleled the effects of electrical stimulation (although this was not the case for the pattern of autotomy onset shown in fig. 3). Mechanical stimulation of nerve trunks has long been known to generate activity in central cells, which may become sensitized by prolonged stimulation. Furthermore, clinical observations indicate that patients undergoing lower limb amputation under an otherwise satisfactory spinal block report discomfort during clamping, cutting and ligation of the sciatic nerve which is relieved when stimulation of the nerve is stopped. Moreover, when the sciatic nerve is infiltrated with a local anesthetic, further discomfort is abolished.²⁵ Taken together, these studies suggest that the mechanical forces exerted on the sciatic nerve in the course of routine surgical procedures may have the capacity to produce relatively long-lasting changes in central neural function which later contribute to post-operative pain.

Although the specific mechanisms by which noxious peripheral stimulation produces central changes are not yet fully established, recent evidence suggests that excitatory amino acids and C-fiber neuropeptides may play a critical role. The excitatory amino acids glutamate and aspartate²⁶ as well as neuropeptides, such as substance P,^{27,28} are released in the dorsal horn in response to peripheral noxious stimulation. Neuropeptides, such as substance P and calcitonin gene-related neuropeptide, are released in response to C-fiber activation and produce slow excitatory potentials.^{29,30} As a result of summation, after repeated or sustained stimulation, these slow potentials give rise to a cumulatively incrementing depolarization. This phenomenon, which has been called "windup," is diminished by selective N-methyl-D-aspartic acid (NMDA) antagonists,^{31,32} suggesting that an action of excitatory amino acids on the NMDA subclass of excitatory amino acid receptors is critical to this effect. It is significant that both neuropeptide (tachykinin) receptor antagonists or a C-fiber neurotoxin and NMDA antagonists have been found to reduce tonic pain following formalin injection in animals³³ and also to reduce behaviors indicative of hyperalgesia in animal models of neuropathic pain.³⁴⁻³⁶

It is expected that the release of these substances, which produces postsynaptic actions lasting from milliseconds to seconds, induces long-lasting changes in neural function by triggering alterations in membrane permeability. For example, NMDA receptor activation would produce an increase in intracellular calcium³⁷ and stimulate second messengers,³⁸ each of which would stimulate protein kinases and modify excitability by phosphorylating substrate proteins.³⁹ Alternatively, NMDA receptor activation may produce even longer-lasting changes by stimulating new gene expression. For example, noxious formalin injection⁴⁰ and peripheral nerve section⁴¹ lead to an increased expression of Fos protein immunoreactivity in the spinal cord of rats. A role for the NMDA receptor is suggested by the finding that the expression of the Fos protein induced by subcutaneous formalin injection is significantly suppressed after intrathecal administration of an NMDA antagonist.⁴²

The implication of these findings for clinical pathologic pain is that the central changes in neural function that are induced by injury (or surgical incision) alter subsequent perception in such a way that noxious inputs may be perceived to be more painful (hyperalgesia) than they would otherwise have been, and that innocuous inputs may give rise to frank pain (allodynia).⁴³⁻⁴⁵ Clinical evidence strongly suggests that noxious stimuli or injury may produce long-term changes in CNS function that are experienced as pain long after the offending stimulus has been removed. The present data are consistent with observations that relief of preamputation pain reduces the incidence of phantom limb pain and stump pain,^{46,47} and that pain after surgical operations^{43,44,48-50} and dental

work^{51,52} is decreased, and in some cases prevented, by a preoperative anesthetic block of the relevant peripheral nerves and/or spinal cord cells involved in the transmission and processing of injury-related nerve impulses.

Finally, in both experiments, the injury prior to neurectomy was induced while the rats were under sodium pentobarbital anesthesia, and they were maintained under the general anesthetic until well after the sciatic and saphenous nerve transections had been performed. Thus, at no time did these rats experience the effects of the noxious stimulus in an awake state. In contrast, previous studies produced the injury without anesthesia,^{7,20} or under brief ether anesthesia³⁻⁵ so that the rats would have had time to experience pain until the neurectomies were performed. The present study makes it clear that the long-term neural activity that underlies the enhanced autotomy occurs even when rats do not experience the noxious stimulus in an awake state.

This conclusion is particularly relevant to the problem of phantom limb pain that resembles a preamputation lesion or injury, since previous attempts to account for these somatosensory pain memories in human amputees have emphasized a psychogenic or psychopathologic basis.^{53,54} The present results suggest that somatosensory memory pains need not represent psychologically important pains, since evidence of persistent central neural activity occurs in the absence of any experience in the awake state, thus supporting the hypothesis that the formation of the somatosensory component of pain memories in human amputees is independent of the conscious experience of pain.¹⁰

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