

Alterations in Ascending Dorsal Horn Neurons by a Surgical Incision in the Rat Foot

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Background: Little is known about the mechanisms of pain caused by a surgical incision. The authors have developed a rat model of postoperative pain characterized by decreased withdrawal thresholds to punctate mechanical stimuli after plantar incision. The present studies examined the response characteristics of dorsal horn neurons receiving input from the plantar aspect of the foot before and after a plantar incision placed adjacent to the low threshold area of the receptive field (RF).

Methods: Individual dorsal horn neurons from the lumbar enlargement were antidromically identified and characterized as low threshold, wide dynamic range (WDR), and high threshold (HT) based on their responses to brush and pinch. Thresholds (in millinewtons), the pinch RF, and stimulus-response functions (SRFs) to von Frey filaments characterized the neurons. SRFs were analyzed using area under the curve. Changes in background activity, punctate mechanical thresholds, SRFs, and RF were recorded after an incision was made adjacent to the most sensitive area of the RF.

Results: In all cells, an incision increased background activity; this remained elevated in 3 of 9 HT and 16 of 28 WDR neurons 1 h later. The SRFs were enhanced in 10 of 27 WDR neurons and in 2 of 8 HT cells after incision. Only the WDR neurons were responsive to filaments that produced withdrawal responses after incision in behavioral experiments. Increases in the RFs outside of the injured area occurred after incision in 15 of 29 WDR and 2 of 9 HT cells.

Conclusion: A plantar incision caused dorsal horn cell activation and central sensitization. Because the threshold of HT neurons did not decrease to the range of the withdrawal responses in behavioral experiments, particular WDR dorsal horn neurons likely contribute to the reduced withdrawal threshold observed in behavioral experiments. Both WDR and HT neurons are capable of transmitting enhanced responses to strong punctate mechanical stimuli after incision. (Key words: Central sensitization; hyperalgesia; pain; postoperative pain.)

MANY remarkable discoveries have been made in pain research over the last 15 yr, yet only a modest effort has been made toward understanding pain from an incision.¹

This article is highlighted in "This Month in Anesthesiology."
Please see this issue of ANESTHESIOLOGY, page 5A.

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Received from the Department of Anesthesia, University of Iowa, Iowa City, Iowa. Submitted for publication January 18, 2000. Accepted for publication July 11, 2000. Supported by a New Investigator Award from the American Society of Regional Anesthesia-Foundation for Anesthesia Education and Research, Rochester, Minnesota; and Grant No. GM55831 from the National Institutes of Health, Bethesda, Maryland (to Dr. Brennan). Presented in part at the annual meetings of the Society for Neuroscience, San Diego, California, November 11, 1995, and the American Society of Anesthesiologists, Atlanta, Georgia, October 21, 1995.

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Not only is surgery a common cause of acute pain, but efficacious postoperative analgesia improves patient satisfaction and reduces morbidity after surgery.² It is imperative that we learn more about the physiology and pharmacology of incisional pain so that postoperative analgesia is effective, safe, easy to administer, has low cost, and potentially improves outcome.³

Pain from a surgical incision occurs at rest and is exacerbated by coughing, ambulation, and mechanical stimulation.⁴ The efficacy of postoperative analgesic treatments must be assessed using evoked responses during function such as movement if outcome is to improve with enhanced analgesia.³ Thus, mechanical hyperalgesia, a decreased pain threshold and an increase in pain response to suprathreshold stimuli, is a most important property of postoperative pain.

We developed and characterized a rat model for postoperative pain^{5,6} that produces reduced withdrawal thresholds suggesting mechanical hyperalgesia, an important quality of incisional pain for patients. In these studies, the median withdrawal threshold decreased from 522 mN before incision to approximately 50 mN and 2 h after incision. Because remarkable pain behaviors suggesting mechanical hyperalgesia occur in our behavioral studies and because little evidence of enhanced mechanical responsiveness of primary afferent fibers after incision exists,⁷ we hypothesized that a surgical incision would produce evidence of activation and enhanced responsiveness of single dorsal horn neurons.

In a previous study, incisions were made in mechanically insensitive areas of the receptive field (RF) of dorsal horn neurons.⁸ Three types of dorsal horn neurons were studied: low-threshold (LT), wide-dynamic-range (WDR), and high-threshold (HT) cells. WDR neurons have areas responding to weak mechanical stimuli and surrounding areas responding to strong mechanical stimuli. HT neurons have areas responding to strong mechanical stimuli. Increases in background activity were rare, whereas expansion of the RF outside the injury was common. The mechanically insensitive area of one third of WDR neurons was converted to low-threshold areas by an incision.

Enhanced responses to von Frey filaments with forces of approximately 50 mN (range, 20-80 mN) could also occur if low-threshold areas of WDR and HT neurons developed enhanced responses after incision. Rather than study the mechanically insensitive area, in this study, the incision was placed adjacent to the low-threshold area, and the change in responsiveness of the

low-threshold area was studied. RF size and background activity were also recorded.

Methods

Experiments were approved by the animal care and use committee at the University of Iowa and were performed on 43 adult (weight, 300–350 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN). Before surgery, the animals were housed in pairs with a 12-h day-night cycle. Food and water were available *ad libitum*. At the end of the experiment, all animals were killed with an intraperitoneal injection of a mixture of pentobarbital and phenytoin.

Preparation

As described previously,⁸ anesthesia was induced in all rats with 4% halothane in a sealed box and maintained with 2% halothane in oxygen *via* a nose cone. The rat was placed on a heating pad to maintain normothermia. A tracheotomy was then performed, and the rats were ventilated artificially (Harvard Apparatus, Inc., South Natick, MA). Anesthesia was maintained *via* a vaporizer calibrated to deliver 1–2% halothane. The internal jugular vein and the common carotid artery were denuded, and catheters were introduced for the administration of intravenous fluids and drugs and the measurement of the mean arterial blood pressure, which was maintained above 90 mmHg, respectively. Muscle paralysis was produced by intermittent intravenous injections of pancuronium bromide (0.2 mg/kg).

Antidromic stimulation and extracellular recordings of dorsal horn neuron activity required limited laminectomies at the cervical and thoracolumbar levels, respectively. As described previously, the first cervical segment and the lumbar enlargement were exposed at the first cervical vertebra. After the laminectomies, the vaporizer was set to deliver 1% halothane and maintained at this level. The dura was removed at both sites, and the spinal cord was covered with mineral oil. A bipolar, concentric stimulating electrode (SNE 100; Rhodes Medical Instruments, Woodhills, CA) was inserted into the right ventrolateral quadrant of the first cervical segment for antidromic stimulation of neurons sending ascending projections to the brainstem and thalamus. Extracellular dorsal horn cell recordings were made using a parylene-coated, tungsten electrode (1–1.5 m Ω impedance; Microprobe Inc., Clarksburgh, MA) inserted in the lumbar enlargement around L5 or L6 near the point where the dorsal root entry diverges from midline. The left hind leg and foot were extended and stabilized. One cell was recorded per rat.

Recording of Dorsal Horn Neurons

Individual dorsal horn neurons with ascending axonal projections through the contralateral first cervical seg-

ment were identified by antidromic stimulation. The search stimulus was 1 mA intensity, 5 Hz rate, and 100 μ s pulse. Only cells that met the following criteria were included: (1) the antidromic action potential occurred at a constant latency after the stimulus artifact; (2) the antidromic action potential followed a high-frequency train of 250 Hz or greater; and (3) the antidromic action potential collided with an orthodromic one⁹ that occurred either spontaneously or was elicited by stimulating the RF. Two HT cells were not collided with an orthodromic spike because this would have required repeated pinching of the left foot at intensities that were potentially tissue damaging.

The recorded action potentials were amplified (Grass Instruments, Quincy, MA; settings: 500 \times , high filter 1 kHz, low filter 30 Hz) and monitored *via* a Tektronix (Beaverton, OR) storage oscilloscope. Individual action potentials were discriminated on the basis of amplitude and waveform with a BAK window discriminator (BAK Electronics, Inc., Germantown, MD). Online analysis was made possible *via* a 1401 Plus Laboratory Interface and Spike2 software (Cambridge Electronic Design Ltd., Cambridge, United Kingdom) installed on a computer. The rate of cell discharge (bin width, 2 s), individual action potential activities (unit trace), and marker were displayed on the computer screen. The oscilloscope trace and marker were also stored on videotape (A.C. Vetter Co, Inc., Rebersburg, MD) for backup and to allow a more detailed analysis later.

Experimental Protocol

Cells were selected for continued study if the activity of the neuron was increased by gently probing, brushing, or pinching the glabrous skin of the foot. Dorsal horn cells were classified based on their responses to mechanical RF stimulation.^{10,11} First, the cell was classified as WDR, HT, or LT based on responses to innocuous brushing with a number-4 camel-hair artist's brush and noxious but nondamaging pinch with a small curved forceps.⁸ The site with the lowest mechanical threshold of the RF was identified by brushing or pinching and marked as a reference for repeated testing. Next, a calibrated series of von Frey filaments (Semmes-Weinstein nylon monofilaments; Stoelting, Wood Dale, IL) was applied in ascending order (6, 11, 14, 30, 42, 65, 73, 98, 149, 247, 637 mN) to the low-threshold site of the RF. If a cell was activated by filaments with a lower bending force than 6 mN, lower filaments were then tested. Each filament was applied once for 3–5 s. The next filament was applied approximately 10–30 s later. The mechanical threshold of the dorsal horn cell was defined as the lowest force causing either activation of the cell if no spontaneous activity was present or an increase in cell activity by at least 2 SDs above background activity. The change in the peak cell activity (2-s bin) was calculated as the peak increase in action potential rate, associated

with the onset of filament application, minus background activity (the 10-s average activity before filament application) and expressed in impulses per second. In addition, the next strength filament must also have excited the cell. If the filament applied appeared to miss the test site or if the background activity was irregular, individual filaments were retested to identify the threshold. The peak increases in cell activity were also measured during the consecutive application of increasing strengths of von Frey filaments to the low-threshold site of the RF in both WDR and HT neurons. All WDR cells were not tested with the 637-mN filament; only data for filaments with forces up to 247 mN are reported for WDR neurons. The peak increases in cell activity were plotted *versus* the increasing strengths of the von Frey filaments applied, forming a stimulus-response function (SRF) for each dorsal horn neuron. In HT neurons, all filaments below threshold were not necessarily tested; a 0 was entered for the increase in cell activity for these filaments well below threshold. In 31 neurons, a second SRF, at the most sensitive area of the RF, was performed 5–10 min after the first SRF. Subsequently, the extent of the RF was mapped using a suprathreshold von Frey filament, or small, blunt, curved forceps were used to pinch the foot. Ring forceps, 1.5 cm in diameter, were used to pinch the skin and deep tissues of the tail, calf, and hamstring and contralateral hind quarters to determine the extent of the RF. The RF was depicted on a drawing of the rat's hind quarter or marked with a felt-tip pen on the hairy skin.

At the end of the initial descriptive phase of the experiment, the baseline spontaneous activity was recorded during a 5-min period and averaged. Subsequently, and similar to the procedure described in our behavioral experiments,⁵ a 1-cm longitudinal incision was made through the skin of the plantar aspect of the left foot adjacent (1–3 mm) to the LT area of the RF previously characterized with von Frey filaments. Depending on the location of the incision in the foot, the underlying muscle was elevated and incised longitudinally, while its origin and insertion were intact. Both skin incision and skin and muscle incision produce similar pain behaviors.^{5,12} Hemostasis was obtained with gentle pressure, and the incision was closed with two interrupted 5-0 nylon sutures. The background cell activity was recorded continuously during the 5–10-min period during foot incision and for 50–60 min after incision. The spontaneous activity was averaged during the last 5 min. Spontaneous activity was considered increased after incision if a neuron increased its activity by more than 2 SDs above preincision background activity. Thereafter, an SRF was repeated adjacent to the incision. A change in von Frey threshold was noted if it increased or decreased by at least 2 filaments. A change in von Frey filament threshold was designated as a change by two filaments because in preliminary studies repeating SRFs

in these cells revealed that the threshold could usually increase or decrease by one filament, but changes greater than one filament were uncommon. It is recognized that HT neurons respond to only a few high-strength filaments so a change of two filaments is less likely than in WDR cells. Finally, the RF was reassessed by applying a suprathreshold von Frey filament or by pinching throughout the hind quarters of the rat as described. The RF was considered expanded within the foot if regions unresponsive to a von Frey filament or pinch with the small forceps before incision became responsive after incision or if the calf, hamstring, or other large area was unresponsive before incision but became responsive.

Area under the Curve

In contrast to other tissue injuries, an incision did not markedly decrease the filament threshold. In addition, the responses to von Frey filaments were not considerably enhanced in all cells. To determine if the responsiveness of the neurons to mechanical stimuli was enhanced after incision, the SRFs (rate *vs.* force) were analyzed using an area under the curve (AUC) analysis. The y-axis was the rate change in impulses per second and the x-axis the log of the bending force of the filament in millinewtons. The log of the bending force was used because the intervals between von Frey filaments were not linear. The AUC (millinewton · impulses per second) was calculated using the area of the trapezoid (the sum of the rectangle and triangle) between the points for each filament. First, the percent difference between the first and second SRF (before incision) was determined in 23 WDR and 8 HT neurons using the following formula: $(AUC2 - AUC1/AUC1) \times 100\%$. The greatest percent increase in the AUC between the two tests was chosen as the cutoff. The percent difference between the first AUC and the AUC after incision was then calculated, and any WDR or HT neuron exceeding the cutoff was considered enhanced to punctate mechanical stimuli by the incision.

Histology

At the end of the experiment, a lesion was made at the antidromic stimulation site (50 μ A, 1 min), the rat was killed, and the cervical spinal cord removed and fixed for at least 2 weeks in 10% formalin containing saturated potassium ferrocyanide and ferricyanide. Frozen serial sections (40 μ m) were cut and viewed under a microscope for the blue reaction, and the lesions were identified. Locations of the antidromic stimulation sites were depicted on a scale drawing of the spinal cord at C1. In six rats, lesions (50 μ A, 2 min) were made at 300, 600, or 900 μ m in the L5–L6 region of the spinal cord. These lesions were identified histologically, and each recording site was depicted on a scale drawing of the lumbar spinal cord based on the depth of the recording.

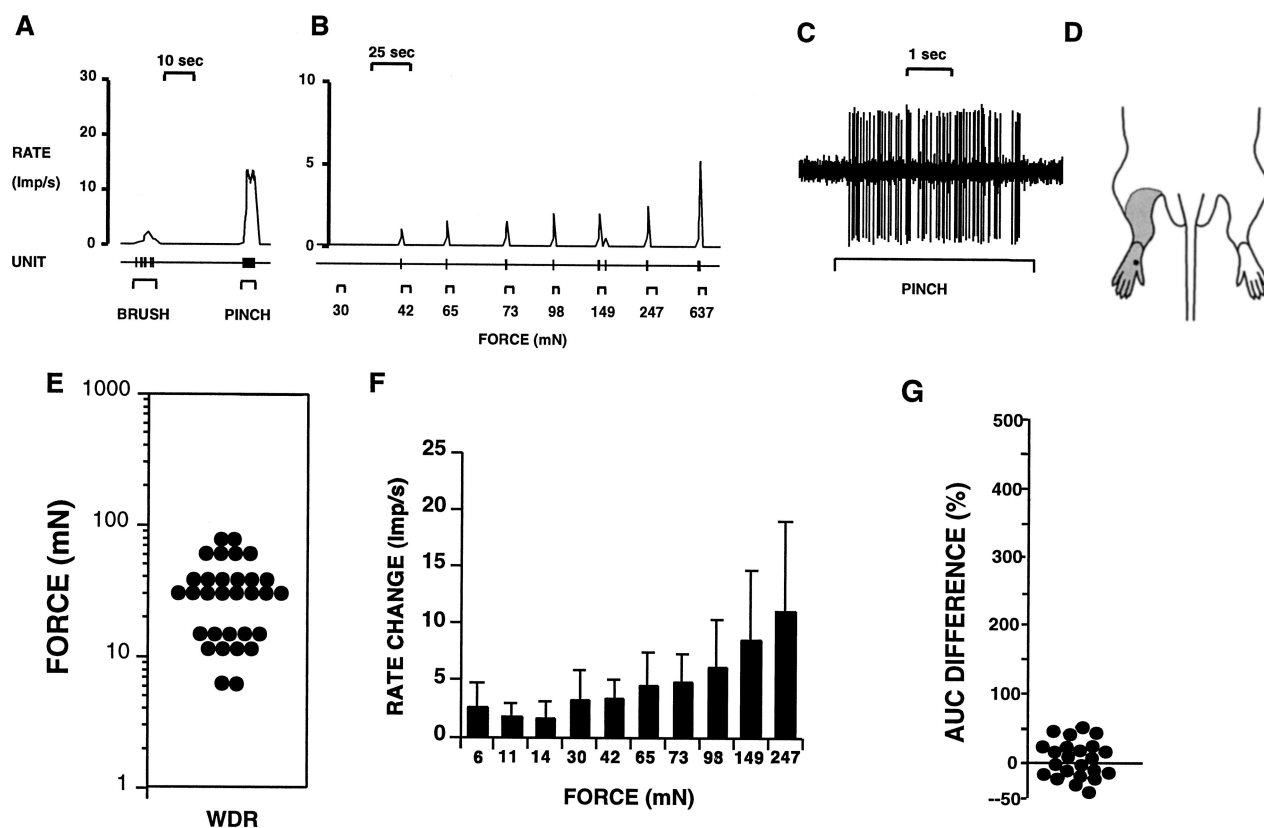


Fig. 1. Characterization of wide-dynamic-range (WDR) neurons and responses to von Frey filaments. (A) Response of the neuron to brush and pinch of the somatic field. (B) Responses to von Frey filaments. (C) Digitized oscilloscope trace of action potentials during pinch. (D) Drawing depicting the somatic field. The gray area is the region responding to pinch of cutaneous and deep structures. The black dot represents the site of application of von Frey filaments. (E) von Frey filament thresholds in 31 WDR cells. (F) Summary of the average peak increase in action potential rate produced by application of von Frey filaments to 31 WDR neurons. The bracket is the SD. (G) The percent difference in the area under the curve (AUC) for two repeated stimulus-response functions in 23 WDR neurons. Rate = rate of action potentials in impulses per second; bin width = 2 s.

Statistical Analysis

The changes in mechanical thresholds before *versus* after incision were compared using a Wilcoxon signed rank test. Mechanical thresholds between cell types were compared using a Wilcoxon-Mann-Whitney test.¹³ Differences in expansion of RFs and changes in background activity after incision between WDR and HT cells were compared using a chi-square test. *P* less than 0.05 was considered significant. SRFs were analyzed before and after incision using two-way analysis of variance for repeated measures, one-way analysis of variance, and a paired *t* test.

Results

Forty-three dorsal horn neurons receiving sensory input from the plantar aspect of the rat foot were recorded. The protocol was incomplete in 3 neurons and completed in the remaining 40. Most of the antidromic stimulation sites were located in the ventral contralateral quadrant at C1 and the intermediate region as described previously.⁸ No clear lesion could be identified in four rats. The average antidromic stimulation threshold was

137 μ A (range, 50–450 μ A). Thirty-one WDR, 9 HT, and 3 LT cells were located at depths from the surface of the spinal cord ranging from 275–1,050, 155–1,001, and 682–1,082 μ m, respectively. The majority of the cells were located in the deep dorsal horn; two were from superficial laminae.

Characteristics of Dorsal Horn Neurons

Thirty-one neurons responded more vigorously to pinching than to brushing; these cells were classified as WDR neurons. An example of a WDR neuron is shown in figures 1A–F. The von Frey filament threshold was 42 mN. The median mechanical threshold of 31 WDR cells was 30 mN (range, 6–73 mN). In general, increasing the force of punctate stimulation to the area with the lowest-mechanical-threshold WDR neurons was accompanied by a greater average increase in cell activity. Nine cells only responded to pinch and were categorized as HT neurons. An HT neuron is shown in figures 2A–F. The von Frey filament threshold was 149 mN. The median mechanical threshold (fig. 2E) of the 9 HT cells was 149 mN (range, 65–637 mN). Stronger filaments produced greater cell activity (fig. 2F), but greater forces

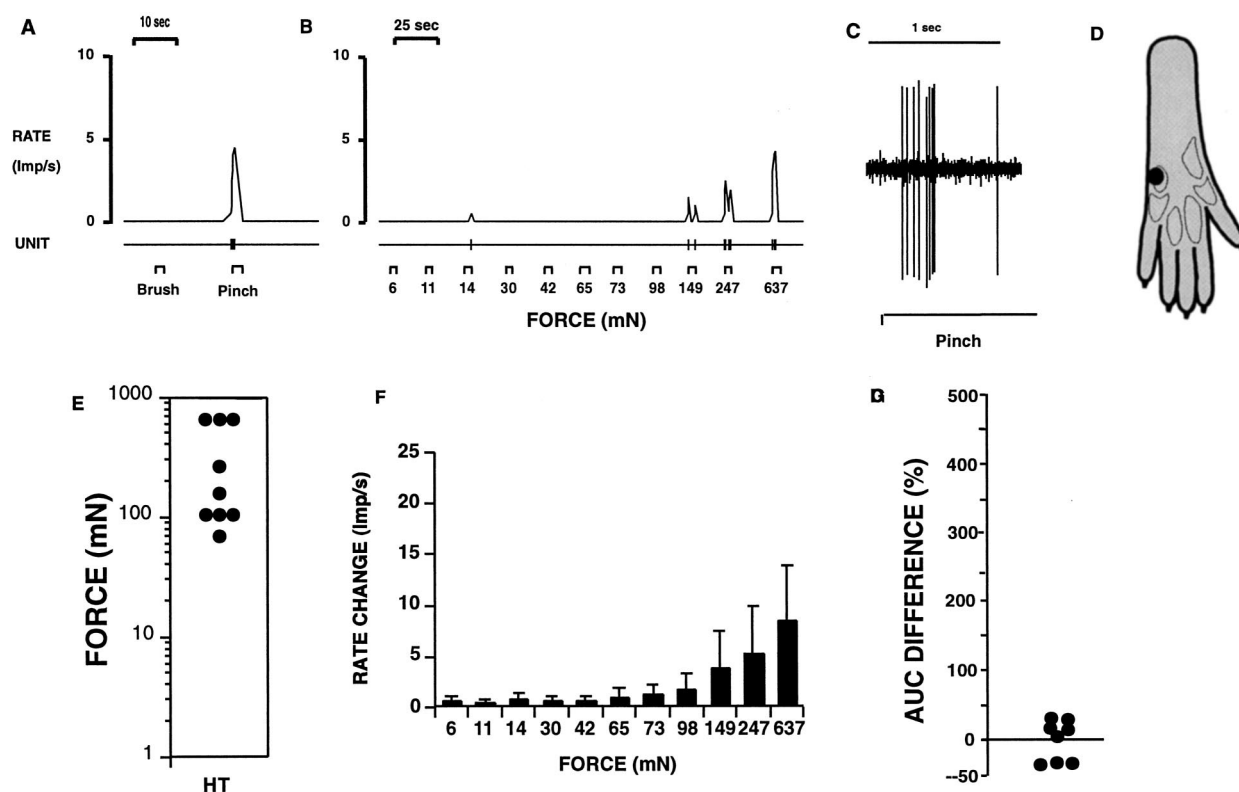


Fig. 2. Characterization of a high-threshold (HT) neuron and responses to von Frey filaments. (A) Response of the neuron to brush and pinch. (B) Responses to von Frey filaments. (C) Digitized oscilloscope trace of action potentials during pinch. (D) Drawing depicting the somatic field. The gray area is the region responding to pinch of cutaneous and deep tissue. The black dot represents the site of application of von Frey filaments. (E) von Frey filament thresholds for nine HT neurons. (F) Summary of the average peak increase in action potential rate produced by application of von Frey filaments to nine HT neurons. The bracket is the SD. (G) The percent difference in the area under the curve (AUC) for two repeated stimulus-response functions in eight HT neurons. Rate = rate of action potentials in impulses per second; bin width = 2 s.

were required to activate the HT neurons ($P < 0.05$ vs. WDR). Three neurons responded more vigorously to brushing than to pinching of the RF and were classified as LT cells. The median mechanical threshold of 3 LT cells was 6 mN (range, 6–14 mN). Application of increasing strengths of filaments to the LT site of the RF did not produce an increase in the response (data not shown).

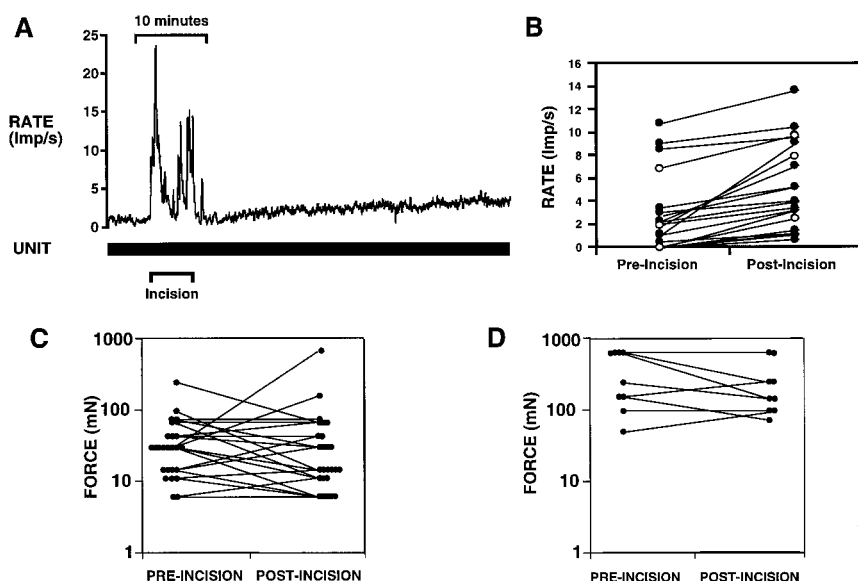
To determine how many cells changed responsiveness to von Frey filaments without an incision, a second SRF (SRF 2) was performed, the threshold determined, and the AUC calculated. It was not uncommon for the threshold to increase or decrease by a single filament between tests; one decreased by more than one filament and two increased by more than one. None of the thresholds of the eight HT neurons changed by more than one filament between the two tests. The median percent change in the AUC for WDR cells was +7% (range, -40% to +54%) and for HT cells was +6% (range, -41% to +33%). The greatest percent increase in the AUC between the two tests was designated the cutoff value to determine enhanced responsiveness to the monofilaments; this was 54% for WDR cells and 33% for HT cells (fig. 2F).

Effect of Incision

The background activity before incision was 2.6 ± 4.5 imp/s (mean ± SD) in 40 cells completing the protocol. In all cells, an incision within the RF produced a burst of activity that persisted throughout the surgery and then slowly decreased (fig. 3A). Activity was increased in 16 WDR and 3 HT neurons 1 h later. An example of a WDR neuron with a sustained increase in activity is shown in fig. 3A. In 19 neurons having a sustained response to incision, the average background activity increased from 2.8 ± 3.4 imp/s before incision to 5.3 ± 3.9 imp/s after incision (fig. 3B). Background was not increased in three LT cells (data not shown). After incision, a decrease in threshold of at least two von Frey filaments was found in six WDR (fig. 3C) and two HT (fig. 3D) neurons. Increases in thresholds of two or more filaments occurred in four WDR neurons (figs. 3C–D). Overall, changes in threshold after incision were not remarkable.

An example of a WDR neuron sensitized to mechanical stimuli after incision is shown in figures 4A–C. Before incision, the mechanical threshold was 42 mN. After incision, the threshold was also 42 mN, and the re-

Fig. 3. Effect of an incision on background dorsal horn cell activity and von Frey filament thresholds. *(A)* Example of an increase in activity produced by an incision. *(B)* Summary of preincision *versus* postincision activity in 18 of 40 neurons in which background activity increased. The dark circles are wide-dynamic-range neurons; the open circles are high-threshold neurons. *(C)* and *(D)* von Frey filament thresholds wide-dynamic-range neurons ($n = 27$) and high-threshold neurons ($n = 8$) before and after incision. Rate = rate of action potentials in impulses per second; bin width = 2 s; AUC = area under the curve.

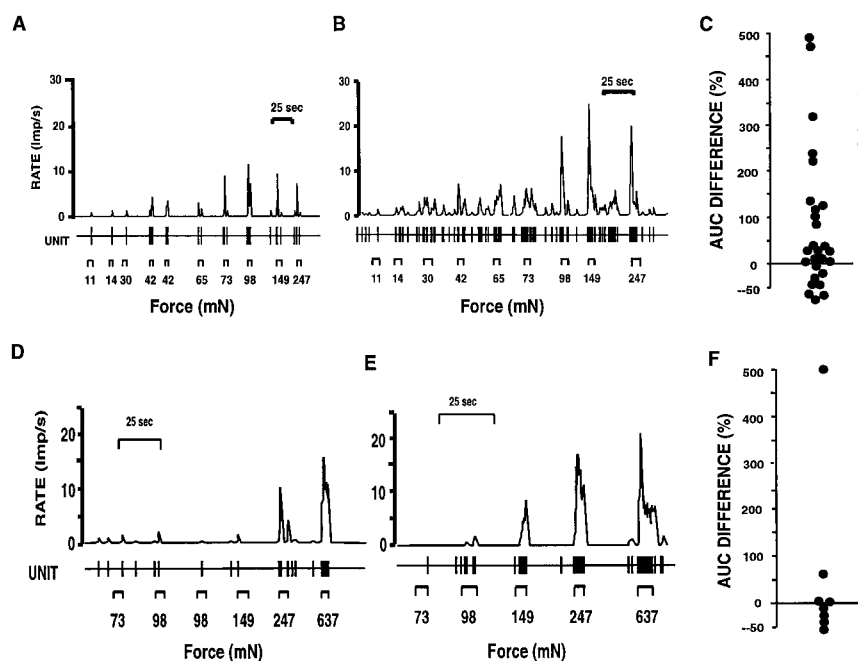


sponses to the von Frey filaments tended to be greater. The median percent change in the AUC after incision for 28 WDR cells was +27% (range, -69%–+485%) and for HT cells was -4% (range, -73%–+512%). Ten of 28 WDR neurons exhibited a greater than 54% increase in the AUC after incision (fig. 4C). The HT neuron with the greatest increase in responsiveness to punctate mechanical stimuli after incision is shown in figures 4D–E. Before incision, the mechanical threshold was 149 mN, and after incision the threshold had decreased to 98 mN. The median percent change after incision in eight HT cells was -4% (range, -73%–+512%). Two neurons increased their activity greater than 33% (fig. 4D). The average changes in activity to application of the von Frey filaments for 10 WDR cells with enhanced responses are

summarized in figure 5A. The changes in activity to application of the von Frey filaments for two HT cells are summarized in figure 5B.

An expansion of the RF outside the area of the injury was found in 17 (15 WDR, 2 HT) dorsal horn neurons, whereas no increase in RF size was detectable in 23 (3 LT, 14 WDR, 7 HT) cells. In nine neurons, parts of the foot previously unresponsive to pinch with small forceps or high-strength von Frey filaments were now responsive (fig. 6). In six other cells, expansion of the RF occurred into previously unresponsive areas of the calf or hamstring. There was spread of the RF to the contralateral side in two neurons. There was no significant difference for expansion of RFs between WDR and HT neurons. RF expansion did not occur in LT cells.

Fig. 4. Stimulus–response functions of wide-dynamic-range (WDR) and high-threshold (HT) neurons enhanced by an incision. *(A)* and *(B)* Example WDR neuron responses before *(A)* and 1 h after *(B)* incision. *(C)* The percent difference in the area under the curve before and 1 h after incision in 27 WDR neurons. *(D)* and *(E)* Example of HT neuron responses before and 1 h after incision. *(F)* The percent difference in the area under the curve for stimulus–response functions before and 1 h after incision in eight HT neurons.



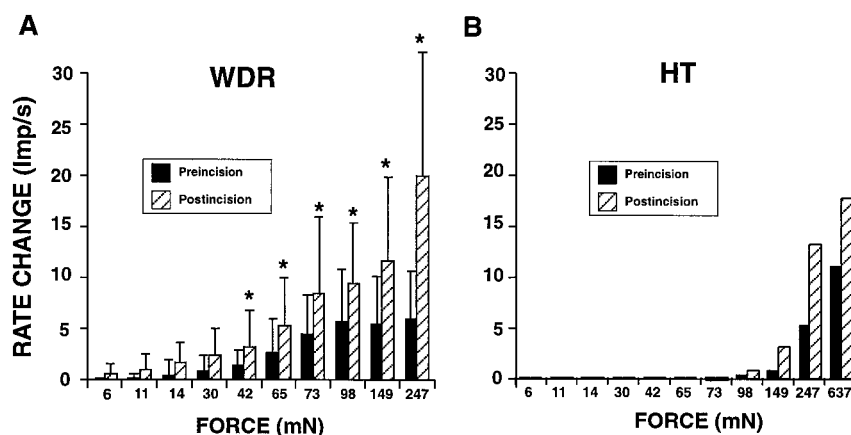


Fig. 5. (A) Summary of the peak increase in action potential rate produced by application of von Frey filaments to 10 wide-dynamic-range neurons with enhanced responses after incision. (B) Summary of the average peak increase in action potential rate produced by application of von Frey filaments to two high-threshold neurons with enhanced responses after incision. Each bar and bracket represent the mean and SD, respectively, preincision (dark bars) and postincision (hatched bars). * $P < 0.05$ versus postincision. Rate = rate of action potentials in impulses per second; bin width = 2 s.

Discussion

In the present study, 51% of dorsal horn neurons had increased background activity, and 45% had expanded RFs outside the area of injury after incision. This increased background activity may signal pain at rest after incisions, and expanded RFs to uninjured areas indicates central sensitization occurred after incision and may further amplify dorsal horn responses. Increased responses to von Frey filaments less than 100 mN are observed in some WDR but not HT cells, suggesting these WDR neurons contribute to the reduced withdrawal threshold observed behaviorally.

Dorsal Horn Neuron Responses to Injuries

Recent studies suggest that the class of dorsal horn neurons sensitized may depend on the particular type of injury applied to the somatic field. Woolf and King¹⁴ demonstrated that some HT dorsal horn neurons with RFs in the plantar aspect of the rat foot sensitized after the application of mustard oil. Unlike the results of the present study, the mechanical thresholds of these sensitized HT neurons approached those of LT and WDR cells. In addition, some of these nociceptive cells now responded to brush and touch stimuli.¹⁴ Activation of HT neurons by LT stimuli has been observed by a number of investigators after a variety of injuries.^{11,15–20} Other investigators have suggested that WDR neurons appear more likely to sensitize or sensitize to a greater extent depending on the injury and the stimulus tested.^{19,21} In a recent review, Cervero and Laird²² proposed that $A\beta$ -mediated touch- and brush-evoked allodynia, as occurs in neuropathic pain and acute inflammation, may be a result of LT mechanoreceptors exciting HT-type neurons. We did not routinely examine brush and touch responses in HT neurons after incision. The inability to convert HT neurons in the present experiments to LT- or WDR-like cells when tested with von Frey filaments may provide an explanation for the absence of allodynia as a prominent feature in postoperative patients compared with those with neuropathic pain or acute inflammation.

The incision used in the present experiments may not be sufficient to excite and sensitize nearly all dorsal horn neurons as occurs after inflammatory injuries or chemical irritation.^{11,18,19} This inability to activate and produce robust dorsal horn neuron sensitization with marked decreases in threshold complicates quantifying the change in responsiveness. Selecting cells that do become more responsive based on the AUC demonstrates that WDR neurons develop greater activity to both low- and high-strength forces, whereas HT neurons only become more responsive to high-strength filaments. This may be a property of incisional pain that is unique compared with inflammatory models. These results are also in agreement with the observation that different patterns of hyperalgesia can be induced depending on the type of injury.²³

Because we made the incision in the low-threshold area, background activity increased after incision in many cells. Few cells had markedly decreased thresholds; perhaps more were decreased, but we cannot detect this during irregular background activity. Background activity may also hinder the assessment of greater responsiveness to von Frey filaments. The same response to the filaments after incision imposed on an increased background activity will produce a greater peak firing rate in response to that particular filament. This could represent greater responsiveness and could be a mechanism for the reduced withdrawal threshold in this model. Because the background activity was subtracted from the peak response produced by the filaments, it is possible that we are minimizing the response of the filaments by subtracting this activity from the response.

In the present study, the RF size was stable before incision, and RF expansion occurred outside the area of the incision. Because the area outside of the incision is uninjured, the input from the pinch stimulus in the uninjured tissue should be roughly the same before and after incision. Therefore, this expansion of RFs indicates that central sensitization, amplified responses to the same peripheral input, occurs after incision. Pain behav-

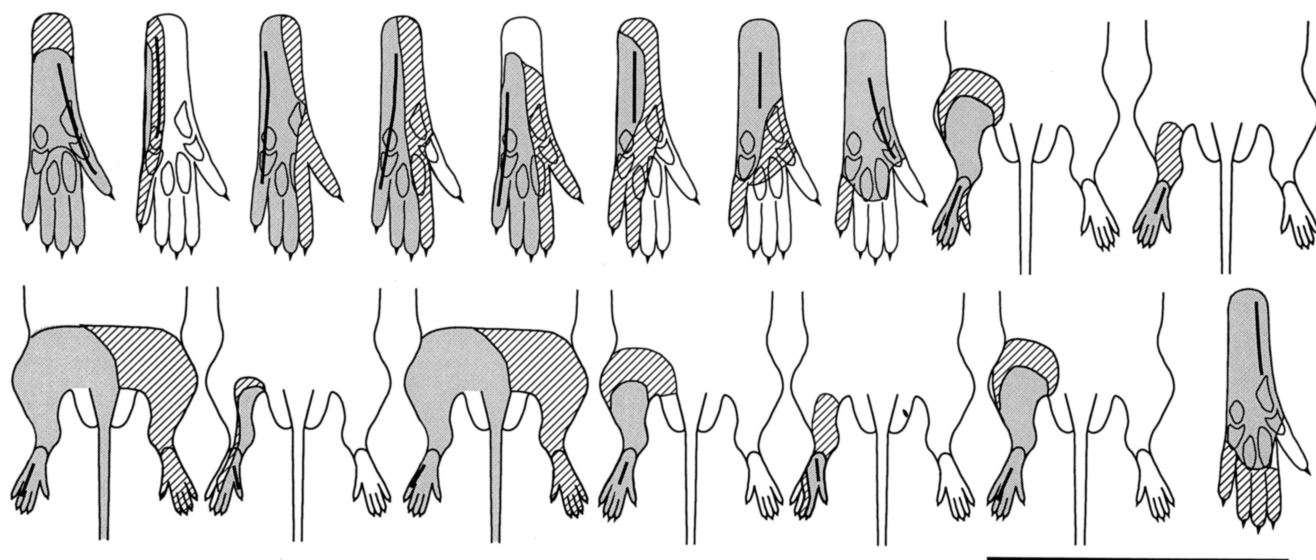


Fig. 6. Examples of increases in receptive field produced by an incision. Overall, an increase was observed in 17 neurons. The gray area indicates the somatic field before incision, the hatched area equals the somatic field after incision, and the incision is depicted by the darkened line on the hind paw. The two receptive fields above the bar are high-threshold neurons.

iors suggest that secondary hyperalgesia, responses caused by stimulation outside the injured tissue, occurs after the same incision in rats.¹² Clinical studies demonstrate that secondary hyperalgesia to von Frey filament application occurs after surgery.^{24,25}

Limitations

All experiments in the present study were performed using 1% halothane in oxygen anesthesia. In rats, administration of 1% halothane causes a reduction in low-threshold RF size of WDR neurons.²⁶ More recently, experiments by Herrero and Headley²⁷ demonstrated that halothane (2%) increased the mechanical thresholds of dorsal horn neurons of sheep, affecting largely WDR neurons. Halothane (2%) increased the cutaneous RF sizes of these neurons and reduced scratch-evoked enlargement of RFs.²⁸ Laird and Cervero²¹ found no difference in the sensitization of rat dorsal horn neurons among three different preparations: halothane anesthetized (1%), pentobarbital anesthetized, and decerebrate unanesthetized.

The goal of the current study was to examine the impact of a plantar incision on the response properties of neurons transmitting sensory information in the spinal cord. One limitation in the current study is that a preparative spine dissection and laminectomy were required to record the neurons. A further limitation of the mechanical stimuli used is that there are nine monofilaments between 3 and 100 mN and only three filaments between 100 and 637 mN. Therefore, small differences are likely detected in the lower range of forces; small changes with HT neurons will be less apparent.

Comparison to Previous Study

In a previous study, a different experimental configuration was used—the incision was placed in a mechanically insensitive HT area of the RF of dorsal horn neurons.⁸ In both the present and previous studies, expansion of pinch RFs outside the injury was common. In contrast, incisions in mechanically insensitive areas of the RF produced little change in background activity compared with the present study. The most remarkable finding of the previous study was that mechanically insensitive areas of some WDR neurons became responsive to lower forces after an incision; little or no changes were found in HT cells. Together, these results suggest that an incision converts a mechanically insensitive area of the RF of some WDR neurons to a mechanically sensitive area; in other WDR cells, increased background activity and enhanced responses to von Frey filament occurs when the incision is placed in the low-threshold area. Together, these changes likely contribute to pain behaviors observed 1 or 2 h after incision.

Conclusion

An incision within the low-threshold area of the RF of dorsal horn neurons resulted in sensitization of both WDR and HT dorsal horn neurons. This sensitization was characterized by an increase in the background activity and enlarged RF. The number of HT cells was limited; however, after incision, the thresholds of most HT cells did not approach the withdrawal thresholds observed in behavioral experiments. Our data suggest that WDR cells with either increased background activities or enhanced responses to punctate stimuli likely code for enhanced withdrawal responses observed behaviorally and suggest unique mechanisms for incisional pain.

The authors thank Gerald F. Gebhart, Ph.D., Professor and Chairman, Department of Pharmacology, University of Iowa College of Medicine, Iowa City, Iowa, for reviewing the manuscript.

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