

## *Incision-induced Changes in Receptive Field Properties of Rat Dorsal Horn Neurons*

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**Background:** To learn more about pain mechanisms produced by surgery, responses of wide dynamic range (WDR) and high threshold (HT) dorsal horn neurons were studied before and after an incision. For this study, an incision was made in a mechanically insensitive area of the receptive field (RF) of the dorsal horn neuron in the plantar aspect of the foot and changes in mechanical response properties were studied.

**Methods:** Action potentials from single dorsal horn neurons were recorded in halothane anesthetized rats and these neurons were characterized as WDR or HT. Changes in background activity and responses to a variety of mechanical stimuli adjacent to the incision, distant to the injury, and in areas throughout the hindquarters were recorded.

**Results:** Fifty neurons were recorded (29 WDR, 21 HT cells); only nine of these had a sustained increase in background activity after incision. Marked decreases in threshold to von Frey filaments applied adjacent to the wound occurred in 9 of 28 WDR neurons but in none of 21 HT cells. Von Frey filament thresholds distant to the incision were largely not changed. A blunt mechanical stimulus activated 18 of 22 WDR neurons when applied directly on the incision. HT cells were largely not excited by this mechanical stimulus after incision. The RF to pinch was enlarged in 31 neurons to include areas outside the injury. Pinch RFs of both WDR and HT cells expanded.

**Conclusion:** These results suggest that incisions in mechanically insensitive areas of the RF of dorsal horn neurons produced little change in background activity; expansion of pinch RFs outside the injury was common. Changing a mechanically

insensitive area of the RF of WDR neurons to a mechanically sensitive area by an incision could contribute to pain behaviors that indicate primary mechanical hyperalgesia in behavioral studies. (Key words: Central sensitization; high-threshold neuron; mechanical hyperalgesia; postoperative pain; rat model; wide dynamic range neuron.)

ALTHOUGH research has increased our understanding of the pathophysiology of pain, surprisingly little effort has been made to understand the basic mechanisms of pain caused by surgical incisions.<sup>1</sup> Mechanical hyperalgesia, exaggerated response to noxious stimuli, occurs directly on or immediately adjacent to surgical incisions in patients, is present in areas outside the injured tissue, and correlates with pain during cough and movement.<sup>2-4</sup> Primary hyperalgesia, hyperalgesia within the area of injury, and secondary hyperalgesia, hyperalgesia outside the injured area, are present after surgery and suggest that both peripheral and central sensitization occur in postoperative pain. In order to learn more about pain mechanisms caused by a surgical incision, we developed a rat model for human postoperative pain.<sup>5</sup> Withdrawal thresholds to von Frey filaments applied immediately adjacent to the incision of the plantar aspect of the rat foot decreased markedly after incision,<sup>5,6</sup> indicating primary hyperalgesia. Punctate stimuli applied outside the injured tissue also caused withdrawal thresholds to decrease, indicating secondary hyperalgesia and suggests central sensitization occurs after plantar incision in this rat model as observed in clinical studies.<sup>5,7</sup>

The importance of dorsal horn neuron sensitization and plasticity caused by surgical incisions to postoperative pain is not understood. Results from electrophysiologic studies demonstrate that the receptive fields (RFs) of dorsal horn neurons include areas responsive to weak mechanical stimuli, areas only responsive to strong mechanical stimuli,<sup>8,9</sup> and in some cases nearly unresponsive zones.<sup>8,10</sup> In previous studies, tissue injury has been used to transform an insensitive area to an area responsive to weak mechanical stimuli using a burn<sup>11</sup> or chem-

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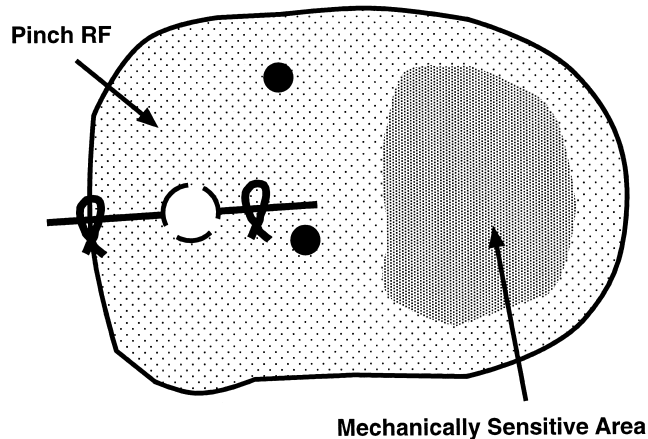
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**Fig. 1.** Schematic of the pinch receptive field (RF) of a dorsal horn neuron. The location of the incision, mechanically sensitive area (*heavily shaded*), and mechanically insensitive area (*lightly shaded*) are depicted. The *open circle* depicts the site of placement of the blunt mechanical stimulus directly on the incision (*dark line*), and the filled circles represent the sites of application of von Frey filaments adjacent and distant to the incision.

ical irritation with mustard oil application<sup>12,13</sup> or capsaicin injection.<sup>14</sup> Results from these studies suggest that central sensitization and plasticity may be important in postoperative pain.<sup>13,15,16</sup>

The purpose of these experiments was to study the responsiveness of wide dynamic range (WDR) and high threshold (HT) neurons to mechanical stimuli before an incision. After characterizing the RFs, incisions were made in areas unresponsive to punctate and blunt mechanical stimuli to determine if these mechanically insensitive areas could become sensitized, responding to weak mechanical stimuli after incision (fig. 1). This process, converting a mechanically insensitive area of the RF to an area responsive to weak mechanical stimuli, could explain, in part, the reduced withdrawal thresholds observed in behavioral studies. Because it has been suggested that WDR and HT neurons respond differently to tissue injury<sup>15</sup>, changes in responsiveness of both groups of dorsal horn neurons to mechanical stimuli after incision were studied. Furthermore, responses of WDR and HT neurons to mechanical stimuli applied distant to the incision were examined to further understand secondary hyperalgesia and central sensitization. In previous studies, a blunt mechanical stimulus applied directly on the incision caused pain behaviors<sup>6</sup>; thus, changes in responsiveness of dorsal horn neurons to this stimulus were also studied. A preliminary report of some of these data has been made.<sup>17</sup>

## Methods

Experiments were performed on 59 adult (weight, 300–350 g) male Sprague–Dawley rats (Harlan, Indianapolis, IN). Before surgery, the rats were housed in pairs under a 12/12 h day/night cycle. Food and water were available *ad libitum*. At the end of the experiment, all rats were euthanized with the intraperitoneal injection of a mixture of pentobarbital and phenytoin.

### Preparation

On the day of the experiment, anesthesia was induced with 4% halothane in a sealed box and maintained with 1–2% halothane in oxygen *via* a nose cone. A tracheotomy was performed and the rats ventilated artificially with oxygen. Anesthesia was maintained *via* a vaporizer calibrated to deliver 1–2% halothane. The rats were placed on a heating pad to maintain normothermia. The internal jugular vein and the common carotid artery were cannulated for the administration of intravenous fluids and drugs and the measurement of the mean arterial blood pressure (maintained above 90 mmHg), respectively.

Antidromic stimulation and extracellular recordings of dorsal horn neuron action potentials required limited laminectomies at the cervical and thoracolumbar level, respectively. The medulla oblongata and first cervical segment were exposed at the first cervical vertebra. The lumbar spinal cord was exposed at the lumbar enlargement between T13 and L3. The halothane concentration was 1–2% during the laminectomies. The rat's head was flexed and fixed in stereotactic head holder and the thoracolumbar spinal cord was stabilized by proximal and distal vertebral clamps. At both sites, the dura was removed and the spinal cord covered with mineral oil in order to prevent drying. After the spinal cord was prepared, the vaporizer was set to deliver 1% halothane and maintained at this level throughout the entire study period. A bipolar, concentric stimulating electrode (SNE 100; Rhodes Medical Instruments, Woodhills, CA) was inserted into the right ventrolateral first cervical (C1) segment for antidromic stimulation. Extracellular dorsal horn cell recordings were made using a tungsten parylene-coated electrode (1–1.5 m $\Omega$  impedance; Microprobe, Clarksburgh, MA) inserted through a small hole in the pia mater of the left lumbar enlargement between L4 and L6. The left hind leg and foot were extended and stabilized by imbedding the dorsum of the foot in clay so that the plantar aspect could be studied. Because the

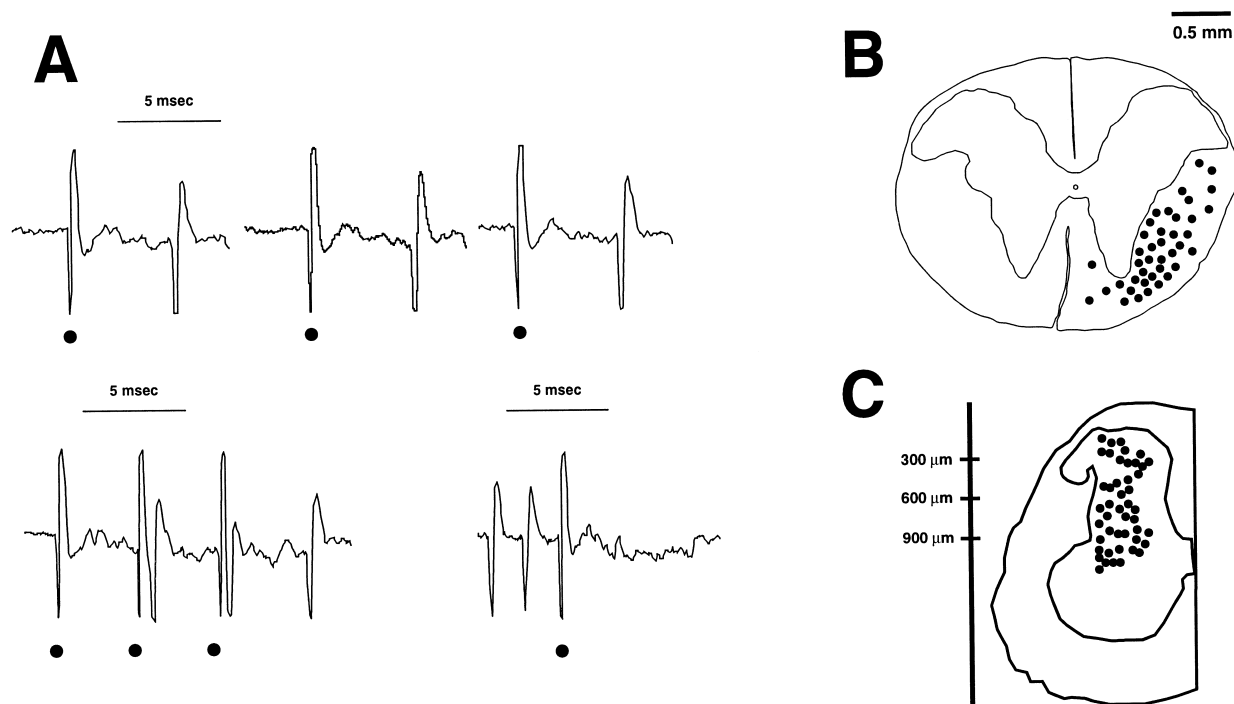


Fig. 2. (A) Criteria for antidromic stimulation. Antidromic action potential occurs at a constant latency following the stimulus artifact. The dot marks the stimulus artifact. The second row shows antidromic action potentials following a high frequency train of 250 Hz and an antidromic action potential collided with an orthodromic stimulus elicited by stimulating the receptive field. (B) Cross-section of the first cervical segment depicting the contralateral stimulation sites for antidromic activation of lumbar dorsal horn neurons. (C) Cross-section of the spinal cord at the ipsilateral side of the fifth lumbar segment depicting the depths for recordings of each dorsal horn neuron. Each filled circle represents one stimulation or recording site for one neuron.

stimulation of C1 produced neck muscle contraction, muscle paralysis was produced by intermittent intravenous injections of pancuronium bromide (0.3 mg/kg). One cell was recorded per rat.

#### Recording of Dorsal Horn Neurons

The search stimulus for dorsal horn neurons recorded from the left side of the spinal cord with ascending axonal projections through the contralateral first cervical segment was 1 mA intensity, 5 Hz, 100  $\mu$ s pulse duration. Antidromically activated cells met the following criteria: (1) The antidromic action potential occurred at a constant latency following 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 one<sup>18</sup> that occurred either spontaneously or was elicited by stimulating the RF (fig. 2A). Six HT cells were not collided with an action potential because this would have required repeated pinching of the left foot with high forces that were potentially tissue damaging. Antidromic stimulation was helpful

in assuring that over prolonged periods of time all recordings were from the same neuron by using action potential shape and antidromic latency. Six cells were recorded that were not antidromic; they were located by tapping the plantar aspect of the foot during electrode advancement. The action potential amplitude and shape were used as guides to assure that the recordings were made from a single neuron throughout the experimental protocol.

Action potentials were amplified (Grass Instruments, Quincy, MA), monitored *via* a storage oscilloscope, and discriminated (BAK Electronics, Germantown, MD) on the basis of amplitude and waveform. On-line analysis was made using a 1401 Plus Laboratory Interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK) installed on a desktop computer. The rates of cell discharge, unit trace (representing an individual action potential), and event marker were displayed on the computer screen. The oscilloscope trace and marker were also stored on videotape (A.C. Vetter, Rebersburg, MD) for backup and to allow more detailed analysis later.

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### *Experimental Protocol*

Cells were selected for continued study if the activity of the neuron was increased by brushing or pinching the left foot. Each cell was classified as WDR or HT based on responses to innocuous brushing (camel hair brush no. 6) and noxious pinch.<sup>19,20</sup> HT cells were identified as cells that only responded to pinch but not to brush. WDR neurons responded to brush and had an even greater response to pinch. Because previous studies showed that low threshold cells did not sensitize following an incision<sup>21</sup> or after other injuries,<sup>12,22</sup> these cells were not studied.

Next, areas of the RF responding to weak mechanical stimuli of all cells were identified by using a brush, pinch, and von Frey filaments with low bending forces (15–98 mN). For HT cells, stronger filaments were necessary. The extent of the brush RF was identified by stroking the ipsilateral and contralateral foot, hamstring, and calf regions. A series of calibrated von Frey filaments (Stoelting, Wood Dale, IL) was applied in ascending order (3, 6, 11, 14, 30, 42, 65, 73, 98, 149, and 265 mN) at the most sensitive site. To perform this stimulus response function, each filament was applied once for 3–5 s, with 10 to 30 s between applications. Forces greater than 265 mN were not used to avoid injury to the RF. If no cell response occurred to 265 mN, we assigned the threshold as greater than 265 mN. The next filament, 637 mN, provides the approximate cut-off force used in previous behavioral experiments. The change in cell activity was calculated as the peak increase in cell activity, associated with the onset of filament application, minus background activity (the 10-s average activity prior to filament application) and expressed in impulses per second (imp/s). The mechanical threshold of the dorsal horn cell was defined as the lowest force that caused either activation of the cell if no spontaneous activity was present or an increase in cell activity by at least two standard deviations above background activity. Also, the next strongest filament must also have excited the cell using the same criteria. The peak increase in cell activity during filament application (using a bin width of 2 s) was plotted versus the forces of the von Frey filament applied to create a stimulus response function.

In this study, we attempted to place the incision in areas responding to strong mechanical stimuli with von Frey filament thresholds greater than 265 mN and unresponsive to the blunt mechanical stimulus. This was typically in a pinch-responsive zone or at the edge of the pinch RF as shown in figure 1. The 265-mN filament was applied to several areas adjacent the intended incision to

identify punctate zones unresponsive to this filament. Anywhere from two to four sites were usually studied. Similarly, one or two punctate sites within the pinch RF or at the edge of the pinch RF at least 1 cm away from the intended incision were studied. The goal was to determine if any of these mechanically insensitive areas could become responsive after incision. The lowest force producing a response adjacent to the incision and the lowest force producing a response distant to the incision at a mechanically insensitive area were also reported. Similar adjacent and distant sites were used to describe zones of primary and secondary hyperalgesia using withdrawal thresholds in behavioral studies.<sup>21</sup>

Next, a blunt mechanical stimulus (5-mm plastic disk attached to 400-mN von Frey filament), as described in our behavioral experiments,<sup>6</sup> was applied once for 3 to 5 s at two or three places directly on the intended incision site and once 1 to 1.5 cm distant from the planned incision. A positive response of the dorsal horn cell caused by the blunt mechanical stimulus was defined as described for von Frey filaments. The greatest increase in cell activity by the blunt mechanical stimulus after incision observed at one site directly on the wound was reported.

The pinch RF of dorsal horn neurons typically extends beyond the border of the area responding to weak mechanical stimuli. The extent of the RF to pinch in the glabrous skin of the foot was mapped by pinching with a small, curved forceps. A ring forceps, 1.5 cm in diameter, was used to pinch the skin and deep tissues of the tail, calf, hamstring, and contralateral hindquarters to determine the extent of the pinch RF. Next, the location of the intended incision was determined, depicted on a schematic of the rat's foot, and if possible, located in a mechanically insensitive area as defined.

At the end of the initial descriptive phase of the experiment, the baseline spontaneous activity was recorded for a 5-min period, and then a 1–1.5-cm longitudinal incision was made as described previously.<sup>5</sup> In most cases, the incision included injury of cutaneous and deep tissues. The cell activity was recorded continuously during the incision and for approximately 1 h after incision. Spontaneous activity after incision was considered increased if the average rate 55 to 60 min after incision increased by more than two standard deviations over preincision activity.

Thereafter, application of brush, the blunt mechanical stimulus, and von Frey filaments was repeated at the same areas tested before. Finally, the size of the pinch RF was reassessed by pinching the foot with a small curved



forceps or pinching throughout the hindquarters with a ring forceps.

#### *Histologic Methods*

At the end of the experiment a lesion was made at the antidromic stimulation site, the rat was euthanized, and the cervical spinal cord was removed and fixed for at least 2 weeks in 10% formalin containing saturated potassium ferro- and ferricyanide. Frozen serial sections (40  $\mu\text{m}$ ) were cut, viewed under a microscope for a Prussian blue reaction,<sup>23</sup> and the lesions identified. Locations of the antidromic stimulation sites were depicted on a scale drawing of the spinal cord at C1.

In a separate group of rats, lesions were made in the lumbar enlargement using a recording electrode placed at depths of 300, 600, and 900  $\mu\text{m}$ . Frozen sections (40  $\mu\text{m}$ ) were cut and viewed under a light microscope, and the lesions were identified and depicted on a schematic of an L5 segment. The approximate location of the tip of the recording electrode for each cell was placed on another drawing of the L5 segment,<sup>24</sup> based on the depth of the micropositioner.

#### *Statistical Analysis*

Data are presented as median or mean  $\pm$  SD. Results from these experiments were analyzed by chi-square analysis to determine differences in response characteristics between WDR and HT cells. Differences in threshold between WDR and HT cells were analyzed by a Mann-Whitney rank-sum test. Differences between background activity before and after incision was analyzed using a Wilcoxon signed-rank test. Comparisons between cells undergoing sham incision versus incision were made using a chi-square test.  $P < 0.05$  was considered statistically significant.

## Results

Fifty dorsal horn neurons, receiving sensory input from the plantar aspect of the rat foot, completed the incision protocol. For some neurons, every test stimulus could not be applied to each cell; the number of cells completing each part of the protocol are reported. All neurons had RFs that included at least part of the plantar aspect of the left foot. The antidromic stimulation sites were located in the ventrolateral and ventromedial part of C1 and also the intermediate region (fig. 2B). In six rats, no clear lesion could be identified. The average antidromic stimulation threshold was  $110 \pm 20 \mu\text{A}$  (range, 40–330

$\mu\text{A}$ ). Twenty-nine WDR and 21 HT were located at depths from the surface of the spinal cord ranging from 260–970  $\mu\text{m}$  and 140–1200  $\mu\text{m}$ , respectively (fig. 2C).

#### *Characteristics of Dorsal Horn Neurons*

WDR neurons ( $n = 29$ ) had an average increase in cell activity by brush and pinch of  $8.4 \pm 5.5$  and  $20.9 \pm 13.4$  imp/s, respectively. Twenty-one cells only responded to pinch and were therefore categorized as HT neurons. The average increase in activity of all HT neurons during a moderate pinch of the RF was  $12.5 \pm 6.4$  imp/s. Eight HT neurons were activated by pinch of cutaneous tissue of the foot; 13 HT cells were not excited by pinch of skin but were activated by pinch that included deep structures.

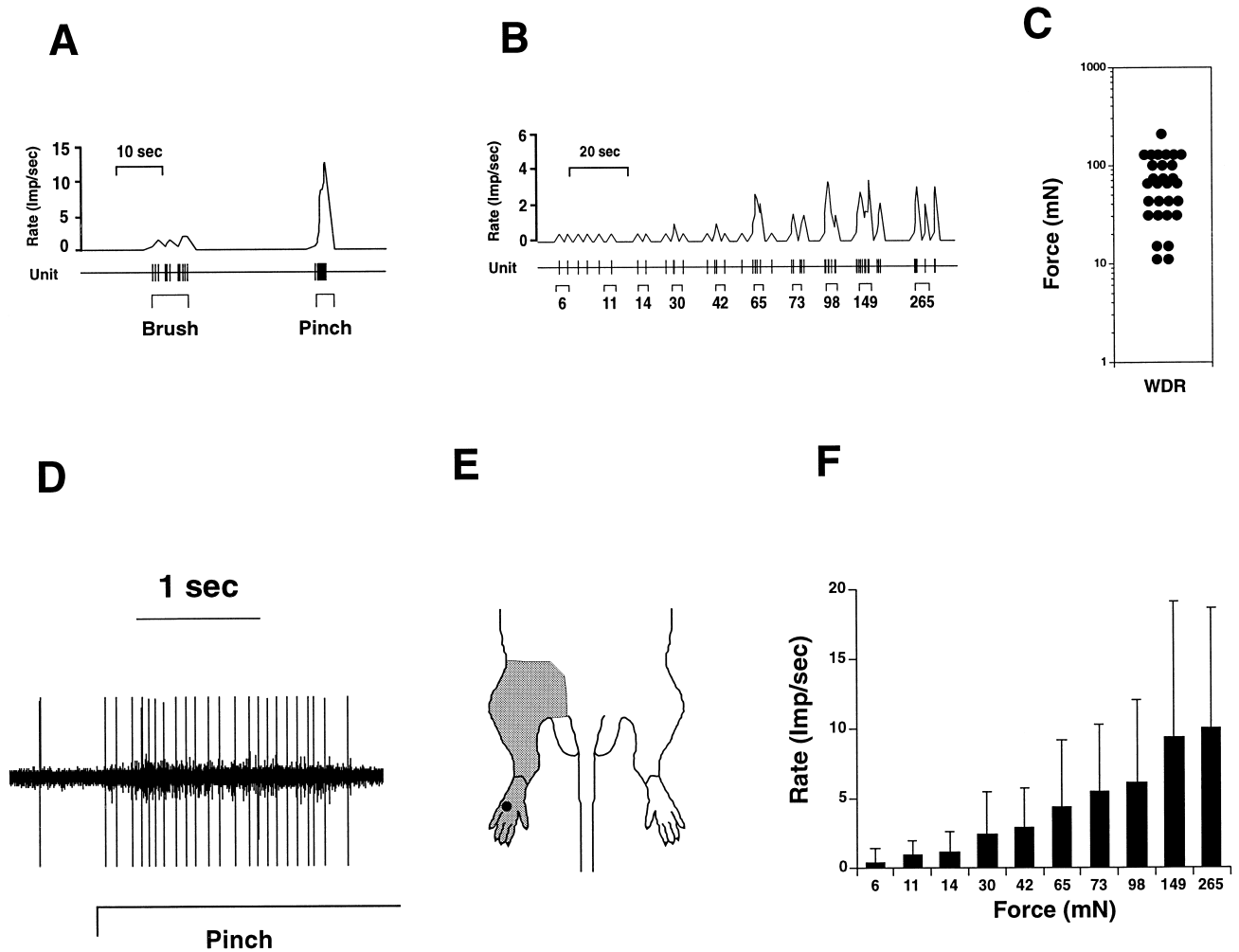
An example of a WDR neuron is shown in figures 3A–3F. This cell responded to both brush and pinch (fig. 3A). The von Frey filament threshold was 30 mN, and greater filament strengths produced increased cell activity (fig. 3B). The median mechanical threshold of 29 WDR neurons was 65 mN (range, 11–149 mN). Overall, in 29 stimulus-response functions from WDR cells, increasing the punctate force was accompanied by greater average increases in cell activity.

Figures 4A–4F show an example of an HT neuron. This cell responded only to pinch (fig. 4A); the von Frey filament threshold was 149 mN (fig. 4B). The median mechanical threshold of these 21 HT neurons was 637 mN (range, 149–637 mN, fig. 4C). The thresholds of HT neurons were greater than WDR neurons ( $P < 0.05$ ). In 21 stimulus-response functions, few responses were observed except for the greatest filaments. Because no differences between HT cells with cutaneous *versus* deep input were apparent after incisions that included cutaneous and deep injury when possible, further evaluations combined HT cells with cutaneous and deep inputs.

#### *Background Activity after an Incision*

An incision produced a burst in activity of most of the neurons, which usually persisted throughout the actual incision and then slowly decreased. In 41 cells (23 WDR, 18 HT), the activity 1 h later did not increase ( $6.3 \pm 8.2$  to  $6.0 \pm 7.6$  imp/s). Activity was increased in only six WDR and three HT neurons ( $0.3 \pm 0.5$  to  $4.6 \pm 5.1$  imp/s); thus, an incision performed in a mechanically insensitive zone of the RF did not produce an increase in background activity of most WDR and HT cells. An example of a WDR neuron with a sustained increase in activity is shown in figure 5A. There was no difference

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**Fig. 3.** Characterization of a wide dynamic range (WDR) neuron. (A) Response of the neuron to brush and pinch of the somatic field. (B) Responses to von Frey filaments; the threshold was 30 mN, and greater filament strengths produced increased cell activity. (C) von Frey filament thresholds of 29 WDR neurons. (D) Digitized oscilloscope trace of the action potentials during pinch. (E) Drawing depicting the somatic field; the gray area is the region responding to pinch. The black dot represents the site of application of the von Frey filaments in (B). (F) Summary of the average peak increase in action potential rate produced by application of von Frey filaments to 29 WDR neurons.

between the cell types (WDR *vs.* HT) that increased or did not increase background activity (fig. 5B).

#### Punctate Mechanical Stimulus

In behavioral experiments before incision,<sup>6</sup> rats did not withdraw to a median punctate force of less than 522 mN (range, 199–522 mN), but after incision the withdrawal threshold was less than 100 mN. If areas of the RF unresponsive to 265 mN become responsive to filaments less than 100 mN after incision, this could represent a withdrawal response in behavioral studies. Responses of 28 WDR and 21 HT neurons were studied after an incision was

made in a mechanically insensitive area unresponsive to filaments of 265 mN or less. Figures 6A–6C show an example of a WDR neuron before and after incision. The most sensitive area of the RF was the heel. An incision in the plantar foot decreased the mechanical threshold at the site marked adjacent to the incision from greater than 265 mN to 73 mN (figs. 6A and 6B). The pinch RF of this WDR neuron expanded from the foot before incision to the ipsilateral calf and hamstring region after incision (not shown). The punctate mechanical threshold of 9 of 28 WDR neurons (fig. 6D) decreased from greater than 265 mN to 98 mN or less; von Frey filament forces of 98 mN or

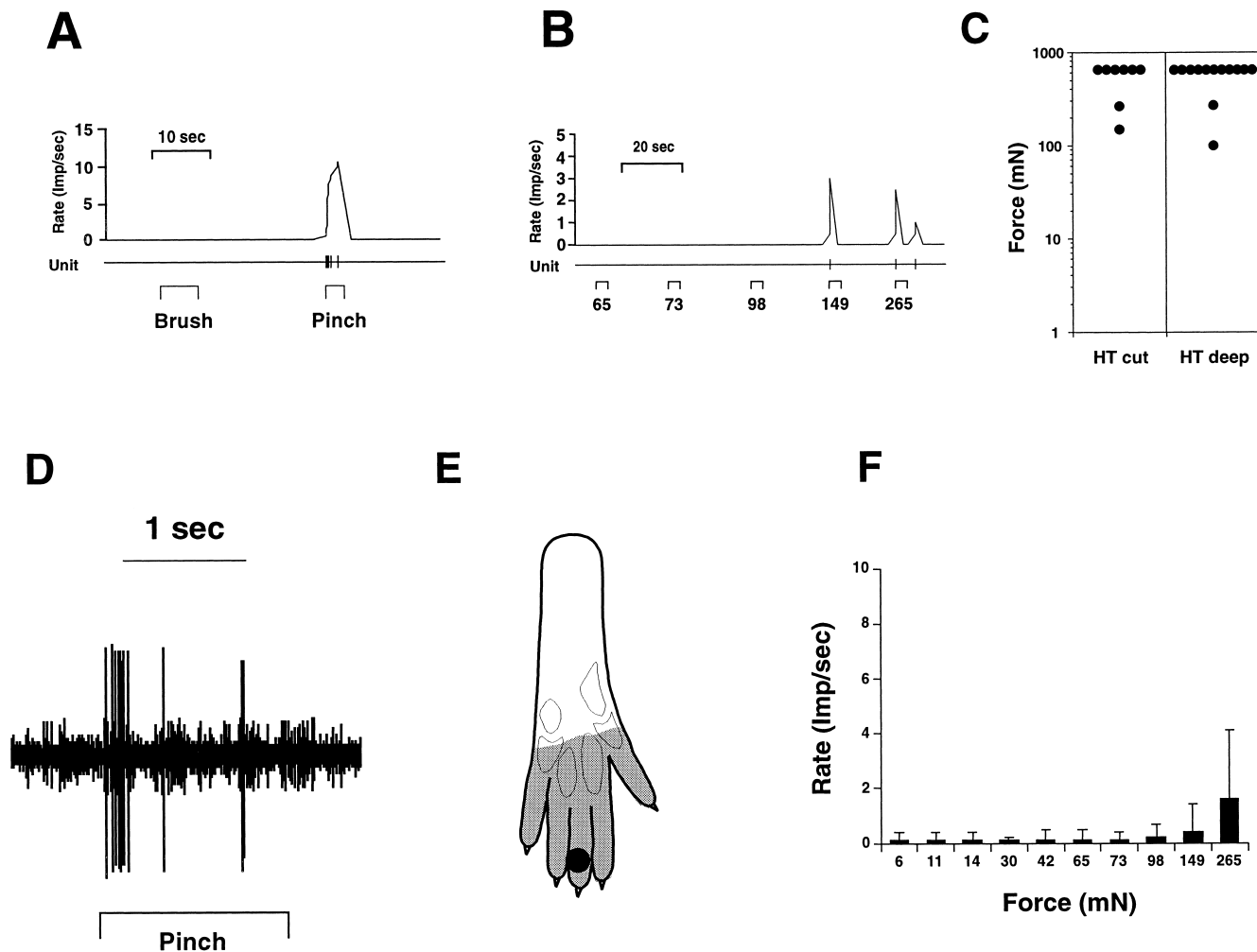


Fig. 4. Characterization of a high threshold (HT) neuron. (A) Response of the neuron to brush and pinch of the somatic field. (B) Responses to von Frey filaments; the von Frey filament threshold was 149 mN. (C) Von Frey filament thresholds of 21 HT neurons. HT cut = neurons responding to pinch of the skin; HT deep = neurons not responding to pinch of skin but responsive to pinch of cutaneous and deep structures. (D) Digitized oscilloscope trace of the responses to pinch. (E) Drawing depicting the somatic field; the gray area is the region responding to pinch. The black dot represents the site of application of von Frey filaments in (B). (F) Summary of the average peak increase in action potential rate produced by application of von Frey filaments to 21 HT neurons; in general, few responses were observed except for the greatest filaments.

less cause withdrawal responses after an incision.<sup>5,6</sup> Only three HT neurons decreased punctate mechanical threshold from greater than 265 mN before incision to 265 mN or less after incision (fig. 6E), none to less than 100 mN. There was a greater tendency for thresholds of WDR neurons to decrease than HT neurons ( $P < 0.05$ ) when tested in this manner.

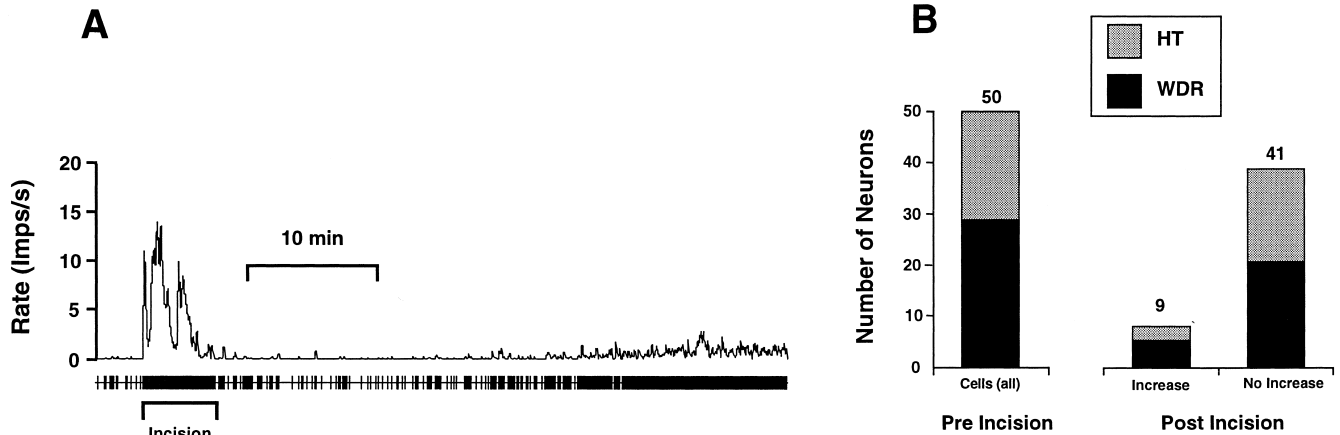
Because reduced withdrawal thresholds occur at sites distant to the incision in behavioral experiments,<sup>5,7</sup> responses of WDR and HT neurons to von Frey filaments applied distant to the incision were studied (figs. 7A-

7E). The punctate mechanical threshold of 10 of 27 WDR neurons decreased from greater than 265 mN to 265 mN or less after an incision in a mechanically insensitive area. Most of the WDR neurons decreased threshold distant to the incision by only one filament. HT neurons were less likely to change their responsiveness ( $P < 0.05$  vs. WDR).

#### Blunt Mechanical Stimulus

Cell responses to the blunt mechanical stimulus applied directly on the intended incision were tested in 22

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**Fig. 5.** Effect of an incision in a mechanically insensitive area of the plantar aspect of the rat hind paw on background dorsal horn cell activity. (A) An example of a wide dynamic range neuron with a sustained increase in activity produced by an incision. (B) Summary of all cells examined for an increase in background activity. HT = high threshold; WDR = wide dynamic range.

WDR and 21 HT neurons (figs. 8A–8E). Eighteen WDR neurons, previously unresponsive to the blunt mechanical stimulus, were activated by this stimulus after the incision. Twenty-one HT neurons did not respond to the blunt mechanical stimulus before incision; only one cell was activated by this stimulus afterward. There was a greater tendency for WDR neurons to respond to the blunt mechanical stimulus after incision ( $P < 0.05$  vs. HT). Cell responses to a blunt mechanical stimulus applied distant to the incision were tested in 30 neurons (12 WDR and 18 HT); a positive response after the incision was found in only one WDR neuron (data not shown).

#### Receptive Field

Prior to incision, the brush RF was mapped with a camel-hair artist's brush and reevaluated after incision in 39 cells (19 WDR and 20 HT). An enlargement of the brush RF into uninjured skin occurred in only three neurons, all of which were WDR cells. HT neurons did not respond to brush outside the injury after incision.

An expansion of the pinch RF outside the area of injury was found in 31 of 49 (20 WDR, 11 HT) neurons. No increase in pinch RF distant to the wound was detectable in 8 WDR and 10 HT neurons. Various patterns of pinch RF enlargement are shown in figure 9.

#### Sham Incision

Ten additional dorsal horn neurons from nine rats were studied that were similar to those undergoing plantar incision. After recording background activity for 5

min, a sham incision was made. This consisted of marking the chart recorder for a 10-min period, the approximate time it would normally take to make an incision. Changes in background activity, RF size, and responsiveness to mechanical stimuli were examined as if the plantar incision had been made. Of these 10 cells, eight were WDR neurons and two were HT. Seven were antidromic from the C1 region and three were identified by touching or tapping the plantar aspect of the foot. The activity of one cell increased from 0.3 to 3.9 imp/s 55 min after sham incision. The remaining cells did not increase their background activity. Overall, the activity before incision was  $6.1 \pm 7.0$  imp/s and after sham incision was  $7.2 \pm 9.2$  imp/s. The blunt mechanical stimulus was applied to three areas along the intended sham incision site; none of the neurons was responsive before incision, and none became responsive after the sham incision. Two of the 10 cells became responsive to the 265-mN filament adjacent to the sham incision. Both were WDR neurons. The RF of one WDR neuron expanded to the contralateral calf and hamstring after sham incision. There was a greater tendency for cells undergoing incision to respond to the blunt mechanical stimulus, decrease their response threshold to less than 265 mN, and expand the pinch RF compared with the sham group ( $P < 0.05$ ).

#### Discussion

The most remarkable finding of this study was that changes in RF properties of some WDR neurons occurred and resulted in mechanically insensitive areas



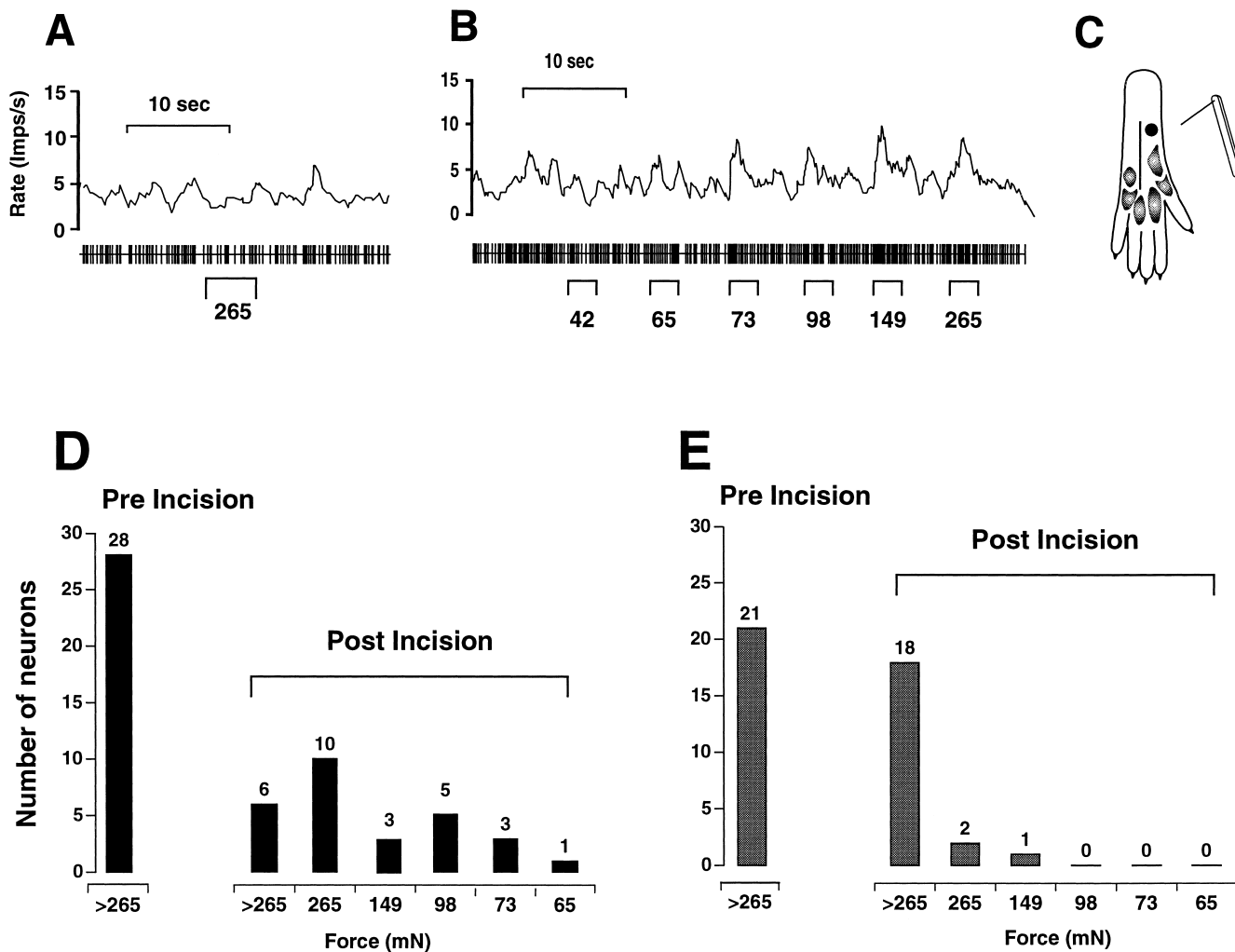


Fig. 6. Response to von Frey filaments applied adjacent to the incision in a mechanically insensitive area of wide dynamic range (WDR) and high threshold (HT) neurons. (A,B) Cell responses to von Frey filaments before and after incision. (C) The location of the incision on the plantar hind paw; the black dot represents the site of application of the von Frey filaments. (D) The number of WDR neurons with reduced thresholds after an incision. (E) The number of HT neurons with reduced thresholds after an incision. There was a greater tendency for WDR cells to have decreased thresholds after incision.  $P < 0.05$  WDR vs. HT.

becoming more responsive to lower forces after an incision; few or no changes were found in HT cells. Similarly, increased cell responses to the blunt mechanical stimulus applied directly on the wound occurred in WDR neurons. Less notably, an incision made in a mechanically insensitive area did not produce an increased background activity in most cells from both groups. Decreased mechanical thresholds to punctate stimuli and increased cell responses to the blunt mechanical stimulus distant to the incision did not occur in most cells. Expansion of the pinch RF into uninjured areas was common and similar in both WDR and HT neurons.

Although it is generally accepted that both classes of

dorsal horn neurons, HT and WDR, are important in the processing of nociceptive information, disparate results have been reported as to which of these cell types become sensitized after tissue injury. Mechanical thresholds of only WDR neurons decreased after prolonged noxious pinch of the tail in rats.<sup>25</sup> Dougherty *et al.*<sup>26</sup> observed increased cell responses to brush and pressure after capsaicin injection in WDR neurons. Both studies reported fewer changes in HT neurons. In contrast other investigators observed a decrease in mechanical thresholds of HT neurons after chemical irritation with mustard oil<sup>12,13</sup> or capsaicin,<sup>14,27</sup> after inflammation,<sup>28-31</sup> and after burn injury.<sup>11,32</sup> These results suggest that conver-

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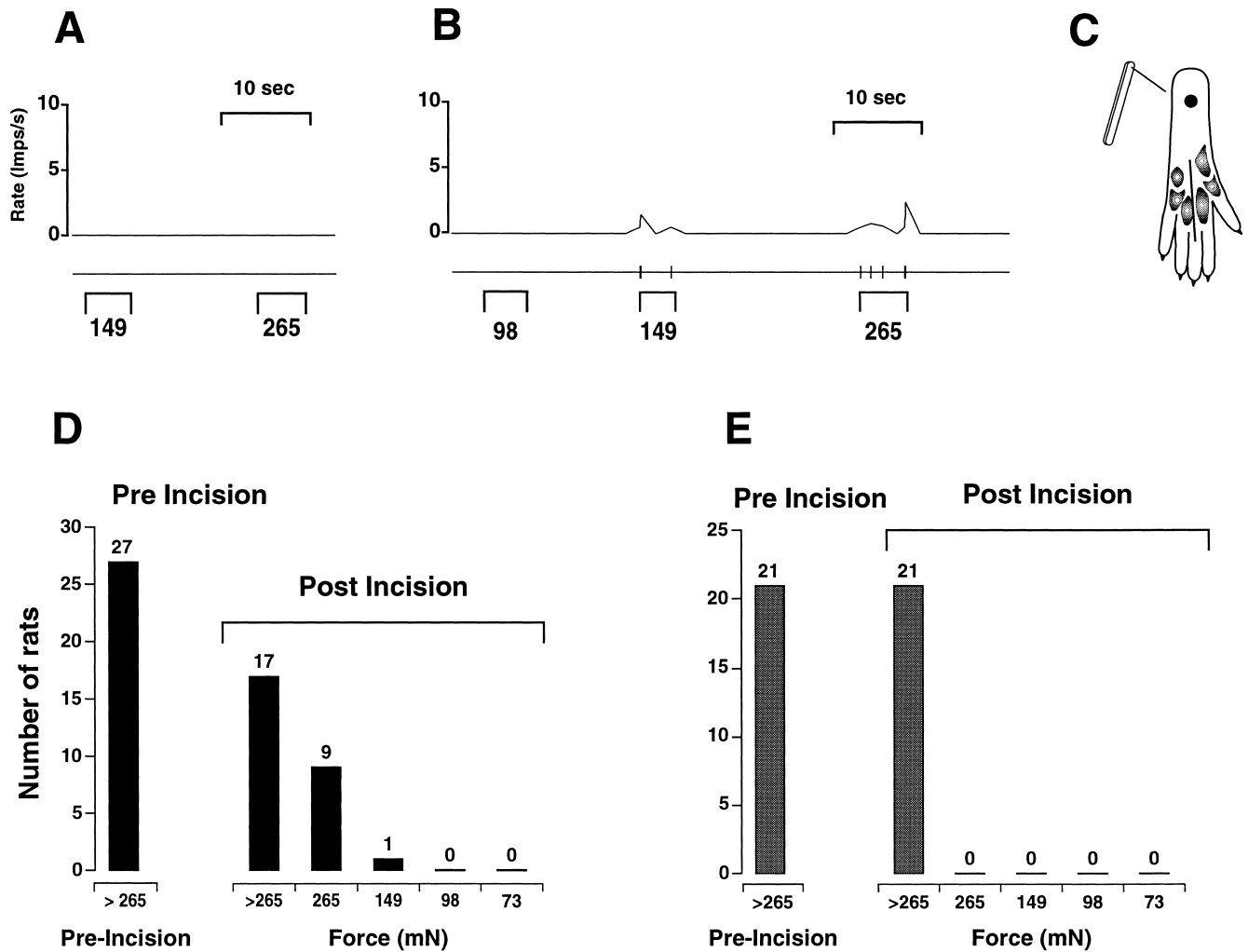


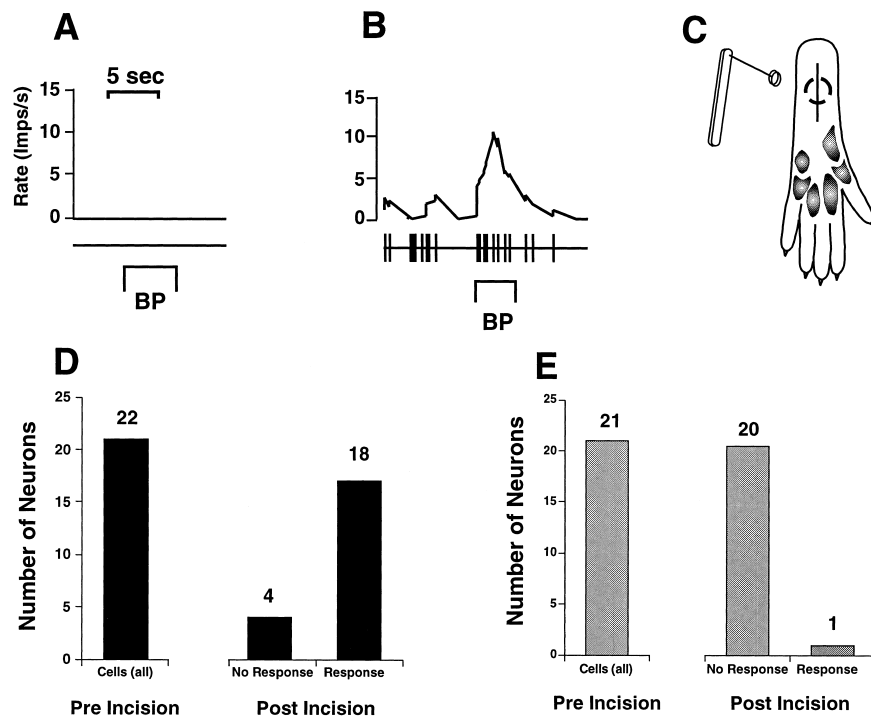
Fig. 7. Responses of von Frey filaments applied distant to the incision in a mechanically insensitive area of wide dynamic range (WDR) and high threshold (HT) neurons. (A,B) Responses to von Frey filaments in a WDR neuron before and after incision. (C) The location of the incision on the plantar hind paw; the black dot represents the site of application of the von Frey filaments. (D) The number of WDR neurons with reduced thresholds at the distant site after an incision. (E) The number of HT neurons with reduced thresholds at the distant site after an incision. There was a greater tendency for WDR cells to have decreased thresholds at the distant site after incision.  $P < 0.05$  WDR vs. HT.

sion of HT to WDR-like neurons occurred by recruitment of input from low threshold mechanoreceptors<sup>12,13</sup> and could therefore transmit pain behaviors to low intensity mechanical stimuli after injury.

Sensitization is the neurophysiologic phenomenon that corresponds to the psychophysical appearance of hyperalgesia. Mechanical hyperalgesia has been demonstrated in postoperative patients.<sup>2-4</sup> This is similar to incision-induced reduction in withdrawal thresholds observed in preclinical behavioral experiments and is likely manifested by activation and perhaps sensitization of dorsal horn neurons innervating the plantar rat foot. One

possible scenario for the reduced withdrawal threshold might be that areas of the RF of WDR neurons with thresholds at least as great or approximating the median withdrawal threshold in our behavioral studies before incision (522 mN) become responsive to forces less than 100 mN (the upper range of the withdrawal threshold) after incision. This is one configuration for testing the neural mechanisms of primary hyperalgesia proposed by Treede *et al.*<sup>33</sup> and used by others to convert HT to WDR-like neurons after other injuries.<sup>11-13,22</sup> Therefore conversion of mechanically insensitive areas of the RF to mechanically sensitive areas occurs in some WDR neu-

**Fig. 8.** Responses to a blunt mechanical stimulus applied on the incision of wide dynamic range (WDR) and high threshold (HT) neurons. (A,B) Responses to the blunt mechanical stimulus before and after incision. (C) The location of the incision; the circle represents the site of application of the blunt mechanical stimulus. (D) The number of WDR neurons with an increase in activity to the blunt mechanical stimulus after incision. (E) The number of HT neurons with an increase in activity to the blunt mechanical stimulus after incision. There was a greater tendency for WDR cells to develop responses to the blunt mechanical stimulus after incision.  $P < 0.05$  WDR vs. HT.



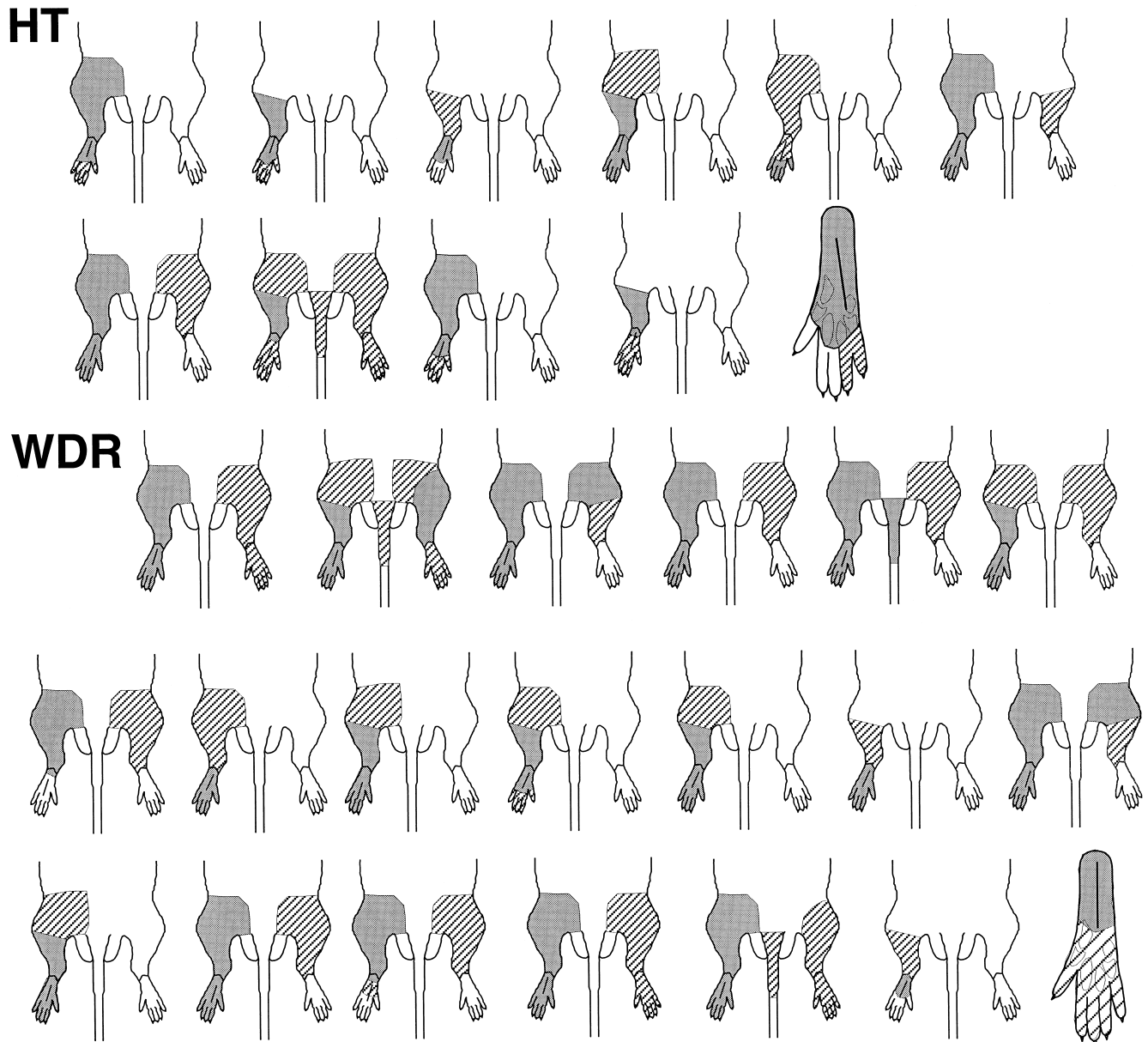
rons after an incision and could transmit the withdrawal response observed behaviorally after incision. Preliminary data also suggest that an incision placed adjacent to areas of the RF of WDR neurons responsive to weak mechanical stimuli can cause enhanced responses of dorsal horn neurons to von Frey filaments less than 100 mN. HT cells did not become responsive to filaments less than 100 mN after incision.<sup>21</sup> Therefore, both processes, insensitive areas of the RF of WDR neurons becoming responsive to weak mechanical stimuli after incision and areas responding to weak mechanical stimuli exhibiting greater responses after incision, could mediate the reduced withdrawal responses observed behaviorally.<sup>21</sup>

Secondary hyperalgesia, characterized by applying the test stimulus distant to the area of injury, is suggested by behavioral studies using the plantar incision,<sup>5</sup> occurs in clinical postoperative states,<sup>4</sup> and has been shown after a variety of other tissue injuries.<sup>3,4</sup> Evidence indicates that secondary hyperalgesia develops as a result of central sensitization. The median withdrawal thresholds to von Frey filaments applied 1–1.5 cm distant to the wound decreased from 522 mN to 198 mN or less, but these withdrawal thresholds tended to be greater than those adjacent to the incision.<sup>7</sup> WDR and HT neurons were tested in an area distant to the incision where thresholds were again at least as great as the cut-off value

used in our behavioral studies (> 265 mN). A decrease in mechanical threshold to less than 265 mN was rare. In addition, an incision sensitized WDR and HT neurons, causing expansion of the RF to pinch, an intense stimulus; the brush RF was rarely extended by the incision. This lack of evidence for greatly reduced thresholds in distant areas of the RF of dorsal horn neurons may occur because the incision is made in a mechanically insensitive area and may not cause sufficient afferent drive to decrease distant responsiveness greatly. Alternatively, a distant incision could increase responsiveness at the sensitive zone—this was not studied in the present experiment.

It has been suggested that hyperalgesia to pressure, observed by several investigators after a variety of tissue injuries,<sup>3,4,35</sup> could be mediated by mechanisms distinct from punctate mechanical hyperalgesia. The force applied is over a larger area and may include spatial summation. In the present study, these mechanically insensitive areas did not respond to the blunt mechanical stimulus before incision; responses after incision were observed in WDR neurons. In behavioral experiments, rats did not respond to this blunt mechanical stimulus before incision, but pain behaviors suggested by decreased weight bearing to this same blunt mechanical stimulus were observed after incision.<sup>6</sup> These results

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**Fig. 9.** Patterns of pinch receptive field (RF) expansion outside the area of injury in 11 high threshold (HT) neurons and 29 wide dynamic range (WDR) neurons after an incision in a mechanically insensitive area of the cell RF. The shaded areas of the rat hind paw depict the pinch RF before an incision (*dark line*) and the hatched zones mark the expansion of the pinch RF after an incision.

suggest that the change of initially mechanically insensitive areas to areas responsive to the blunt mechanical stimulus could be one mechanism for primary pressure hyperalgesia after incision. No cell responses were observed when the blunt mechanical stimulus was applied distant to the incision. These results are in agreement with findings from our behavioral studies<sup>7</sup> and observations by others after a variety of tissue injuries<sup>36,37</sup> that

pain or pain behaviors to a blunt mechanical stimuli do not occur distant to an injury.

An increase in background activity, a manifestation for activation of central neurons, has been observed after a variety of injuries. In the present study, an increased background activity after incision was observed in only 9 of 50 dorsal horn neurons. In a preliminary study,<sup>21</sup> approximately 40% of dorsal horn neurons increased

background activity after an incision made adjacent to the area of the RF responding to the weakest mechanical stimulus. This indicates that an incision made in a mechanically insensitive zone tended to increase background activity in fewer cells probably because there is less primary afferent activation.

The experimental set-up used in the present study to assess responses of dorsal horn neurons to incision has some limitations. First, all experiments were performed using 1% halothane in oxygen anesthesia. Yamamori *et al.*<sup>38</sup> reported that the administration of 1% halothane reduced low threshold RF size of WDR neurons without changing the RF responding to HT stimuli; other investigators found that greater halothane concentrations (approximately 2%) increased mechanical thresholds of WDR neurons in sheep.<sup>39,40</sup> No difference in sensitization of rat dorsal horn neurons to prolonged pinch of the tail occurred among three different preparations, halothane anesthetized (1%), pentobarbital anesthetized, and decerebrated-unanesthetized rats.<sup>25</sup> In the present experiments, 1% halothane anesthesia was chosen because it is similar to that which patients receive during most surgeries. A second limitation may be that possible changes in responsiveness of dorsal horn neurons to incision are underestimated because halothane is continued, whereas behavioral experiments used to correlate with the neurophysiologic phenomenon are performed in unanesthetized rats. Third, a preparative spine dissection and laminectomy are required; it is possible that this preparation already sensitizes dorsal horn neurons or may enhance inhibitory inputs.<sup>41</sup> The size of the laminectomy was limited, and no injury was present in the RF of the recorded neurons before plantar incision. Finally, some of the HT neurons received input from deep tissue like muscle and joint. Despite an incision of skin, fascia, and muscle at the plantar aspect of the foot, these neurons may be difficult to sensitize to mechanical stimuli.

## Conclusion

These data suggest that conversion of mechanically insensitive areas of WDR neurons to areas responsive to weak mechanical stimuli could contribute to pain behaviors caused by punctate and blunt mechanical stimuli. The experimental configuration, placing an incision in a mechanically insensitive area and testing distant to the incision, does not clarify pain behaviors to stimuli ap-

plied distant to the incision (secondary hyperalgesia); other scenarios must be examined. In the present experiments, HT neurons did not convert to WDR-like cells when tested with von Frey filaments, blunt mechanical stimuli, and brush, indicating that an incision has distinct sensitization characteristics compared with other injuries.

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