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Comparison of the Immediate Effects of Surgical Incision on Dorsal Horn Neuronal Receptive Field Size and Responses during Postnatal Development

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Background: **Pain behavior in response to skin incision is developmentally regulated, but little is known about the underlying neuronal mechanisms. The authors hypothesize that the spatial activation and intensity of dorsal horn neuron responses to skin incision differ in immature and adult spinal cord.**

Methods: **Single wide-dynamic-range dorsal horn cell spike activity was recorded for a minimum of 2 h from anesthetized rat pups aged 7 and 28 days. Cutaneous pinch and brush receptive fields were mapped and von Frey hair thresholds were determined on the plantar hind paw before and 1 h after a skin incision was made.**

Results: **Baseline receptive field areas for brush and pinch were larger and von Frey thresholds lower in the younger animals. One hour after the incision, brush and pinch receptive field area, spontaneous firing, and evoked spike activity had significantly increased in the 7-day-old animals but not in the 28-day-old animals. Von Frey hair thresholds decreased at both ages.**

Conclusions: **Continuous recording from single dorsal horn cells both before and after injury shows that sensitization of receptive fields and of background and afferent-evoked spike activity at 1 h is greater in younger animals. This difference is not reflected in von Frey mechanical thresholds. These results highlight the importance of studying the effects of injury on sensory neuron physiology. Injury in young animals induces a marked and rapid increase in afferent-evoked activity in second-order sensory neurons, which may be important when considering long-term effects and analgesic interventions.**

ACUTE pain resulting from surgery in patients of all ages is commonly encountered in clinical practice. Advances in understanding mechanisms of acute postoperative pain in adults have brought about a better understanding of treatment options and their impact on morbidity. Despite these advances, acute pain, and postoperative pain in particular, continues to result in increased healthcare costs in children and adults by increasing morbidity and prolonging hospital stay, directly effecting health care resources.[1] Postoperative pain and its treatment in infants and children are of particular concern because they have received much less attention, and our understanding of acute pain mechanisms during development remains poor.

In the United Kingdom, there are approximately 500,000 surgical procedures necessitating anesthesia in children each year, whereas in the United States, it is estimated to be well over 3 million.[2] A direct result of children undergoing procedures is the subsequent acute pain associated with tissue trauma. Many of these children are very young at the time of tissue injury. Children who undergo painful procedures have been shown to have altered responses to stimuli long after the initial procedure.[3] This occurs as a generalized response and is not necessarily restricted to the area where the previous pain was initiated.[4,5] Other important potential effects of acute pain in early life may include long-lasting changes in behaviors such as have been observed in animal models and which may be permanent.[6–8] There is no known correlate of these sequelae in adults.

The study of central nociceptive processing has highlighted important differences in the neurobiology of nociceptive circuits in the young.[9,10] Developmental differences in the pattern and intensity of dorsal horn cell responses to noxious and inflammatory stimuli have been observed,[11,12] and it is becoming clear that in many cases, pain and analgesia in young patients are likely to differ substantially from those in adults. Whether this holds true for the pain of surgical trauma, which is a major source of noxious nociceptive input in children, is not clear. Surgical trauma is initiated by the scalpel incision, which immediately activates and damages cutaneous nerve terminals in the region, thereby altering the pattern of postsynaptic activity in central sensory circuits. This pattern of activation can be analyzed by studying the receptive field (RF) properties and the spike activity of individual dorsal horn sensory neurons that receive input from the skin in and around the area of damage. Understanding the developmental profile of this pattern of activation is likely to improve postoperative pain management in children.

The paw incision model has been extremely useful in the study of incision pain.[13–15] In this model, pain from incision, as measured by an increased behavioral response to mechanical and thermal stimuli, occurs in all ages.[13] However, the duration of mechanical hyperalgesia is much shorter in young when compared with adults, and there are substantial differences in the sensi-

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tivity to preoperative local anesthetic block and cyclooxygenase-1 inhibition.[16–18] These findings suggest a postnatal developmental regulation of key factors involved in the initiation, maintenance, and resolution of nociceptive processing after skin incision that requires investigation. In adults, the activity of individual primary afferents and dorsal horn cells has been studied at various times after incision in rats.[19–24] The first hours after incision are marked by a modest sensitization in mechanosensitive Aδ and C fibers,[22] accompanied by increased background activity of subpopulations of dorsal horn cells,[23] changes that are likely to underlie the observed pain behavior.[13] This postincisional central sensitization differs from other types of central sensitization[25] in that it requires a continuous afferent barrage from the periphery[21] and is not dependent on *N*-methyl-D-aspartate.[26] Here, we have used this model of incisional pain to study the postnatal developmental regulation of dorsal horn activity after skin incision in young and adult rats. We have focused on the dorsal horn cell cutaneous RF size and mechanical-evoked activity in the first hours after incision. By performing defined incisions within a known RF, we have been able to analyze how dorsal horn cell activity is altered in different spatial regions of the field. The data provide important insights into how the immediate postsynaptic effects of incision in dorsal horn nociceptive circuits differ with postnatal age and illustrate important developmental differences in responses to different stimulus modalities after surgery.

Materials and Methods

Sprague-Dawley rats of both sexes and aged 7 postnatal days (P7; 1 week) and 28 postnatal days (P28; 4 weeks) were used in this study. All electrophysiology experiments were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Behavior experiments were performed after approval from the Animal Care and Use Committee (Wake Forest University, Winston-Salem, North Carolina).

In Vivo *Electrophysiology*

Rats were anesthetized with a single inhalational anesthetic, 3.5% isoflurane, *via* nose cone. The animals were tracheotomized, placed on a ventilator with 95% O_2 and 5% CO_2, placed in a stereotaxic frame, and held with ear and hip bars. Temperature was maintained with a heated blanket, and the heart rate was monitored throughout by electrocardiogram. The lumbar spinal cord was exposed by laminectomy and held stable with a rostral vertebral clamp, and the dura was removed. After the animal was stabilized, the isoflurane was reduced and kept constant at 1.4%.

Extracellular recordings of dorsal horn cell activity were made in the L4–L5 spinal cord with 10-μm-tip glass-coated tungsten microelectrodes, lowered onto the cord surface under microscopic vision. Vertical tracks were made through the dorsal horn in 2- or 10-μm steps using a microdrive. The average depths of cells in this study were 216 μm from the surface of the spinal cord in the P7 animals and 353 μm in the P28 animals and were therefore classified as cells from deep laminae III, IV, and V.[12] Figure 1 shows a scatter plot of the depth of the cells at the different ages. Recordings were fed into Chart software (AD Instruments, Chalgrove, Oxfordshire, United Kingdom), and data were analyzed using a Chart software spike histogram. All cells were wide-dynamic-range (WDR) neurons responding to the initial search stimulus of stroking the paw and subsequently to both brush and pinch. Single spikes were isolated, and background activity was noted. Any cell that was not a WDR cell (cell responding to only brush or only pinch) was discarded, and any cell that could not be carried throughout the entire testing paradigm was omitted. Throughout the recording period, spike shape was scrutinized to ensure that the same cell was still being recorded. Only one cell was studied per animal. Animals were killed during isoflurane anesthesia with an overdose of sodium pentobarbital at the end of the experiment.

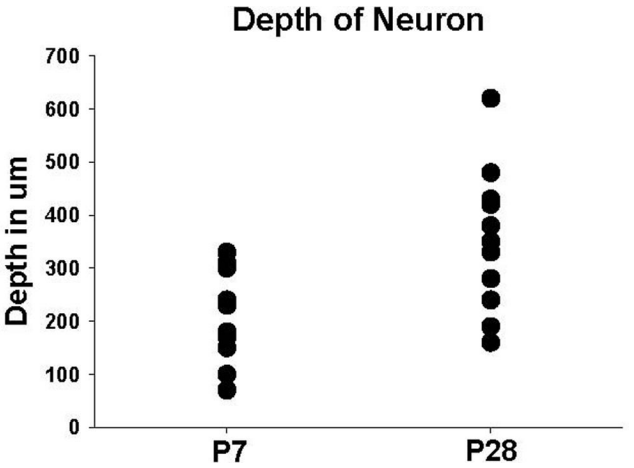

Fig. 1. Scatter plot of the depth of the cells from the surface of the spinal cord at the different age animals. The average depth of the neuron from the postnatal day 7 (P7) animals was 216 μm from the surface of the spinal cord and 353 μm in the postnatal day 28 (P28) animals.

Establishing RF Boundaries. All RFs were located in the middle of the plantar hind paw in both P7 and P28 animals so the center of the RF was as close to the center of the paw as possible (fig. 2). The spatial extent of the cutaneous RF was mapped with a standard fine artist paintbrush for light touch and rounded blunt forceps for pinch. The edge of the field was defined as the area where no spikes were evoked by skin stimulation.

Quantitative Spatial RF Mapping. To map the gradient of cutaneous sensitivity across the RF, pinch and brush stimuli were applied at two defined sites within the RF, and the spike activity was recorded. One site was

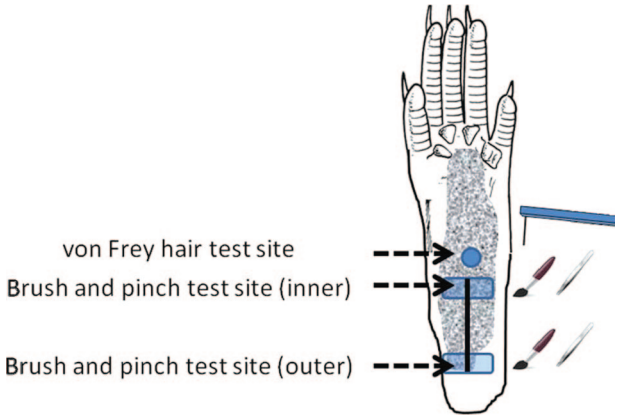

Fig. 2. The paw of the rat and the experimental protocol. After isolation of the cell with the entire receptive field (RF) in the plantar surface of the paw, the RFs to low-threshold brush and high-threshold pinch were mapped. A marker was used to draw the length of the incision (*straight black line* down the center of the foot) relative to the size of the foot from the center of the RF to just outside the RF. The threshold and the responses to threshold and suprathreshold were determined at the center of the RF adjacent to the incision. Brush and pinch RFs were mapped, and responses were recorded in the center of the RF across the incision and at the extent of the incision inside at the outer edge of the RF (*small circles* for the thresholds and *arrows* for the brush and the pinch at the limits of the incision). An incision was made along the line and closed with one suture. One hour later, the same testing paradigm was repeated.

near the RF center (inner), and the other was at the edge of the RF (outer); both were across the skin mark for the incision (fig. 1). The number of spikes evoked by a 1-s pinch and three consecutive brush strokes was recorded at each site.

Measuring Responses to von Frey Hairs. Mechanical RF thresholds were established by sequential application of calibrated von Frey hair filaments of increasing strength to the center of the RF (fig. 1). The RF mechanical sensitivity was also tested at this site by measuring spike activity evoked by a 3-s skin application of both threshold and suprathreshold (three hairs above threshold) von Frey hair filaments.

Experimental Procedures

Upon isolation of single unit spikes, the background activity was noted for 15 min before mapping the boundaries of the cutaneous RF. When the RF boundaries had been established, a line was drawn on the skin that extended from the center of the field to just beyond the outer edge of the pinch field (fig. 2). Quantitative spatial mapping of the RF was then performed by measuring the sensitivity to brush and pinch at two defined site near this line, one in the center of the RF (inner) and one on the RF boundary (outer). A skin incision was then made along the line of the skin mark, such that it went across the center of the RF and reached the outer edge of the RF boundary (fig. 2). The incision was standardized to be one half the size of the original incision used in a previous study, or slightly less than half of the distance from the toe pads to the heel, and was closed using one inverted mattress suture with 5.0 braided silk on an FS2 needle.[15] One hour later, the background activity was again noted, and the above stimulation procedure was repeated.

Behavioral von Frey Hair Testing of Mechanical Threshold

Animals were placed on a mesh floor in a plastic cage and acclimatized to the environment for 20 min before testing. Withdrawal to mechanical stimulation was assessed on the hind foot with application of calibrated von Frey filaments to the foot pad just anterior and lateral to the incision until the filaments bent. The von Frey filaments used were 3.84, 4.08, 4.31, 4.56, 4.74, 4.93, 5.18, 5.46, 5.88, and 6.10, corresponding to 0.5, 0.9, 1.7, 3.7, 5.5, 8.0, 12.4, 21.5, 53.0, and 84 g, respectively. This was done three times, with a positive response determined by brisk withdrawal of the paw. The force resulting in withdrawal with a 50% probability was determined using the up–down method. Withdrawal threshold was determined before surgery and 1 h after surgery. All animals were included in the data analysis, and no animal in the study had a wound dehiscence or infection during the study.

Data Analysis

Baseline and background spikes were counted with a fixed window duration of 30 s. The total number of spikes fired in response to each stimulus was counted, with the duration of the window for spike counting kept at the duration of the stimulus so as to exclude after discharge. This time was consistent over all cells; the window durations were approximately 3 s for von Frey hair filament threshold and suprathreshold, 0.5 s for pinch, and 1 s for brush. Recordings were fed into Chart software, and data were analyzed using a Chart software spike histogram. RF areas were mapped on paper and then digitized, and the area was calculated as percent of the surface area of the paw (surface area being age dependent). The average absolute area of the RF at baseline in P7 animals was 72 mm^2, whereas in the P28 animals it was 220 mm^2.

Data were analyzed using the paired *t* test and adjusted for multiple comparisons using the Bonferroni correction where appropriate for within-groups comparison. For comparison between groups, a change score was calculated by taking the difference between the responses before and after incision. This was analyzed using an unpaired *t* test and is presented as the change score with a 95% confidence interval (CI) where significant. There is inconsistency in the literature regarding presentation and analysis of von Frey filament withdrawal thresholds. Under many circumstances, nonparametric assumptions should be considered with von Frey filament withdrawal threshold analysis and presentation.

This is the case for the von Frey filament threshold response based on WDR neuron response, and therefore threshold is a given filament where the median and range are presented. The Wilcoxon signed rank test was used to test for differences in threshold from incision. This is different from the behavioral withdrawal threshold. The behavior 50% withdrawal threshold in grams is derived from patterns of withdrawal to different von Frey filaments using the up–down method for behavior, and these data achieve interval (continuous) level measurement status. In addition, the results for the inference testing were confirmed using nonparametric testing, Wilcoxon signed rank test and Mann–Whitney test where appropriate, and significance holds up under these circumstances. Therefore, we consider our data on 50% withdrawal threshold to satisfy the assumptions for parametric statistics based on normal distribution and interval (continuous) measurement, and as a result, means and SEs of the means are presented and analyzed with parametric statistics. A paired *t* test was used for analysis of mechanical thresholds, and the group differences were compared using a change score and an unpaired *t* test. Statistics were calculated using JMPIN Version 5.1 (SAS Institute, Cary, NC). No formal power, calculation was performed before this study because the effects of incision had not been studied before. Effect size was determined using standard estimates of the Cohen *d*, whereby the average mean difference was divided by the SD. Significance is $P < 0.05$. Data are presented as the mean ± the SE of the mean.

Results

These experiments required stable extracellular recording of single WDR deep dorsal horn cells for over 2 h to map RF properties before and after skin incision with precision. This was achieved in 12 cells at P7 and 11 cells at P28. Spike shape and amplitude were compared before and 1 h after the skin incision to ensure that the same WDR cell was recorded throughout the experiment. Representative spike traces from P7 and P28 WDR neurons are shown in figure 3.

Background Firing Increases after Skin Incision in P7 but Not P28 Rats

Table 1 shows the mean background firing frequency of WDR neurons before and 1 h after skin incision at P7 and P28. Whereas there was no difference in background firing frequency at the two ages before the incision, at P7 there was a significant (approximately 10-fold) increase in background spike activity 1 h after the incision. This was in contrast to P28, where skin incision had no effect on background firing.

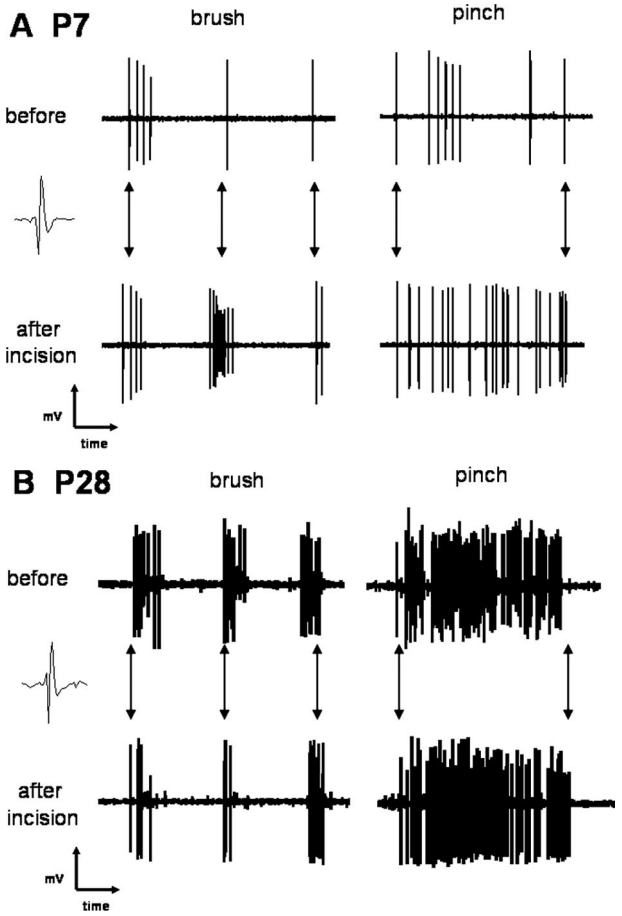

Fig. 3. Representative raw tracings of elicited responses from a single cell in postnatal day 7 (P7) and postnatal day 28 (P28) animals before and 1 h after incision. Multiple spikes are elicited from the stimuli at both ages. The baseline response was more robust in the older animals. A greater increase can be seen for both the brush and the pinch after incision in the P7 cell (*A*), but only a slight difference if any can be seen in the P28 cell (*B*). *Arrows* show the three brush strokes, and *arrows* for the pinch show the beginning of the stimulus and the end of the pinch. The responses of a single cell can readily be seen to pinch with multiple spikes, while the pinch is in place, and then an end to spikes with removal of the stimulus (*A* and *B*) and the multiple spikes elicited with three strokes of the brush (*A* and *B*).

Innocuous Brush and Noxious Pinch RFs Enlarge 1 h after Skin Incision at P7 but Not at P28

The mean brush RF size of WDR neurons at the two ages, at baseline and 1 h after incision, are shown in figure 4A. Consistent with previous reports,[12,27] we observed that the mean baseline RF size for innocuous brush is significantly larger at P7 (31.6 ± 5% of the surface area of the foot) than at P28 (17.5 ± 3.4%). Despite this, 1 h after incision, the brush RF had significantly increased in the P7 rats to 55.5 ± 7% of the surface area of foot. In contrast, there was no significant difference in the brush RF in P28 rats (20.8 ± 3.9%) after the incision.

The same increase was observed in pinch RF size. Figure 4B shows that, like brush RFs, the mean baseline RF size for noxious pinch in P7 WDR cells is significantly

Table 1. Background Firing and Evoked Responses to Brush and Pinch Stimulation before and 1 h after Incision at P7 and P28

	Background	Pinch Inner	Pinch Outer	Brush Inner	Brush Outer
P7, n = 12					
Before	0.2 ± 0.1	15.2 ± 3.9	3.15 ± 1.5	6.7 ± 1.4	0.9 ± 0.3
After	1.9 ± 1.2	37.3 ± 9.3	20.3 ± 4.8	18.9 ± 6.4	9.5 ± 3.6
P value	0.02	0.05	0.01	0.04	0.03
P28, n = 28					
Before	0.3 ± 0.1	46.0 ± 11.3	13.2 ± 3.6	15.1 ± 6.2	1.8 ± 0.6
After	0.4 ± 0.2	49.4 ± 6.5	19.3 ± 4.6	19.0 ± 6.8	4.6 ± 1.1
P value	NS	NS	NS	NS	0.03
P value for change score between ages	NS	0.03	NS	0.04	NS

All numbers are spikes per second during stimulus.

NS = not statistically significant; P7 = postnatal day 7; P28 = postnatal day 28.

larger (64 ± 5% of the surface are of the foot) than that of P28 rats (45.7 ± 8%; $P < 0.05$). One hour after incision, the pinch RF size in P7 pups increased significantly to 83 ± 5% ($P < 0.05$) of the surface area of the foot after the incision, whereas the mean pinch RF size at P28 after incision was not significantly different from baseline (53.6 ± 5%).

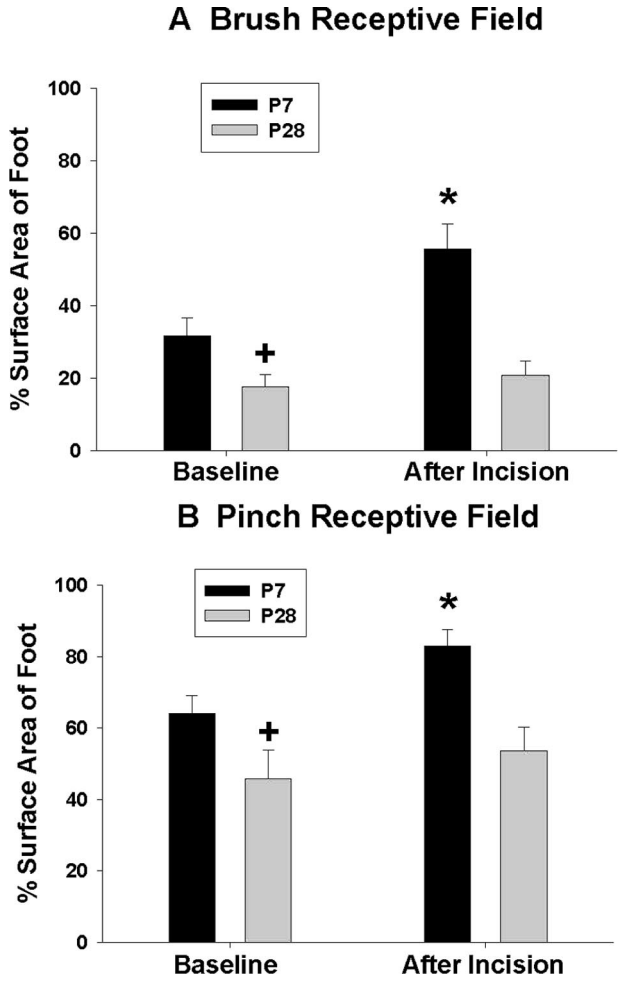

Fig. 4. Receptive field (RF) size for brush (low threshold) and pinch (high threshold) before and 1 h after incision in postnatal day 7 (P7) and postnatal day 28 (P28) animals. (*A*) Brush RF is larger in the P7 animals at baseline (+ $P < 0.05$). Brush RF increases 1 h after the incision in the P7 animals (* $P < 0.05$) but does not increase significantly in the P28 animals. (*B*) Pinch RF size before and 1 h after incision in P7 and P28 animals. Pinch RF is larger in the P7 animals at baseline (+ $P < 0.05$). Pinch RF increases 1 h after the incision in the P7 animals (* $P < 0.05$) but does not increase significantly in the P28 animals.

A change score was calculated for both brush and pinch to directly compare the differences between the P7 and the P28 RF. The increase in RF for brush and pinch were significantly larger in the P7 animals, with a change score in the P7 animals for brush of 22.6 (95% CI, 14.7–30.5), compared with 2.9 (95% CI, 2.6–3.2) in the P28 animals, and a change score in the P7 animals for pinch of 19.2 (95% CI, 15.1–23.3), compared with 7.9 (95% CI, 5.9–9.9) in the P28 animals.

Spatial Analysis across RFs Reveals a Marked Increase in Brush- and Pinch-evoked Spikes at the Edges of the RF 1 h after Skin Incision at P7

To test whether the increase in RF size after skin incision at P7 was accompanied by increased cutaneous sensitivity at the RF boundaries, neuronal spike activity evoked by low-threshold brush and the high-threshold pinch was measured at inner and outer zones in the RF before and 1 h after skin incision. The results are shown in table 1. At both ages, the WDR cells show a gradient across their RF, such that testing in the inner part of the RF always produces more spikes than testing in the outer part, and this gradient is maintained after skin incision. However, table 1 also shows that in P7 animals, both brush and pinch responses at both sites increased significantly 1 h after skin incision. This increase was especially marked at the outer site, where brush responses increased approximately 10-fold and pinch responses increased approximately 6-fold, thereby significantly reducing the sensitivity gradient between the inner and outer RFs. In P28 animals, skin incision had no effect at 1 h on pinch-evoked spike activity at either the inner or the outer site. There was also no difference in the neuronal response to the low-threshold brush inside the RF next to the incision (inner). The only increase in evoked activity observed at P28 animals was to brush next to the incision at the edge (outer) of the initial RF (from 1.8

spikes/s before the incision to 4.6 spikes/s after the incision).

Magnitude of RF Effects

The magnitude of the effect was analyzed using the Cohen *d*. The effect size was very large in the change in the RF size in the P7 animals, with a Cohen *d* value of 1.0 for the low-threshold brush RF and 0.7 for the high-threshold pinch RF. However, the effect size in the P28 was small in the older P28 adult animals, with a Cohen *d* of 0.2 for the low-threshold brush RF and 0.3 for the high-threshold pinch RF. This relation was similar across all outcome measures reported. The significance of this is that there is a much greater amount of afferent activity generated on any single WDR from nociceptive input in the young animals because the area causing activation is much greater. Although there seems also to be increased activity in the older animals after incision, the study is not powered to detect the difference. In addition, the relative change in electrical signal with more spikes arriving at the WDR from a given stimulus in the RF of any given peripheral neuron after the incision leads to a large relative increase in electrical activity in the WDR in the P7 that is not matched at P28, at least in the initial hour after incision. It is the ubiquity of the response in the P7 animals that is of note when considering the increased amount of neural input arriving at the spinal cord with a given injury and the effect size of the changes after incision.

Mechanical von Frey Hair Responses in the Center of the RF Increases 1 h after Skin Incision at Both Ages

Whereas the increases in WDR cell spontaneous activity and RF size were significantly greater at P7 compared with P28, this was not true of responses to single punctuate von Frey hair stimulation. The von Frey hair thresholds and spike responses in the center of the RF before and after a skin incision are shown in table 2. The baseline mean von Frey hair threshold at P7 was lower than at P28 (4.0 [0.6–8.5] *vs.* 7.5 [0.4–8.5] g), although this difference was not statistically significant. One hour after incision, the threshold decreased significantly at both ages ($P < 0.05$), but the effect was greater in the P28 animals. The number of spikes evoked by neurons to both threshold and suprathreshold von Frey hair stimulus was significantly increased 1 h after incision in both the P7 and the P28 animals. The threshold spike response and the suprathreshold responses nearly doubled at P7 and at P28.

Table 2. Mechanical Thresholds and Responses to Threshold and Suprathreshold von Frey Hair Stimulation before and 1 h after Incision

	Threshold, g	Threshold Response, spikes/s	Suprathreshold Response, spikes/s
P7			
Before	4 (0.6–8.5)	4.8 ± 1.5	13.7 ± 2.9
After	2.5 (0.4–8.5)	8.2 ± 1.4	23.1 ± 5.7
P value	<0.05	<0.05	<0.05
P28			
Before	7.5 (0.4–8.5)	3.4 ± 0.8	11.0 ± 2.4
After	1.8 (0.4–6.4)	8.7 ± 1.7	22.4 ± 6.2
P value	<0.05	<0.05	<0.05
P value for change score between ages	NS	NS	NS

Threshold and suprathreshold responses are elicited spikes to threshold von Frey hair and suprathreshold von Frey hair.

NS = not statistically significant; P7 = postnatal day 7; P28 = postnatal day 28.

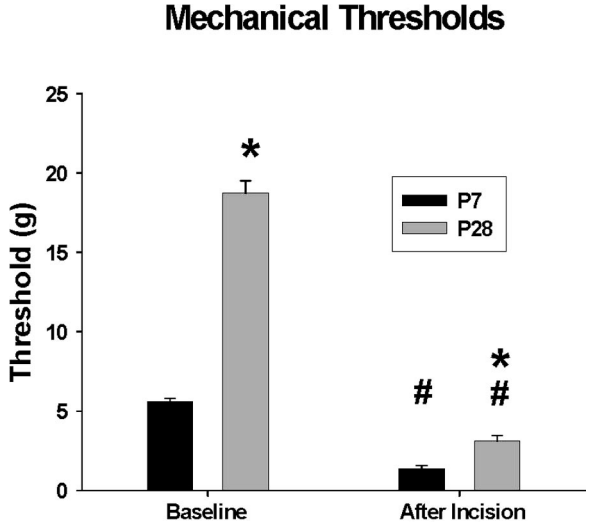

Fig. 5. Mechanical thresholds in grams for postnatal day 7 (P7) and postnatal day 28 (P28) animals before surgery and 1 h after incision. Baseline and 1-h thresholds are different between the two ages (* $P < 0.05$). Both ages had a significant decrease in threshold 1 h after incision (# $P < 0.05$). However, there was a greater decrease in threshold in the P28 animals compared with the P7 animals (83.4% *vs.* 72.4%, respectively). The difference between the two ages was significant ($P < 0.05$), with a change score for the P7 of 3.9 (95% confidence interval, 3.8–4.0), compared with 20.7 (95% confidence interval, 16.4–25.1) in the P28 animals.

This result was reflected in behavioral studies. Withdrawal thresholds in awake animals before and 1 h after incision are presented in figure 5. As with individual WDR cells, mechanical thresholds at baseline were lower in young animals (5.7 + 0.1 g in P7 and 18.8 + 0.8 g in P28; $P < 0.05$). After surgery, the threshold decreased significantly at both ages ($P < 0.05$), with the thresholds being different between the ages ($P < 0.05$), but the effect was greater at P28 than at P7 ($P < 0.05$). The difference between the two ages was significant ($P < 0.05$), with a threshold change score in grams for the P7 of 3.9 (95% CI, 3.8–4.0), compared with 20.7 (95% CI, 16.4–25.1) in the P28 animals.

Discussion

In this article, we have shown that there is a marked difference in the acute initial effects of surgical incision

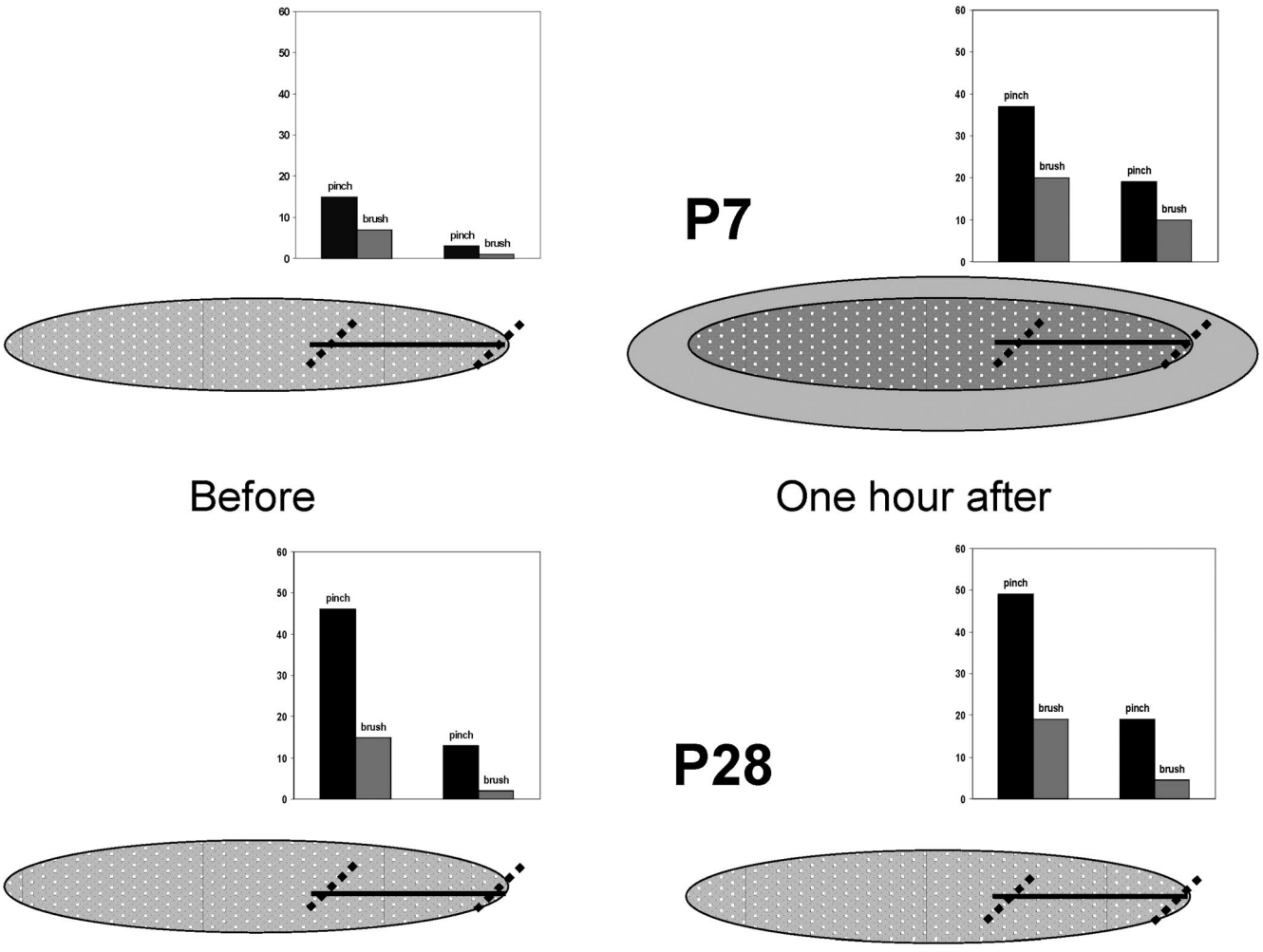

Fig. 6. Diagram illustrating the change in brush and pinch responses across the inner and outer receptive field zones (*dotted lines*) at postnatal day 7 (P7) and postnatal day 28 (P28) before and 1 h after an incision is made in the receptive field (*solid line within oval field*). Average data are plotted as spikes per second.

on RF size and evoked responses of dorsal horn WDR cells in young and mature rats. As might be expected, dorsal horn WDR cells at both ages rapidly become more sensitive to mechanical von Frey hair stimulation at the center of the RF after an incision across the field, presumably as a result of peripheral afferent terminal sensitization. Aδ and C fibers adjacent to a skin incision are known to become sensitized after 1 h in adults,[21] and although this has not been directly tested in newborns, the ability of nociceptors to sensitize has been reported from early stages of development.[28,29] In contrast, other changes, such as in RF size and RF sensitivity gradient to brush and pinch, which result from central integration of afferent input across wider areas of the RF, were substantially different at the two ages. At P7, there is a rapid expansion of both brush and pinch RF size that is not observed at P28. In addition, brush- and pinch-evoked activity at the inner and outer edges of the incision is markedly increased at P7, reducing the sensitivity gradient across the RF. This is in contrast to the modest change in brush responses at the outer edge only at P28. Evidently, then, the response to tissue trauma from surgical incision in young animals has a different temporal and spatial pattern from that in adults. The results suggests a more immediate integration of afferent input leading to an intense activation across the RF of the WDR neurons in the young spinal cord, which is not observed in older animals. Figure 6 summarizes these differences in the initial RF response of WDR cell to skin incisions at P7 and P28.

The results were obtained using precise skin incisions within the RF of individual dorsal horn neurons combined with careful analysis of responses to standardized cutaneous stimuli at different sites within the RF. As reported elsewhere, baseline RF sizes to brush stimuli were relatively larger in the paw of the younger animals when compared with the older animals.[12,27] We show that this is also true of pinch RFs. Despite their relatively larger size, the RF of the P7 WDR neurons increased to both the high-threshold and the low-threshold stimuli in response to incision, whereas in the P28 animals, the RF size did not acutely change in response to the incision.

Previous studies in adult animals have shown an increase in RF in 15 of 29 of WDR neurons 1 h after an adjacent skin incision.[23] There are differences in methodology from our study. These studies did not map the entire cutaneous RF; rather, the increase in RF was characterized as a response in an area that was previously unresponsive, with examples of increases in RF provided. In our current study, the actual size of the RF was calculated before and after incision from the same WDR neuron. The difference in the brush-evoked response at the edge of the RF in the P28 animals, the only RF parameter that was different for the P28 animals, suggests that RF changes were taking place but that these were modest in comparison with those at P7. It is important to note that the response seems to increase after incision in the older animals in the other modalities tested, but the study is not powered to evaluate this. The Cohen *d* presented suggests that the magnitude of the effect is greater in the young animals on the RF. There is, in fact, an effect on RF in the older animals from the Cohen *d*, but the smaller effect size is not powered to pick up this difference either.

The mechanism behind the rapid and marked change in RF size and responsiveness in the young animals is unclear. Rapid expansion of RF size and increased sensitivity occurs because dorsal horn WDR RFs are surrounded by "low-probability" firing zones, where stimulation normally results in subthreshold depolarization. In adult rats, intense nociceptor stimulation outside the RF causes rapid inclusion of these zones into the RF, thus increasing their spatial extent, amplifying their responsiveness, and reducing their thresholds.[30] The fact that these rapid RF changes after skin injury are restricted to younger animals suggests that young WDR neurons are subject to much greater injury-induced nociceptor barrage than are adult WDR neurons.

One possibility is that more afferent activity is evoked from the damaged area in young animals, but this is unlikely because afferent firing frequencies in immature Aδ and C fibers are, if anything, lower in immature animals compared with adults.[31] In addition, the comparable increase in von Frey hair filament–evoked activity at P7 and P28 suggests similar peripheral sensitization at the two ages. A more likely reason, therefore, lies in the immaturity in central processing in dorsal horn circuits, especially in inhibitory interneurons. Stimulation of both low- and high-threshold afferents excites both excitatory and inhibitory interneurons in the dorsal horn, and the balancing action of inhibition is an essential part of sensory processing. Much evidence suggests that central inhibitory processing is less effective in young dorsal horns, leading to larger baseline RFs and sensitization to repetitive low-threshold stimulation.[10,32] This may be due to immature connections at the circuit level[33] or to functional immaturity of inhibitory synapses.[34,35] The relative lack of brainstem inhibitory descending controls of spinal nociceptive circuits is also likely to play a role.[36,37] One explanation, therefore, for the early exaggerated sensitization of P7 RFs after skin incision is that the balancing action of inhibitory interneuronal activity that is normally rapidly recruited upon noxious stimulation in adults[38] is not present at younger ages. Consistent with this proposal is the relative delay in the maturation of glycinergic synaptic activity in the newborn dorsal horn compared with γ-aminobutyric acid–mediated synaptic activity.[34] Strong tonic glycinergic inhibition with characteristic fast kinetics is a feature of the adult dorsal horn but is absent in the newborn, where slower γ-aminobutyric acid inhibition dominates.[39]

The RF response to skin incision at P7 differs from the response to experimental carrageenan inflammation, where increases in RF were not found until after the second postnatal week.[12] Pain signaling differs depending on the nature of the injury, and it is clear that that the mechanisms of surgically induced tissue trauma pain have unique qualities[40] and may represent a complex combination of both inflammatory and neuropathic pain, as suggested for postoperative pain.[41]

The behavioral von Frey hair withdrawal data demonstrated a decrease in threshold after incision in both age animals, the decrease being greater in the older animals, as previously reported in slightly older animals 2–4 h after incision.[15–17] Previous studies have suggested that enhanced dorsal horn responses to punctate mechanical stimuli code for the decreased von Frey hair withdrawal thresholds,[21,23] and this is supported here. What is also clear from this study, however, is that a different picture of sensory sensitization emerges when examining integrated responses to brush and pinch across whole neuronal RFs compared with simply measuring von Frey thresholds (with either behavior or electrophysiology). RF analysis shows that surgical incision has a greater impact on the sensory neuronal traffic in young dorsal horns than is apparent from von Frey hair threshold tests alone.

The findings presented in this article have revealed fundamental differences that occur during development in response to tissue trauma from surgical incision. Sensory circuits in young dorsal horns respond more rapidly to the afferent barrage from skin incision than do adult circuits, leading to a striking enlargement of RFs within an hour after the tissue damage. Further studies to determine the mechanisms of the rapid changes, the time course, and the long-term implications of early spinal cord activation will be essential. With greater understanding of the underlying differences in responses during development, better and more directed interventions can be designed to reduce unwanted short- and long-term consequences of skin damage in young patients.

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