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Electrophysiologic Characteristics of Large Neurons in Dorsal Root Ganglia during Development and after Hind Paw Incision in the Rat

Douglas G. Ririe, M.D., Baogang Liu, M.D.,† Bridgette Clayton, B.S.,‡ Chuanyao Tong, M.D.,* James C. Eisenach, M.D.§*

Background: **Withdrawal thresholds in the paw are lower in younger animals, and incision further reduces these thresholds. The authors hypothesized that these differences result in part from changes in intrinsic electrophysiologic properties of large neurons.**

Methods: **Using isolated whole dorsal root ganglion, current clamping was performed to determine the electrophysiologic properties of large neurons before and after incision in animals aged 1 and 4 weeks. Mechanical withdrawal thresholds were used to follow paw sensitivity.**

Results: **After paw incision, withdrawal thresholds decreased to a similar degree at both ages, but returned to control threshold at 72 h only in the 1-week-old animals. The resting membrane potential was less negative and the rheobase and the resistance of the membrane were lower at baseline in the 1-week-old animals ($P < 0.05$). After incision, the membrane potential became more depolarized and the rheobase was less in both ages. These changes remained 72 h after the incision in both ages.**

Conclusion: **These findings suggest that lower mechanical thresholds in the younger animals may be partially attributed to the intrinsic electrophysiologic properties of the larger-diameter afferent neurons. The lack of resolution of the electrophysiologic changes in the young despite the resolution of the withdrawal response suggests that continued input from large fibers into the central nervous system may occur at this age despite the apparent resolution of behavioral changes. Further studies are needed to determine the etiology of these differences, their impact in the central nervous system, and whether theses changes can be prevented.**

DIFFERENCES in response to mechanical stimulation in the periphery exist both in animals and in humans during development.[1–3] In the young, the peripheral afferent nerve has a larger receptive field and is more excitable, with a larger response to a given stimulus, including long-lasting afterdischarges.[4,5] In addition, spontaneous activity can occasionally be seen in the immature afferent fibers, a finding not commonly observed in later development unless the nerve or spinal cord is damaged.[4,6]

Surgery is performed in patients of all ages, including extremes of immaturity. Surgical tissue trauma results in a cascade of events that culminate in spontaneous pain and hypersensitivity to mechanical stimuli.[2,7,8] Initially, there is local activation of peripheral nerves from disruption of the integrity of the skin and transection injury to small nerve endings. This is followed by release of inflammatory mediators and further increase in neural signaling. In addition, healing is associated with hyperinnervation to the previously injured skin that is greater in the younger animal.[7] These peripheral events occur simultaneously with changes in central processing of afferent input over time.[9]

After incision, there is a more rapid resolution of hypersensitivity to mechanical stimuli in the young.[2] Different responses to the incision at the neuronal level may partially underlie short- and long-term effects of tissue injury. The signal from the local injury passes through the dorsal root ganglion (DRG) on its way to the dorsal horn of the spinal cord. Alterations in intrinsic excitability of DRG cells have been previously shown in nerve injury and in DRG compression.[10–12] The majority of the nerve cell's transcriptional machinery resides here, making this a crucial part of the peripheral nerve cell. Peripheral activity may induce changes, and development may modulate this signaling. Examination of intracellular and membrane electrophysiology of peripheral afferents is possible at the cell body without dissociating cells, and the state of excitability of this part of the neuron's membrane may reflect changes at peripheral and central terminals.

Noxious and innocuous stimuli early in development can have potentially adverse effects.[13] Repetitive innocuous stimuli can result in abnormal signaling. Innocuous sensation or light touch is primarily carried by large myelinated or Aβ fibers and can be assessed by response to von Frey filaments.[14] The large neuronal cells in the DRG have been shown to be large myelinated fibers by conduction velocity and likely represent light touch under normal circumstances.[15] In the rat, physiologic function of the C fibers is not fully established until 2 weeks postnatally, but Aβ fibers function normally.[16] However, anatomically Aβ fibers extend into more superficial laminae in the young, and input from these fibers may be activating dorsal horn structures normally reserved for noxious signaling.[17] Although Aβ fibers in the adult do not seem to play a large role in responses to incisional pain after surgery, this may be different in the young due to the altered anatomic connections.[17–19] Therefore, un-

* Associate Professor of Anesthesiology, † Research Fellow, ‡ Research Technician, § Professor of Anesthesiology.

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Address correspondence to Dr. Ririe: Department of Anesthesiology, Wake Forest University School of Medicine-Baptist Hospital and Brenner Children's Hospital, Medical Center Boulevard, Winston-Salem, North Carolina 27157-1009. dririe@wfubmc.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue.

derstanding of both the activation of the structures in the dorsal horn by Aβ fibers and also the activity of these neurons during development and in response to incision is important. With a better understanding of the peripheral sensitivity and activity of these neurons during development, targeting of these nerves to prevent adverse sequela of surgery early in life may prove to be beneficial by reducing the impact incision has in the spinal cord and brain.[13,20] In this study, we hypothesized that the electrophysiologic nerve cell properties that govern neuronal excitability differ during development and after incision, and that these developmental differences parallel differences in the behavioral response to surgical incision at different stages of development.

Materials and Methods

Animal Behavior and Surgery

After approval from the Animal Care and Use Committee (Wake Forest University, School of Medicine, Winston-Salem, North Carolina), male Sprague-Dawley rats at 1 and 4 weeks of age were studied. One-week-old animals are preweanling, and 4-week-old animals are postweanling. After baseline testing, all animals were anesthetized with 2% halothane in oxygen during spontaneous ventilation through a nose cone. As previously described,[2,8] the plantar aspect of the left hind paw was prepared in a sterile manner with a 10% povidone-iodine solution. A midline incision from the heel to the base of the toes was performed with a No. 11 blade using sterile technique. Rather than a fixed-length incision, this created an incision of a fixed proportion of the size of the paw at different ages.[2] A small forceps was used to elevate the flexor tendon from the heel to the toes. The incision was closed with 5.0 nylon on an FS-2 needle using two inverted mattress sutures, and the sutures were left in place. Control animals underwent anesthesia with prep, but no incision.

Mechanical Stimulation Testing

Animals were placed on a solid surface in a plastic cage. They were acclimated to the environment for 20 min before testing. Withdrawal to mechanical stimulation was assessed by holding the animal with the hind feet resting on the solid surface with a temperature of 37°C and application of calibrated von Frey filaments to the dorsum of the foot 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 as previously described.[21] Withdrawal thresholds were determined before surgery and at 6, 24, and 72 h afterward and in animals with no surgery at the same time points. All animals were included in the data analysis, and no animal in the study had a wound dehiscence or infection during the study.

Microelectrode Intracellular Recording from DRGs

Animals underwent general anesthesia with halothane and spontaneous ventilation for removal of DRGs. After surgical dissection, the left L5 DRG was removed from 18 animals at each age. This was 6 animals at each time for control and 24 and 72 h after incision. DRGs were placed in a recording chamber and mounted on the stage of an upright microscope (BX50-WI; Olympus America, Inc., Center Valley, PA). A U-shaped stainless steel wire on which three to four fine nylons fibers spanned the two sides was used to gently hold the ganglion immersed at the bottom of the chamber. The chamber was continuously perfused with oxygenated artificial cerebrospinal fluid containing 130 mM NaCl, 3.5 mM KCl, 1.25 mM NaH_2PO_4, 24 mM $NaHCO_3$, 10 mM dextrose, 1.2 mM $MgCl_2$, and 1.2 mM $CaCl_2$ (pH = 7.3) at a rate of 2 ml/min, and the temperature was maintained at 37° ± 1°C as described previously.[11,22]

Dorsal root ganglion cells were visualized under differential interference contrast through a digital camera (Hitachi Kokusai Electric, Inc., Tokyo, Japan) attached to a standard display monitor. Intracellular electrophysiologic recordings were obtained from each neuron in the study with a sharp microelectrode filled with 2.5 M potassium acetate (pH = 7.2). Satisfactory recordings were obtained with electrodes of 50–80 MΩ. Before electrode penetration, the DRG soma was visually classified according to its diameter as large (≥ 45 μm). Cell size was measured directly from oscilloscope in two dimensions and averaged, and cell size was expressed as average cell diameter. The electrophysiologic data were collected with the use of a single-electrode continuous current clamp (AxoClamp-2B; Axon Instruments, Foster City, CA) and analyzed with Clampex 8 software (Axon Instruments).

After a stabilization period of 10 min, a large neuron was isolated. Criteria used for acceptable neurons are a resting membrane potential (V_m) of less than −45 mV and a peak action potential (AP) height greater than 0 mV regardless of the V_m, *i.e.*, overshoot of the AP height over 0 mV. After a period of stabilization of the V_m of approximately 3 min, a current clamp protocol of increasing current injection into the cell was begun. During the absence of any external stimulus, any pattern of spontaneous activity (regular, bursting, or irregular) was noted. From each DRG, two to five cells were studied. To compare the sensitivity of neurons before and after paw incision in the different age animals, various membrane properties were measured as previously described.[23,24] V_m was first measured 3 min after a stable

recording was obtained and was measured again after the end of the protocol. The current clamp protocol consisted of depolarizing currents of 0.05–4.0 nA (100-ms pulse duration) delivered in increments of 0.05 nA until an AP was evoked. The threshold current (rheobase) was defined as the minimum current required to evoke an AP. The AP and afterhyperpolarization (AHP) were determined from the trace generated by the rheobase depolarization. The AP voltage threshold was defined as the first point on the rising phase of the spike at which the change in voltage exceeded 50 mV/ms. The duration of the AP was measured at the AP threshold level. The AP amplitude was measured between the peak and the AP threshold. The input resistance (R_{in}) for each cell was obtained from the slope of a steady state current–voltage plot in response to a series of hyperpolarizing currents of 100 ms in duration, delivered in decreasing steps of 0.05 nA from 0.2 to −2 nA. The AHP amplitude was measured from the valley peak to the baseline, and the AHP duration was measured at amplitude halfway between.

Statistical Analysis

Data were normally distributed and are presented as mean ± SE. Withdrawal thresholds and electrophysiologic parameters were analyzed using analysis of variance between groups of similar ages for time and treatment and using Fisher protected least significant differences. Repeated measure was used for withdrawal thresholds. Between-age differences were analyzed for baseline values only, except for rheobase, V_m, and R_{in}. Multiple comparisons were adjusted for by using the Bonferroni correction where appropriate. The Fisher exact test was used for differences between spontaneous oscillations between ages. All results were considered significant if the *P* value was less than 0.05.

Results

Changes in Withdrawal Threshold

Withdrawal thresholds before surgery were lower in the 1-week-old animals (5.7 ± 0.11 g; n = 10) when compared with the 4-week-old animals (46.2 ± 1.5 g; n = 10) ($P < 0.05$; fig. 1). After incision, the mechanical withdrawal threshold decreased significantly in both ages ($P < 0.05$). The relative decrease for the two ages was not different with a percent decrease in threshold of 27% in the 1-week-old rats and 33% in the 4-week-old rats at 6 h after surgery. The threshold in the 1-week-old animals increased at 24 h and by 72 h was no different from control threshold (100% of control). However, the threshold in the 4-week-old animals at 24 and 72 h remained below the control (48% and 48% decreased from control, respectively).

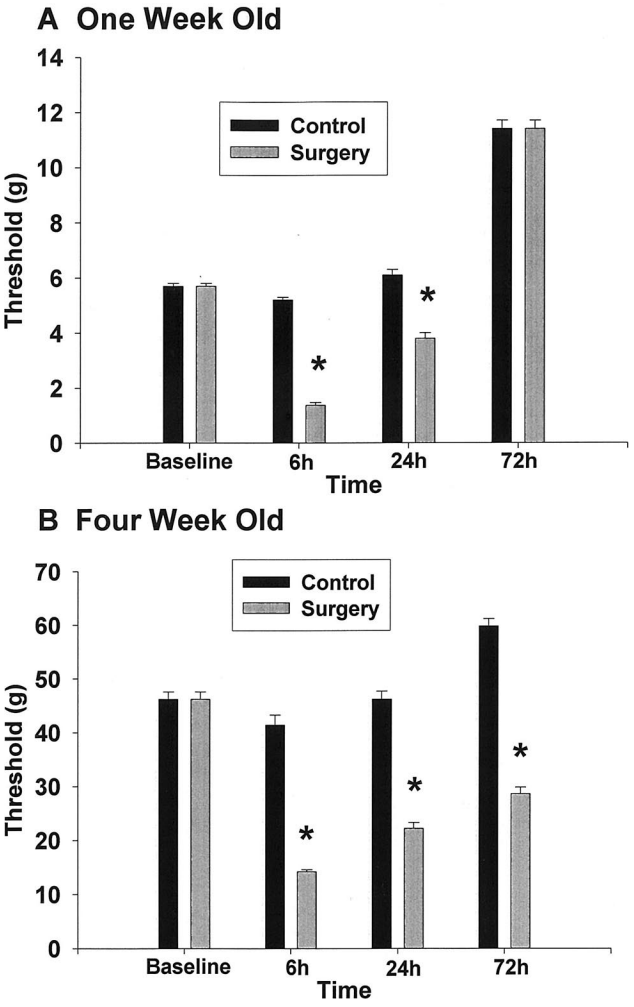

Fig. 1. Mechanical withdrawal thresholds in grams *versus* time in 1-week-old (*A*) and 4-week-old (*B*) rats. Baseline withdrawal thresholds are different ($P < 0.05$). Thresholds decreased significantly after incision in both age animals ($P < 0.05$). However, whereas thresholds remained significantly different from control in the 4-week-old animals, thresholds were not different from control by 72 h in the 1-week-old animals. * Difference between control and surgery.

Spontaneous Activity of Neurons

None of the neurons in the study had spontaneous activity during the observation period. Representative tracings of the AP in neurons from 1- and 4-week-old animals before surgery are shown in figure 2A. Five of 68 neurons from the 1-week-old animals displayed spontaneous oscillations of V_m and multiple APs generated at threshold, reflecting increased excitability in these neurons (7.4%; confidence interval, 1.3–13.5%) (fig. 2B). One of these cells was in the no-surgery control group, and there were two cells in each of the 24- and 72-h postincision groups. None of the 68 neurons from 4-week-old animals demonstrated spontaneous oscillations (0%; confidence interval, 0–4.4%). The difference between the young and the old is not significant. However, the confidence intervals are provided to provide more information. Because this is a rare finding and the

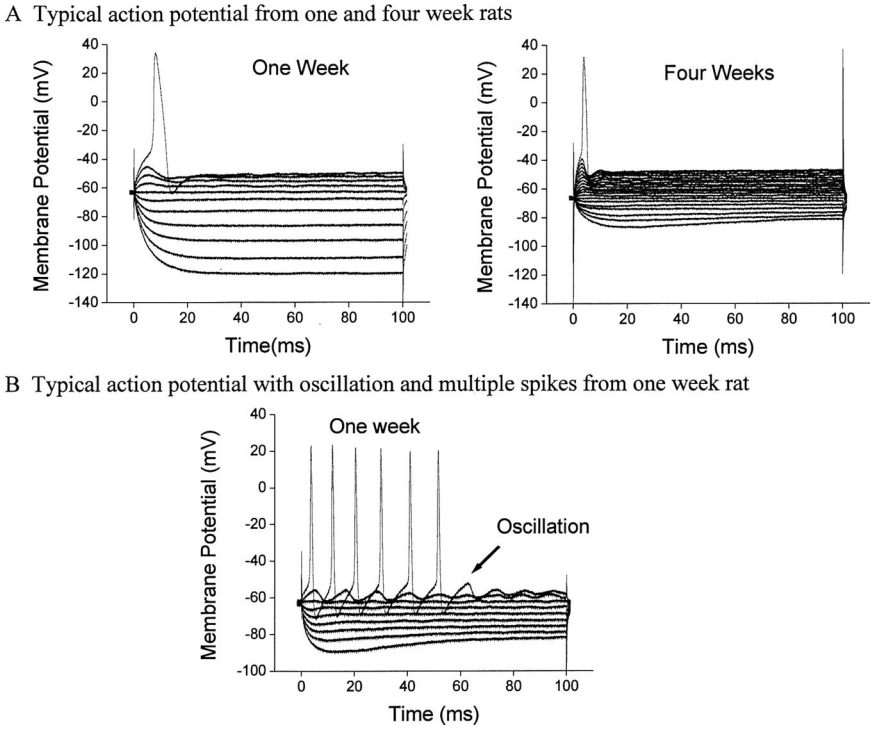

Fig. 2. Action potential activation during current clamp step protocol. Representative action potentials elicited in 1- and 4-week-old animals are shown in *A*. Note the much greater change in membrane potential deflection from a fixed current in the 1-week-old animals when compared with the 4-week-old animals at baseline. This is consistent with the greater resistance in the younger animals. *B* demonstrates oscillations and multiple spikes in a cell from a 1-week-old animal. This was not seen in cells from the older animals.

study was not designed to evaluate this finding, it may be an important difference.

Differences in Large DRG Neurons at Baseline in the Different Ages

The V_m, rheobase, and R_{in} all differed at baseline between the 1- and 4-week-old animals ($P < 0.05$; fig. 3). V_m at baseline was less negative (more depolarized) in the 1-week-old animals (-60.5 ± 1.3 mV) when compared with the 4-week-old animals (-64.5 ± 1 mV) ($P < 0.05$; fig. 3A). Rheobase of the 1-week-old animals was less (0.9 ± 0.1 nA) when compared with the 4-week-old animals before surgery (1.16 ± 0.14 nA) ($P < 0.05$; fig. 3B). R_{in} was greater in the 1-week-old animals before surgery (43.7 ± 1.8 MΩ) than in the 4-week-old animals (19 ± 1.5 MΩ) ($P < 0.05$; fig. 3C). Large neuron size was larger in the 4-week-old animals when compared with the cells from the 1-week-old animals ($P < 0.05$; table 1).

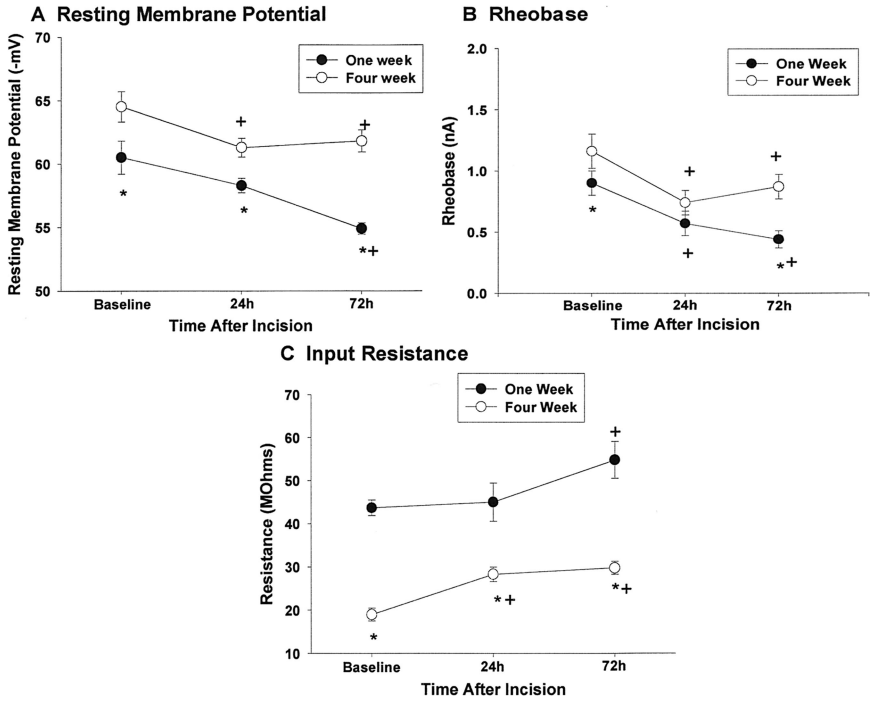

Fig. 3. (*A*) Incision induced changes in resting membrane potential. (*B*) Rheobase (threshold current). (*C*) Input resistance during development. In *A*, resting membrane potential is less negative in the 1-week-old animals at baseline when compared with the 4-week-old animals ($P < 0.05$). Incision produced a significant change in resting membrane potential in both ages ($P < 0.05$). In *B*, rheobase was lower in the 1-week-old animals at baseline compared with the 4-week-old animals ($P < 0.05$). Incision produced changes in the rheobase for both age animals ($P < 0.05$). In *C*, input resistance was higher in the 1-week-old animals at baseline when compared with the 4-week-old animals ($P < 0.05$). Incision increased the input resistance in both ages, suggesting increased excitability in the neurons after the incision. * Difference between 1- and 4-week-old animals. + Difference from baseline.

Table 1. Electrophysiologic Characteristics of Large Cells from L5 Dorsal Root Ganglia after Incision at 1 and 4 Weeks of Age

Age	n	AP Threshold, mV	AP Amplitude, mV	AP Duration, ms	AHP Amplitude, mV	AHP Duration, ms	Cell Diameter, μm
1 Week							
Control	22	−34.5 ± 3.4*	57.4 ± 2.0*‡	0.9 ± 0.1*‡	8.2 ± 0.9*‡	3.0 ± 0.2*‡	49.4 ± 0.7*
24 h	26	−40.0 ± 2.1†	66.1 ± 1.9†	2.2 ± 0.2†	12.1 ± 0.9†	3.1 ± 0.1	50.8 ± 1.4
72 h	20	−33.4 ± 3.0	50.9 ± 1.7	2.8 ± 0.2†	9.0 ± 0.7	4.5 ± 0.3†	49.3 ± 0.9
4 Week							
Control	24	−43.2 ± 1.5‡	65.1 ± 2.2	1.7 ± 0.1	13.2 ± 1.4‡	2.1 ± 0.1	54.4 ± 0.6
24 h	20	−48.9 ± 1.1†	59.2 ± 1.4	1.8 ± 0.1	8.7 ± 0.9†	2.6 ± 0.2	51.8 ± 1.5
72 h	24	−43.5 ± 1.4	60.8 ± 2.2	1.7 ± 0.1	11.1 ± 0.8	2.8 ± 0.2	56.1 ± 1.8

All values are mean ± SEM. n is the total number of cells recorded from six animals at every treatment or time for each age.

* Difference between ages. † Difference at baseline. ‡ Difference within age after incision.

AHP = afterhyperpolarization; AP = action potential.

Overall differences between groups exist for AP threshold, AP amplitude, and AHP duration ($P < 0.05$; table 1).

Differences in Large DRG Neurons after Incision at Different Ages

Incision produced a decrease in rheobase, a less negative (more depolarized) V_m, and an increase in R_{in} at both ages ($P < 0.05$; fig. 3). After incision, the V_m became less negative in both ages ($P < 0.05$). In the 4-week-old animals, the V_m decreased at 24 h when compared with baseline, but by 72 h after the incision, the V_m was the same as at 24 h. However, in the 1-week-old animals, V_m continued to become even less negative (more depolarized) at 72 h. Rheobase decreased after incision at both ages ($P < 0.05$). However, rheobase was similar between the 1- and 4-week-old animals at 24 h, whereas at 72 h, the largest difference was found between ages. Incision produced an increased in R_{in} in DRG neurons of both ages. However, the effect on R_{in} was greatest at 72 h in the 4-week-old animals, whereas in the 1-week-old animals, the R_{in} was greatest at 24 h and remained at the same level at 72 h. Cell size was not different within each age (table 1). For the 1-week-old animals, incision altered AP amplitude, AP duration, AHP amplitude, and AHP duration but did not produce any significant change in AP threshold (table 1). For 4-week-old animals, incision produced significant effects only on AHP amplitude and AP threshold.

Discussion

Differences in response to pain from various injuries occur as a function of age and stage of development.[2,25–27] Understanding the etiology of these differences may help to define changes that can either accentuate the pain signaling from the periphery or reduce it.[28] Noxious input from the periphery may provide greater input to the immature spinal cord and may result in long-term alterations of neural processing after transient acute painful stimuli. The data presented in this study demonstrate differences between 1- and 4-week-old animals in the electrophysiologic characteristics of large neurons from isolated DRGs. Specifically, there is a less negative (more depolarized) V_m in the younger animals. This, in conjunction with the smaller rheobase, or threshold current needed to cause activation of the nerve, may result in greater excitability. In addition, incision in the hind paw in the dermatome of neurons in the study results in changes in neuronal characteristics in all ages, suggesting that peripheral neuronal activity changes may in part drive changes in the spinal cord and elsewhere in the central nervous system in response to incision. Of possibly greater importance, the electrophysiologic changes that occur persist in the younger age group despite the withdrawal threshold returning to normal. This is a significant divergence between the ages. This suggests that changes are occurring in the young to mitigate the acute behavioral findings of altered thresholds. However, persistent input into the central nervous system may result in continued changes in processing of afferent stimuli, which is masked by behavioral assessment. Therefore, the opportunity to alter the input is ignored and may result in long-term changes in the spinal cord or even the brain leading to more subtle behavioral alterations and responses.[13]

Previous reports of evoked firing properties of the dorsal horn cells of the spinal cord in rat suggest that there is no significant difference in firing frequency, rheobase, adaptation, or regularity of action potential discharge at different ages after birth up until the third postnatal week.[29] However, this does not address differences in neuronal characteristics in the peripheral neurons. Peripheral neuronal input may be critical to normal development at the level of the spinal cord. It may also be responsible for altered development if input is out of proportion to that required for establishing and reinforcing normal connections. The synaptic reorganization and strengthening that occur over the course of development may be related to the maturation of intrinsic neuronal characteristics.[16] Reorganization occurs postnatally in the dorsal horn with Aβ fibers being in lamina I and II initially and withdrawing over time.[17] Work in this

area suggests the changes that occur postnatally may be an activity-dependent process.[30] This potential enhanced activity in the peripheral neuron from the lower V_m and the lower rheobase in the peripheral neuron may allow the immature neurons to contribute to the maturational organization in the dorsal horn. However, increased firing beyond this may be detrimental by providing an inordinate amount of signaling into the dorsal horn setting up circuits that may alter sensation and sensory processing remotely from the time of the surgery or tissue trauma. These same changes in the older animal, if they become established, such as in nerve injury, whereby there is ongoing activity from even innocuous stimulation as a result of reduced thresholds, may alter circuits in the central nervous system and lead to chronic pain.

In this study, the DRG neurons were randomly selected, and many of these do not actually innervate the location of the incision (previous unpublished results in our laboratory, 2006). However, a difference after incision is clearly demonstrated. This suggests changes in neurons from the injury may alter nearby neurons in the DRGs not innervating the area of injury, because changes found in this study are too profound to attribute to one or two neurons in the DRG.[16] The large neurons in the DRG likely represent the large myelinated Aβ fibers based on conduction velocity characterization in a previous study.[15] von Frey filaments test in large part Aβ fibers, and the electrophysiologic data closely correlate with the mechanical withdrawal thresholds reported here for the older animals. The divergence of the mechanical thresholds and the electrophysiologic changes in the younger animals suggests that the input to the spinal cord is still present in the young, but the responses are changed. This difference may be from where the large neurons synapse in the young spinal cord, modulation of the connections through descending pathways, or more rapid adaptation to the activity.[17]

Sensory hyperinnervation can occur in the periphery in the young, which is more intense than that seen in response to the same injury later in life.[7,31] These sprouts are still coming into the same soma of the afferent neuron into the DRG and may alter sensitivity in the area of the wound through ongoing increases in signaling into the cell body and subsequent alterations in channels, receptors, or other related mediators of the neuron responses. Although A and C fibers are both involved in this localized, peripheral sprouting in the skin, it is the enhanced sprouting of the A fibers, and in particular the large A fibers in this preparation, that are of greatest interest in the younger animal. In our preparation, these large neurons have characteristics that may render them more excitable. With input of these A fibers into more superficial lamina in the young, input from these fibers may be activating dorsal horn structures normally reserved for noxious signaling.[17]

The underlying differences inside the neurons responsible for the phenotypical differences in electrophysiologic characteristics in our studies are not known. Although these are still *in vitro* changes and the exact implications are not entirely clear, especially during current clamp depolarization, new evidence suggests that the cellular characteristics from *in vitro* preparations agree with the more natural states for most neuronal classes.[32] Changes in calcium and sodium ionic currents have been found during development.[33,34] More specifically, the neurons from the younger animals may be more likely to have different types of inward currents from both calcium and sodium, and as maturation occurs, greater numbers of neurons with fewer inward currents exist. In addition, in the younger cells, low-threshold calcium currents are in greater abundance, with fewer high-threshold calcium currents.[35] This may explain the increased likelihood of multiple action potentials and oscillations in the younger neurons. Another possibility is the differential expression of ion channel subtypes as a function of development and even in response to the injury, or even regulated by changes in neuronal activity from the injured area.[36,37] Differences in $Na_V1.9$ between 1-week-old and adult animals with respect to A fibers have been shown, because normally $Na_V1.9$ is not present in adult A fibers and is present at 1 week.[36] This is contrary to $Na_V1.9$ in C fibers, which seems not to change during development. Postnatal increases in other sodium channel subtypes or ratios of other channels have also been described that may influence excitability.[38,39] These and other changes in ion channels, ratios, and expression will be crucial to understanding the basis for our findings and their implications.

Limited changes in the Aβ-fiber electrophysiologic characteristics have been described after incision in adult animals, but these changes were only assessed within the first 45 min after incision.[40] Aδ fibers were sensitized in this study, but only Aδ and C fibers were assessed for sensitization further in time after incision.[41] However, these were all adult animals, and further studies during development will provide knowledge of the changes in neuronal activity from incision to understand the impact in the young.

The data presented here demonstrate differences in electrophysiologic phenotype of large neurons from DRGs as a function of development. The effects of incision on altering the underlying electrophysiologic phenotype of the large neurons are also presented. Further studies using voltage clamping will be needed to establish the role different ion currents may play in these findings, both at baseline during development and in response to incision. Studies will also be needed to determine the developmental regulation of these ion currents through protein and gene expression during development and in response to surgical incision. Most importantly, defining the mechanism of the divergence

in the behavior and the neuronal characteristics after incision in the young and understanding the impact of ongoing neuronal input at both ages will be critical in understanding postoperative pain. Through further study, the impact development has on peripheral neuronal responses to incision will allow more developmentally appropriate interventions to reduce the impact of surgical incision in the young.

References

1. Fitzgerald M, Shaw A, MacIntosh N: Postnatal development of the cutaneous flexor reflex: Comparative study of preterm infants and newborn rat pups. Dev Med Child Neurol 1988; 30:520–6
2. Ririe DG, Vernon TL, Tobin JR, Eisenach JC: Age-dependent responses to thermal hyperalgesia and mechanical allodynia in a rat model of acute postoperative pain. ANESTHESIOLOGY 2003; 99:443–8
3. Holmberg H, Schouenborg J: Postnatal development of the nociceptive withdrawal reflexes in the rat: A behavioural and electromyographic study. J Physiol 1996; 493:239–52
4. Fitzgerald M: The post-natal development of cutaneous afferent fibre input and receptive field organization in the rat dorsal horn. J Physiol 1985; 364:1–18
5. Torsney C, Fitzgerald M: Age-dependent effects of peripheral inflammation on the electrophysiological properties of neonatal rat dorsal horn neurons. J Neurophysiol 2002; 87:1311–7
6. Liu B, Eisenach JC: Hyperexcitability of axotomized and neighboring unaxotomized sensory neurons is reduced days after perineural clonidine at the site of injury. J Neurophysiol 2005; 94:3159–67
7. Reynolds ML, Fitzgerald M: Long-term sensory hyperinnervation following neonatal skin wounds. J Comp Neurol 1995; 358:487–98
8. Brennan TJ, Vandermeulen EP, Gebhart GF: Characterization of a rat model of incisional pain. Pain 1996; 64:493–501
9. Torsney C, Fitzgerald M: Spinal dorsal horn cell receptive field size is increased in adult rats following neonatal hindpaw skin injury. J Physiol 2003; 550:255–61
10. Stebbing MJ, Eschenfelder S, Habler HJ, Acosta MC, Janig W, McLachlan EM: Changes in the action potential in sensory neurones after peripheral axotomy *in vivo*. Neuroreport 1999; 10:201–6
11. Zhang JM, Song XJ, LaMotte RH: Enhanced excitability of sensory neurons in rats with cutaneous hyperalgesia produced by chronic compression of the dorsal root ganglion. J Neurophysiol 1999; 82:3359–66
12. Song XJ, Hu SJ, Greenquist KW, Zhang JM, LaMotte RH: Mechanical and thermal hyperalgesia and ectopic neuronal discharge after chronic compression of dorsal root ganglia. J Neurophysiol 1999; 82:3347–58
13. Anand KJ, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM: Long-term behavioral effects of repetitive pain in neonatal rat pups. Physiol Behav 1999; 66:627–37
14. Kandel ER, Schwartz JH, Jessell TM: Principles of Neural Science. New York, McGraw-Hill, 2000
15. Ma C, Shu Y, Zheng Z, Chen Y, Yao H, Greenquist KW, White FA, LaMotte RH: Similar electrophysiological changes in axotomized and neighboring intact dorsal root ganglion neurons. J Neurophysiol 2003; 89:1588–602
16. Fitzgerald M, Gibson S: The postnatal physiological and neurochemical development of peripheral sensory C fibres. Neuroscience 1984; 13:933–44
17. Fitzgerald M, Butcher T, Shortland P: Developmental changes in the laminar termination of A fibre cutaneous sensory afferents in the rat spinal cord dorsal horn. J Comp Neurol 1994; 348:225–33
18. Hämäläinen MM, Gebhart GF, Brennan TJ: Acute effect of an incision on mechanosensitive afferents in the plantar rat hindpaw. J Neurophysiol 2002; 87:712–20
19. Pogatzki EM, Gebhart GF, Brennan TJ: Characterization of Aδ- and C-fibers innervating the plantar rat hindpaw one day after an incision. J Neurophysiol 2002; 87:721–31
20. Ririe DG, Barclay D, Prout H, Tong C, Tobin JR, Eisenach JC: Preoperative sciatic nerve block decreases mechanical allodynia more in young rats: Is preemptive analgesia developmentally modulated? Anesth Analg 2004; 99:140–5
21. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL: Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53:55–63
22. Liu B, Li H, Brull SJ, Zhang JM: Increased sensitivity of sensory neurons to tumor necrosis factor alpha in rats with chronic compression of the lumbar ganglia. J Neurophysiol 2002; 88:1393–9
23. Czeh G, Kudo N, Kuno M: Membrane properties and conduction velocity in sensory neurones following central or peripheral axotomy. J Physiol 1977; 270:165–80
24. Pellegrino M, Nencioni B, Matteoli M: Response to axotomy of an identified leech neuron, *in vivo* and in culture. Brain Res 1984; 298:347–52
25. Ririe DG, Eisenach JC: Age-dependent responses to nerve injury-induced mechanical allodynia. ANESTHESIOLOGY 2006; 104:344–50
26. Howard RF, Walker SM, Mota PM, Fitzgerald M: The ontogeny of neuropathic pain: Postnatal onset of mechanical allodynia in rat spared nerve injury (SNI) and chronic constriction injury (CCI) models. Pain 2005; 115:382–9
27. Walker SM, Meredith-Middleton J, Lickiss T, Moss A, Fitzgerald M: Primary and secondary hyperalgesia can be differentiated by postnatal age and ERK activation in the spinal dorsal horn of the rat pup. Pain 2007; 128:157–68
28. Vega-Avelaira D, Moss A, Fitzgerald M: Age-related changes in the spinal cord microglial and astrocytic response profile to nerve injury. Brain Behav Immun 2007; 21:617–23
29. Baccei ML, Fitzgerald M: Intrinsic firing properties of developing rat superficial dorsal horn neurons. Neuroreport 2005; 16:1325–8
30. Beggs S, Torsney C, Drew LJ, Fitzgerald M: The postnatal reorganization of primary afferent input and dorsal horn cell receptive fields in the rat spinal cord is an activity-dependent process. Eur J Neurosci 2002; 16:1249–58
31. Aldskogius H, Hermanson A, Jonsson CE: Reinnervation of experimental superficial wounds in rats. Plast Reconstr Surg 1987; 79:595–9
32. Boada MD, Woodbury CJ: Physiological properties of mouse skin sensory neurons recorded intracellularly *in vivo*: Temperature effects on somal membrane properties. J Neurophysiol 2007; 98:668–80
33. Fedulova SA, Kostyuk PG, Veselovsky NS: Ionic mechanisms of electrical excitability in rat sensory neurons during postnatal ontogenesis. Neuroscience 1991; 41:303–9
34. Ogata N, Tatebayashi H: Comparison of two types of Na+ currents with low-voltage-activated T-type Ca2+ current in newborn rat dorsal root ganglia. Pflugers Arch 1992; 420:590–4
35. Kostyuk P, Pronchuk N, Savchenko A, Verkhratsky A: Calcium currents in aged rat dorsal root ganglion neurones. J Physiol 1993; 461:467–83
36. Benn SC, Costigan M, Tate S, Fitzgerald M, Woolf CJ: Developmental expression of the TTX-resistant voltage-gated sodium channels Nav1.8 (SNS) and Nav1.9 (SNS2) in primary sensory neurons. J Neurosci 2001; 21:6077–85
37. Klein JP, Tendi EA, Dib-Hajj SD, Fields RD, Waxman SG: Patterned electrical activity modulates sodium channel expression in sensory neurons. J Neurosci Res 2003; 74:192–8
38. Felts PA, Yokoyama S, Dib-Hajj S, Black JA, Waxman SG: Sodium channel alpha-subunit mRNAs I, II, III, NaG, Na6 and hNE (PN1): Different expression patterns in developing rat nervous system. Brain Res Mol Brain Res 1997; 45:71–82
39. Rush AM, Brau ME, Elliott AA, Elliott JR: Electrophysiological properties of sodium current subtypes in small cells from adult rat dorsal root ganglia. J Physiol 1998; 511:771–89
40. Hamalainen MM, Gebhart GF, Brennan TJ: Acute effect of an incision on mechanosensitive afferents in the plantar rat hindpaw. J Neurophysiol 2002; 87:712–20
41. Pogatzki EM, Gebhart GF, Brennan TJ: Characterization of Aδ- and C-fibers innervating the plantar rat hindpaw one day after an incision. J Neurophysiol 2002; 87:721–31