Age-dependent Responses to Thermal Hyperalgesia and Mechanical Allodynia in a Rat Model of Acute Postoperative Pain

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Background: Developmental differences in short- and long-term responses to pain, especially surgical pain, have received minimal attention. The purpose of the present study was to examine postoperative responses in rats of developmental ages paralleling the infant to young adult human.

Methods: The withdrawal threshold to von Frey filament testing and withdrawal latency to hind-paw radiant heating were determined before and for various times after hind-paw incision in rats 2, 4, and 16 weeks of age. Control rats of these ages were observed serially without surgery.

Results: In control animals, younger rats were more sensitive to mechanical stimulation and less sensitive to thermal stimulation. Paw incision resulted in similar changes to both types of stimulation in all age groups, peaking 4 h after surgery. However, the return to normal sensitivity to mechanical stimulation, as measured by return of threshold to 80% of normal, occurred more quickly in 2-week-old than in 4- and 16-week-old animals. In contrast, there was no age difference for time to return to normal sensitivity to thermal stimulation after surgery.

Conclusions: The more rapid recovery of the younger animals from the mechanical allodynia but not thermal hypersensitivity after surgery suggests the presence of developmental differences in modulation of A-fiber sensitization after surgery. However, the lack of age difference in recovery of thermal hypersensitivity after surgery suggests that sensitization of C-fiber input has a similar time course of resolution of pain over the ages studied in this model. The neural bases for these developmental differences are under study and may lead to a better understanding of pain during development and altered approaches to treatment of postoperative pain in neonates and infants.

ACUTE pain and chronic pain occur in all age groups. Neonates, infants, and children are often exposed to noxious stimuli ranging from repetitive stimuli in the neonatal period (heel lancing) to vaccinations or major surgery. Considerable research effort has focused on pain and pain mechanisms, but only recently has the importance of pain during development been recognized.1-4 As a result, research has begun to be focused on the impact of development, particularly the developmental influences surrounding the neonatal period.1 However, developmental differences in response to pain in the period from the neonate to the adult have received less attention. Furthermore, our knowledge of the cause of the differences in the pain pathways during this developmental period and the role they may play in differential age-dependent behavioral responses remains limited.

In 1996, a model of acute incisional pain was developed by Brennan et al.5 Incision results in hypersensitivity to mechanical and thermal stimuli in these rats, as it does in postoperative patients. No previous studies have examined the role of development in this model of postoperative pain, despite the common occurrence of surgery at younger ages. However, developmental differences have been demonstrated in response to persistent noxious stimulation in inflammatory models of pain by use of carrageenan, mustard oil, and formalin.6-8 In addition, developmental differences in response to specific nerve fiber stimulation have been demonstrated.9 In human infants, mechanical hypersensitivity has been demonstrated after surgery, but developmental changes have not been examined.10 The objectives of this study are to characterize the hypersensitivity after surgery from the neonatal period to early adulthood in the rat. Specifically, we hypothesize that there are differences in behavioral responses to mechanical and thermal hyperalgesia as a function of age in the acute postoperative pain model.

Materials and Methods

After approval from the Animal Care and Use Committee, male Sprague-Dawley rats were used to study the effects of paw incision on withdrawal thresholds. Three groups of animals were studied (2, 4, and 16 weeks of age), representing preweanling, postweanling, and sexually mature animals. Different animals were used for the thermal and mechanical testing, and controls were littermates of the treated animals.

After baseline testing, animals were anesthetized with 2% halothane in oxygen with spontaneous ventilation. As described previously,5 the plantar aspect of one hind paw was prepared in a sterile manner with a 10% povidone-iodine solution and draped. Using sterile technique, a midline linear incision was made with a No. 11
scalpel from the heel to the base of the toes. A fixed length of incision was not used, because this anatomic distance increases with age. The incisions were therefore comparable as a function of the size of the foot of animals of different ages. The incision was made in the middle of the foot longitudinally from the proximal toe pad to the heel so that the heel end of the incision was equidistant from the lateral, medial, and proximal portions of the foot surface. A small forceps was used to elevate the flexor tendon from the heel to the toes. The incision was then closed with two inverted mattress stitches using 5-0 nylon sutures on an FS2 needle. The sutures were left in place; 90% of the sutures were gone by 1 week, and all sutures were gone at 2 weeks (this was similar across age groups). The animals were allowed to recover from general anesthesia for 2 h. For the preweanling animals, the animals were allowed to recover in the cage with their mothers. No animal in the study had a wound dehiscence or infection during the study; therefore, all animals were included in the data analysis.

**Mechanical Threshold**

Mechanical withdrawal threshold was determined before surgery, then at 4 h and 1, 2, 3, 7, 11, and 14 days after surgery. This duration was determined by following all animals until the youngest animals had all been back to baseline thresholds for at least 1 week. The threshold to withdrawal with a 50% probability to testing with calibrated von Frey monofilaments was determined by use of the up-down method as previously described.11 These thresholds were determined distal to the incision 1–2 mm into the foot pad. Withdrawal thresholds to mechanical stimulation are presented as mean ± SEM and were analyzed within each age group over time using repeated-measures ANOVA with the Fisher protected least-squares difference. The withdrawal response thresholds ipsilateral to surgery were compared with those for the contralateral hind paw and for control animals that did not receive surgery on either paw. Time for withdrawal threshold to return to within 80% of that of the nonoperated hind paw in the same animal was determined. These times to 80% recovery are presented as median with ranges and were compared across age groups using Kruskal–Wallis analysis and then the Wilcoxon rank sum test.

**Thermal Threshold**

Thermal testing was performed after the animal had been placed in a clear plastic box on a glass surface maintained at 30°C. A calibrated radiant heat source was focused on the hind paw, and the latency to withdrawal was recorded, using a 30-s maximum exposure to avoid tissue injury. Withdrawal latency was measured three times in each foot just lateral to and anterior to the incision in the middle of the footpad. These three observations were averaged for each animal. Thermal withdrawal latencies were determined before surgery and then at 4 h and 1, 2, 3, 7, 11, 14, 21, 28, 35, and 42 days after surgery. This duration was determined by following all animals until the youngest animals had all been back to baseline latencies for at least 1 week. Withdrawal latency to thermal stimulation is reported as mean ± SEM and was analyzed within each age group over time by repeated-measures ANOVA with the Fisher protected least-squares difference. The withdrawal latency ipsilateral to surgery was compared with that for the contralateral hind paw and for control animals that did not receive surgery on either paw. Time for withdrawal latency to return to within 80% of that of the nonoperated hind paw in the same animal was determined. These times to 80% recovery are presented as median with ranges and were compared across age groups by Kruskal–Wallis analysis and then the Wilcoxon rank sum test.

**Results**

**Mechanical Thresholds**

The withdrawal threshold to punctate mechanical testing with von Frey filaments increased as a function of age, with the younger animals having a significantly lower mechanical threshold than the older animals. Thus, in control animals without surgery, the withdrawal threshold to mechanical stimulation was lowest in 2-week-old (5.9 ± 0.8 g; n = 12), intermediate in 4-week-old (19 ± 0.8 g; n = 12), and greatest in 16-week-old (29 ± 2.5 g; n = 10; P < 0.05, all groups differ) animals (fig. 1). Two weeks later in control animals, the 2-week-olds exhibited increases in withdrawal threshold, which was then not different from the 4-week-olds (18 ± 0.8
and 18 ± 2.1 g, respectively), but both were still lower than in the 16-week-olds (31 ± 4.7 g; P < 0.05 compared with 2- and 4-week-olds). Further testing demonstrated that the thresholds of the 2- and 4-week-olds continued to increase over time and reached thresholds similar to those of the adult (16-week-old) animals by approximately 6–10 weeks of age.

The withdrawal threshold to von Frey filament testing decreased significantly after surgery in animals of all ages, with a peak 4 h after the incision (fig. 2). At this time, the percent decreases in thresholds from presurgery values were similar for all ages (67% decrease for 2-week-olds, 79% for 4-week-olds, and 72% for 16-week-olds). Despite this similarity in degree of reduction in withdrawal threshold, the duration of reduced withdrawal threshold to tactile stimulation varied as a function of age (fig. 2). The median time from surgery until the withdrawal threshold of the injured paw returned to within 80% of the contralateral paw was 2 days (range, 4 h to >14 days) in the 2-week-olds, 5 days (4 h to >14 days) in the 4-week-olds, and 8.5 days (4 h to >14 days) in the 16-week-olds (P < 0.05, 2-week group differs from 4- and 16-week groups).

**Thermal Withdrawal Latency**

In control animals without surgery, withdrawal latency to thermal stimulation was greater in 2-week-olds than 4- or 16-week-olds (18.8 ± 1.1 s in the 2-week-olds [n = 8], 11.2 ± 0.7 s in the 4-week-olds [n = 8], and 12.2 ± 0.5 s in the 16-week-olds [n = 8]) (fig. 3). This difference was still present 1 week later in these controls but was gone 35 days later (fig. 3). As with mechanical testing, paw incision resulted in hypersensitivity to thermal stimulation, which peaked 4 h after surgery and was similar across all ages (78% in 2-week-olds, 70% in 4-week-olds, and 75% in 16-week-olds) (fig. 3). Unlike mechanical testing, however, recovery of the injured paw to within 80% of the contralateral paw to thermal testing occurred with a similar time course in all age groups (fig. 4) (median of 7 days [range, 1–35 days] in 2-week-olds, 7 days [1–42 days] in 4-week-olds, and 10 days [1–35 days] in 16-week-olds).

**Discussion**

In this study, developmental differences in response to mechanical threshold withdrawal responses were demonstrated, with age-dependent increases in mechanical thresholds from 2 to 16 weeks of age. This is similar to previous studies using rats demonstrating increasing withdrawal thresholds as a function of age. This is also consistent with studies in humans examining developmental differences in withdrawal thresholds.
However, our results extend the age range over which differences in responses have been reported. Furthermore, the present study also examined differences in mechanical thresholds in response to a surgical stimulus, demonstrating a more rapid decrease in mechanical allodynia after surgery in very young animals.

All age groups demonstrated development of mechanical allodynia immediately after the incision. In our study, the mechanical allodynia in the 2-week-old animals diminished much more rapidly than in the 4- and 16-week-old animals and by 2 days was within 80% of control. During this time in the normal course of development, the mechanical threshold for withdrawal is increasing, possibly in relation to A-fiber withdrawal from deeper lamina in the dorsal horn. After the surgical insult, the increase in threshold that normally occurs seems to overcome the mechanical allodynic response rapidly. Whether this phenomenon is an effect of more rapid healing of the wound itself or results from developmental changes or modulations in the peripheral and/or central nervous system remains unclear.

We also report differences in thermal withdrawal thresholds as a function of age, with the 2-week-old animals having a greater threshold to the thermal stimulus than either the 4- or 16-week-old animals. This is in contrast to the results with mechanical thresholds. Although it might be anticipated that the younger animals would have a shorter latency to the thermal stimulus, thermal latency has been reported to vary widely and may depend on not only age but also heat source, distance from the heat source, location of the thermal stimulus, and the heat absorption of the tissue itself. Our results may be related to our assessment of the thermal withdrawal latency at the foot pad just anterior and lateral to the incision. However, consistent with our results with the 14-day-old animals having a higher latency threshold, others have seen an increase in thermal latency in the tail and forepaw between 9 and 21 days. Because the thermal latency is a measure of primarily C-fiber function in other models, this may be consistent with the C-fiber input not developing fully until the second postnatal week, as previously suggested.

In our study, the same intensity of thermal stimulus was used in the animals of different ages, such that the difference at baseline withdrawal is different, possibly because of the size of the foot and possibly of differences in radiant heat absorption. We thought that it was important to use the same intensity of thermal stimulus across the age groups and not change the thermal threshold to achieve a fixed withdrawal threshold across the age ranges. Although having a longer withdrawal threshold in the younger animals compared with the older animals may make the younger more sensitive to changes in thermal latency from pain, if anything, this would increase the likelihood of finding a difference between the younger animals and the older animals. Because we did not find a difference, use of the same intensity allowed us to demonstrate yet another difference in behavior in the younger animals, the difference in baseline thermal withdrawal latency.

This is the first study to examine the thermal threshold changes as a result of surgical injury over different ages. The lack of age-dependent differences in the thermal withdrawal latency return to baseline suggests that over this age range, the C-fiber modality is functionally the same as for the older animals. This is in contrast to the A-fiber modality measured by use of mechanical threshold. The reason for the differences over age for the different modalities is unclear. Differences could be from effects of inhibition on the different nerve fibers. This could be either from anatomic development at this time, whereby descending inhibitory pathways that are known to still be immature at 2 weeks may have a greater effect on modulation of the spinal signals from
one fiber or another,21 or from developmental differences in other inhibitory neurotransmitters.22

The possibility exists that rapid healing is responsible for the return to control levels for the mechanical threshold. However, the wound at 24–48 h grossly appears the same in all age groups. The incision still has the sutures at that time, and the skin is still incompletely healed. Nevertheless, decreased inflammation in the deeper tissues or a more rapid reduction in inflammation in the younger animals is possible. If this were the case, one might expect to see differences in both thermal and mechanical testing. Another possibility is that the changes reflect maturation in the central nervous system during this time of development. In previous experiments with this model, the mechanical threshold reductions were found to be mediated by wide-dynamic-range neurons.23 In particular, this may be a result of differentiation and development, whereby establishment of more permanent connections in the dorsal horn from the peripheral nerve fibers is altered or accelerated in the injured animal to return the mechanical threshold to control levels in the 2-week-old animals more rapidly, possibly via wide-dynamic-range neurons.

Previous work has been done looking at the age of the initiation of the chronic painful insult with the spinal nerve ligation model.24 In this study, differences in onset and duration of allosthyria were reported as a function of age in development between 1 and 3 weeks. In the 3-week-old rats, allosthyria developed to an extent similar to that seen in adult rats. For the younger rats, the signs of mechanical allodynia lasted for a shorter period of time. This once again is consistent with the results in this acute pain model, suggesting that a mechanism extinguishes this response or that another maturational event occurs between the second and third postnatal weeks in the rat to permit the vulnerability to maintenance of pain. However, no further studies have been done using this chronic model to define the changes that occur between postnatal weeks 2 and 3 that may be responsible for these observations.

Another pain model, the formalin model, has also been used to study differences in responses during development. This is an acute model of inflammatory pain that may be closer to the acute postoperative model than the previous chronic pain model. In this model, using formalin injection into the paw, hypersensitivity was greatest in the younger animals, reaching adult levels by 4 weeks of age.5,25 The animals’ achievement of a response to the pain stimulus similar to that of adults by 4 weeks of age is consistent with our results. We did not specifically examine the type of response to the surgery qualitatively and therefore cannot draw conclusions about the younger animals possibly being more sensitive or having a greater response, as was done in the preceding study.26

The 2-week-old rats also show a very important difference from the other ages; they are still breast-feeding and continue to do so until 3 weeks of age, when they are weaned. The mere interaction of the 2-week-old with the nurturing of the mother may provide some effects that might alter behavioral responses.27 Furthermore, breast-feeding may have analgesic properties and therefore may alter responses to behavioral testing of postoperative pain.28 We would anticipate that if breast-feeding or social interaction modulated the hypersensitivity responses, both thermal and mechanical thresholds would be affected similarly. Because the mechanical allodynia returned to normal more rapidly than the thermal hyperalgesia, we do not believe that the interaction of the 2-week-old animals with the mother explains our findings, although it is still possible that a differential nerve fiber effect of socialization could occur.

One of the limitations of this study is the use of animals at different stages of development and the attempt to determine the developmental correlate or stage of developmental equivalency in other animal models or in humans. The newborn rat pup is similar in neurologic development to the 25-week premature human infant, the 1-week-old rat pup is equivalent to the newborn human infant, and the 3-week-old rat similar to the toddler.25 However, correlation between other ages of rats in weeks and humans in years and neurologic and anatomic stages of development is unclear. Because the average rat weans between 3 and 4 weeks of age and the average rat is sexually mature at about 10 weeks of age, we assume that the 16-week-old rat is similar in developmental stage to the adult human and the 4-week-rat is similar to the small child between the ages of 2 and 4 yr. Along these lines, the 2-week-old rat pup is probably similar to the human infant between the ages of 6 months and 1 year.

Another limitation of this study is the use of mechanical allodynia and thermal hyperalgesia to assess pain. The use of mechanical allodynia is an elicited response and a measure of pain. This may be akin to movement or physical therapy–like pain after surgery but does not give a true reflection of ongoing painful input into the central nervous system from the injured site. Nevertheless, it provides objective information regarding the sensitivity of the nerves in the area of injury, although it may be slightly different from the pain signals at rest. The use of thermal hyperalgesia is a measure of C-fiber-mediated pain,18 but the correlation between postoperative pain sensation and thermal hyperalgesia is unclear.

In this study, we have demonstrated differences in mechanical allodynia as a function of age and that the resolution of postoperative mechanical allodynia but not thermal hyperalgesia is developmentally regulated. Many different systems are changing in the first several weeks of maturation in the rat, both peripherally and centrally. Differences in immune function that may alter the in-

Anesthesiology. V 99, No 2, Aug 2003
flamatory response to tissue trauma from surgery; developmental differences in opioid, NMDA, and other receptor systems; and neuroanatomic changes are all occurring.\textsuperscript{15,21,29–51} Our results help define the role of development in responses to acute surgical pain and will allow further studies of the anatomic and biologic mechanisms. Further examination of the developmental responses of C-fiber- and A-fiber-mediated hypersensitivity may help in understanding the differences between children and adults in response to pain and surgery. These further studies may help clarify differences between acute pain responses during development, allow improved treatment, and possibly lead to prevention or reduction in duration of hyperalgesia and allodynia.

References