

Postoperative Models of Nociception

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Introduction

Postoperative pain is a common form of acute pain, yet it is a costly, poorly understood problem (Kehlet 1994). It is generally thought that postoperative pain can be adequately treated by administration of sufficient parenteral opioids. However, studies of patients after major surgeries demonstrate that postsurgical pain induced by activities is poorly responsive to opioids compared with pain at rest (Gould and others 1992). In particular, pain measures during ambulation and with coughing and movement (mechanical stimuli) may be severe even with sufficient parenteral opioid use in patients after major surgery. Additional drug dosing can be limited by respiratory depression and sedation; other side effects such as nausea and vomiting, delayed gastric emptying, and prolonged ileus are common. Better pain control with activity can be achieved with combinations of drugs administered epidurally (Dahl and others 1992); however, this therapy is labor intensive, is not without risk, and has not yet proven to be cost effective. Thus, despite the simple nature of pain caused by tissue injury after incision, uncomplicated, effective, inexpensive treatments are not yet available (Kehlet 1994).

Even though pain research has resulted in many remarkable discoveries in the last 15 yr, a small proportionate effort has been made toward understanding pain caused by an incision (Kalso and Rosenberg 1995). If we learn more about mechanisms of acute incisional pain and the sensory processes that intensify pain after surgery, new treatment methods can be advanced. It has been suggested that it is necessary to study the efficacy of postoperative analgesic treatments using pain scores during evoked responses such as movement and coughing if outcome is to improve (Kehlet 1994). Thus, mechanical hyperalgesia—a decreased pain threshold and an increase in pain response to suprathreshold stimuli—is a most important property of incisional pain. Research on mechanical hyperalgesia caused by an incision is important for developing therapies for postoperative pain.

Previously, the experimental pain community has lacked an animal model for incision-induced pain. To understand mechanisms of sensitization caused by surgery and investigate new therapies for postoperative pain in humans, col-

leagues and I developed and characterized a rat model for human postoperative incisional pain (Brennan and others 1996). This new model has several unique properties compared with other animal models of pain. Importantly, it is caused by an incision, is profound and persistent, is characterized by reduced withdrawal thresholds suggesting that mechanical hyperalgesia is present, and results in behaviors that are timed similarly to pain measures in postoperative patients.

A Rat Model for Postoperative Pain

Anesthesia and Surgery

Colleagues and I have performed our experiments on adult male rats. Anesthesia is induced with 1.5 to 2% halothane in 100% oxygen in a sealed box; then after induction, the same halothane concentration is delivered via a nose cone. Each animal subsequently receives an intramuscular injection of penicillin (30,000 IU of Flocillin) in the triceps muscle. The plantar aspect of either hind paw is prepared in a sterile manner with a 10% povidone-iodine solution, and the foot is placed through a hole in a sterile cloth drape. A 1-cm longitudinal incision is made with a number 11 blade through skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. In most animals, the plantaris muscle is elevated and incised longitudinally (Figure 1A). The muscle origin and insertion remain intact. After hemostasis with gentle pressure, the skin is apposed with two mattress sutures of 5-0 nylon on an FS-2 needle (Figure 1B). The wound site is covered with a mixture of polymixin B, neomycin, and bacitracin ointment. The sutures are removed under halothane anesthesia at the end of postoperative day 2. Typically the wounds are well healed within 5 to 6 days.

Emergence and Early Postoperative Period

After surgery, the rats are housed individually in clean bedding consisting of organic cellulose fiber such as Cellu-Dri (Shepherd Specialty Papers, Inc, Kalamazoo, Michigan) or Tek-Fresh (Harland, Madison, Wisconsin). The incisions are checked daily for any sign of wound infection, dehiscence, or hematoma that would exclude rats from further study. We make every attempt to avoid infection because of the poten-

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Figure 1 Photographs of the different stages of the surgery of the rat foot. (Left) A 1-cm longitudinal incision is made through the skin and fascia starting 0.5 cm from the proximal edge of the heel and extending toward the middle of the foot. The plantaris muscle is elevated and incised longitudinally. (Right) After hemostasis, the wound is apposed with two mattress sutures.

tial for increased inflammation at the incision. Although this has not occurred, it could confound the assessment of pain behaviors. Dehiscence can occur if the rats remove the suture during emergence or in the early postoperative period. Poor apposition of the wound could prolong the time course or intensify pain behaviors. Approximately one in 12 animals is excluded for wound dehiscence. Hematoma formation is rare.

There appears little evidence for severe pain and distress since spontaneous pain behaviors like vocalization or persistent *flinching behavior* have not been observed postoperatively. We have observed limping and some guarding during the recovery period. Typically the animal licks the antibiotic ointment that has been applied at the end of the surgery. It is not known whether this is a pain-related licking behavior as in other pain models. We allow 2 hr for recovery before we observe the rats' behavior, although in some cases, pain-related behaviors have been examined as soon as 1 hr after the incision and termination of anesthesia.

Pain-related Behaviors

After emergence and recovery from anesthesia, unrestrained rats are placed beneath a clear plastic chamber (21 × 27 × 15 cm) on an elevated plastic mesh floor (ADPI Enterprises, Philadelphia, Pennsylvania) and allowed to acclimate until they are resting undisturbed. Withdrawal responses to mechanical stimulation are determined using calibrated Semmes Weinstein von Frey filaments (Stoelting, Wood Dale, Illinois). These filaments are nylon monofilaments with similar lengths and varying diameters; as the diameter of the filament increases, the force necessary to bend the filament increases. These monofilaments are applied individually from under the cage through openings in the plastic mesh floor (12 × 12 mm grid) to an area adjacent to the wound (Figure 2). Most often they are applied medial to the incision near the heel. Each filament is applied once starting with 15 milliNewtons (mN) and continuing in an ascending order until a withdrawal response occurs or 522 mN (the

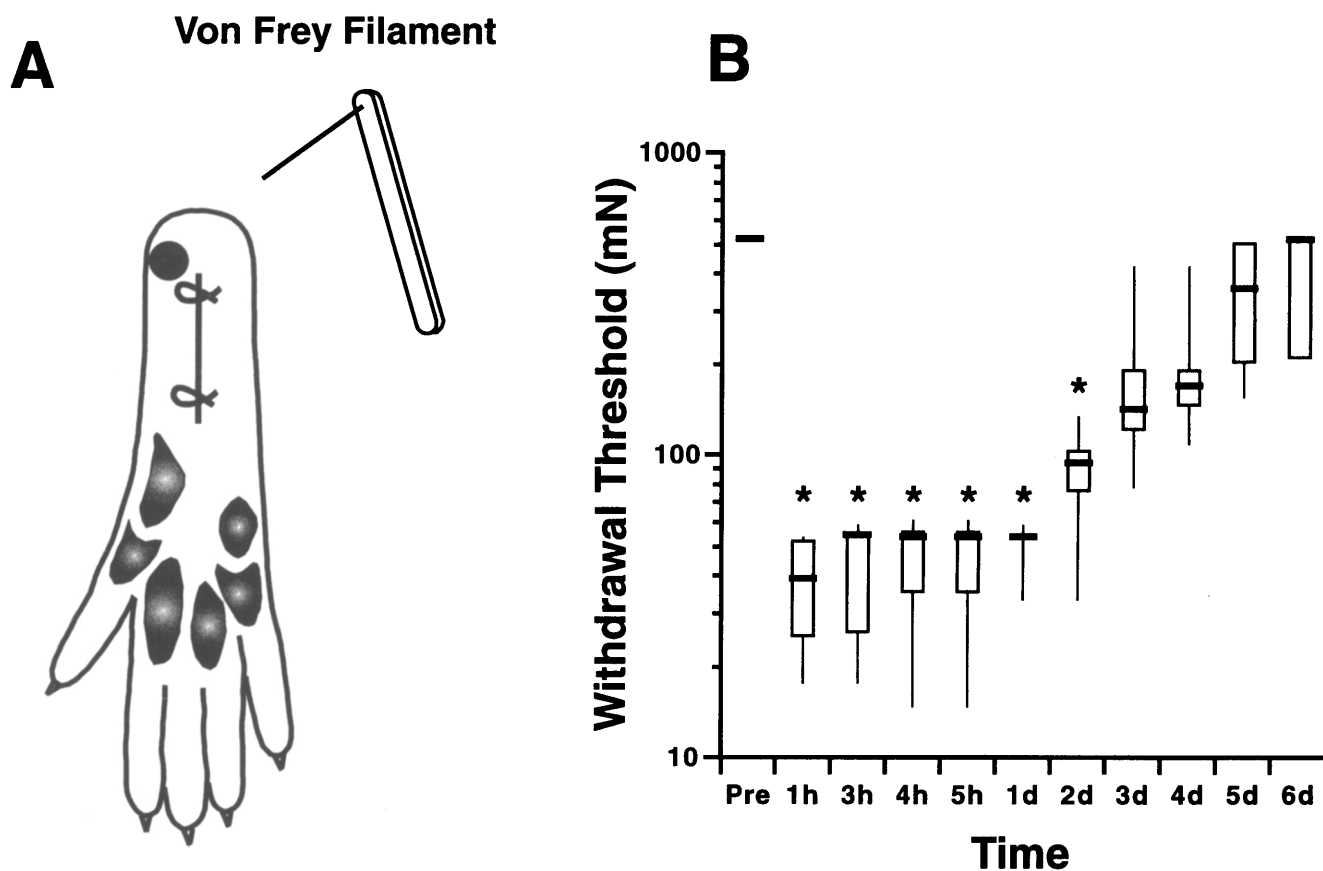


Figure 2 Time course of withdrawal thresholds to punctate mechanical stimulation after plantar incision. Results are expressed as medians (horizontal line) with 1st and 3rd quartiles (boxes), and 10th and 90th percentiles (vertical lines). (A) Diagram of the plantar aspect of the rat foot showing the incision and the site of application of von Frey filament (small darkened circle). (B) Withdrawal thresholds after incision in vehicle-treated rats. Pre represents time before foot incision. * $p < 0.05$ versus preincision values by Friedman and Dunnett's test. Reprinted from *Anesthesiology*, Vol. 87, T.J. Brennan, E. Umali, and P.K. Zahn, "Comparison of pre- versus post-incision administration of intrathecal bupivacaine and intrathecal morphine in a rat model of postoperative pain," p 1517-1528, 1997, with permission from Lippincott Williams & Wilkins.

cutoff value) is reached. When these filaments are applied to the unincised normal rat foot near the heel, withdrawal is usually not elicited until the cut-off filament is applied. The withdrawal responses are brisk after the punctate filament application and are not accompanied by vocalization. The rats can be more active after the withdrawal and require a few minutes to acclimate themselves before further testing is possible. Typically, after a 5-min test-free period, each filament is again applied once starting with 15 mN until a withdrawal response is elicited. This is repeated a third time 5 min later. The lowest force from the three tests producing a response is considered the withdrawal threshold. The cutoff value—522 mN—is recorded even if there had been no withdrawal response to this force. Typically the withdrawal threshold in the incised foot decreases after incision to less than 100 mN for 2 to 3 days (Figure 2). A gradual return toward preincision withdrawal thresholds occurs over the next 5 to 6 days. A reduced withdrawal threshold to punctate mechanical stimuli occurs at other sites around the wound as

well. A similar time course has been shown in a number of studies and demonstrates that this incisional injury causes reproducible, quantifiable, reduced withdrawal thresholds to mechanical stimuli, indicating that mechanical hyperalgesia occurs. This mechanical hyperalgesia persists for several days after the incision and then gradually decreases. Similar filaments have been used to characterize the area of mechanical hyperalgesia in patients after surgery (Richmond and others 1993; Stubhaug and others 1997; Wilk and others 1996).

In additional studies, another mechanical stimulus has been used to characterize the pain behaviors after incision (Zahn and others 1997). We have observed that after incision, rats do not allow a blunt mechanical stimulus (a plastic disk attached to a nylon monofilament) to be applied to the wound (Figure 3). When this plastic disk is applied directly on the wound, the rats withdraw as with the punctate von Frey filaments, or they lift the foot, not allowing the 400-mN filament to bend. This blunt mechanical stimulus evokes no

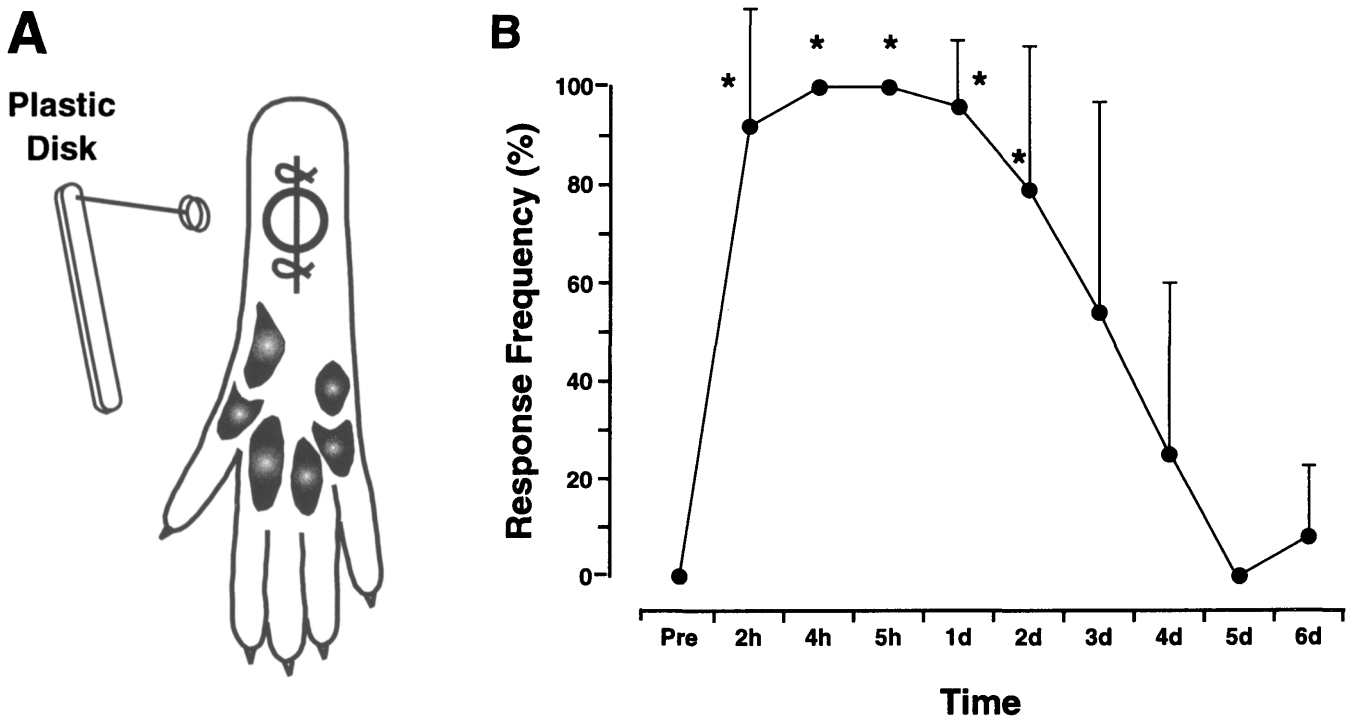


Figure 3 Time course of responses to a blunt mechanical stimulation (plastic disk attached to a von Frey filament) after plantar incision. (A) Diagram of the plantar aspect of the rat foot showing site of application of the plastic disk (larger, darkened circle). (B) Response frequency after incision in vehicle-treated rats. Pre represents time before foot incision. * $p < 0.05$ versus preincision values by Friedman and Dunnett's test. Reprinted from *Anesthesiology*, Vol. 87, T.J. Brennan, E. Umali, and P.K. Zahn, "Comparison of pre- versus post-incision administration of intrathecal bupivacaine and intrathecal morphine in a rat model of postoperative pain," p 1517-1528, 1997, with permission from Lippincott Williams & Wilkins.

response in normal rats; blunt mechanical stimuli applied to an incision have also been used to measure hyperalgesia in patients after surgery (Inagaki and others 1993; Moiniche and others 1997; Tverskoy and others 1990).

In this model, no measure of spontaneous pain behaviors like biting, scratching, licking, or vocalization have been utilized because these activities have not been observed. We have observed that rats do not bear weight on the incision in the postoperative period and have developed a cumulative pain scale that relies on the position in which the foot is placed during a 1-min period. This nonevoked weight-bearing pain behavior may be another measure of mechanical sensitivity since our scoring is based on distortion of the wound by the mesh cage floor and likely represents nonevoked rather than spontaneous pain behavior (Zahn and others 1997).

Again, after emergence and recovery from anesthesia, unrestrained rats are placed beneath a clear plastic chamber ($21 \times 27 \times 15$ cm) on a smaller elevated plastic mesh floor (8×8 mm grid; ADPI Enterprises). Using an angled magnifying mirror, the incised and nonincised feet are viewed. Both feet of each animal are closely observed during a 1-min period repeated every 5 min for 1 hr. Depending on the position in which each foot is found during the majority of the 1-min scoring period, a 0, 1, or 2 is given. Full weight bearing of the foot (when the wound has been blanched or

distorted by the mesh) is assigned a score of 0. If the foot is completely off the mesh, a score of 2 is recorded. If the area of the wound touches the mesh without blanching or distorting, a 1 is given. The 12 scores (0 to 24) obtained during the 1-hr session for each foot are summed, and the difference between scores from the incised and nonincised feet is taken as the cumulative pain score for that 1-hr period. This pain behavior is increased after incision, and a gradual return toward preincision values occurs over the next few days (Figure 4).

We have followed the time course of pain-related behaviors and have observed the rats for 5 to 6 days in a number of studies. Throughout the experimental period, the animals remain well groomed and maintain normal food and water intake. The weights of rats undergoing incision were recorded throughout a study period ($n = 6$ per group). The weights of a sham group and a group undergoing skin, fascia, and muscle incision before surgery were 346 ± 5 and 355 ± 18 g (mean \pm SD), respectively. There was a small decrease in weight during the first 3 days after surgery (or sham operation) but no significant difference between the two groups (Figure 5). The respective weights of the sham and the incision groups at the end of the protocol were 351 ± 7 and 356 ± 24 g (Brennan and others 1996). Except for impaired weight bearing on the area of the incision early in the postoperative period, gait appears normal.

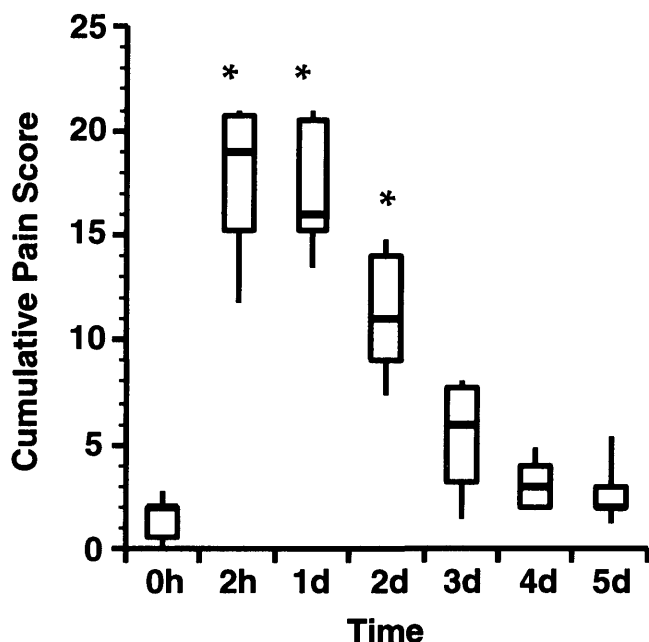


Figure 4 Summary of the cumulative weight-bearing scores before and after skin, fascia, and muscle incision. Results are expressed as medians with 1st and 3rd quartiles, and 10th and 90th percentiles. * $p < 0.05$ versus preincision values (0h) by Friedman and Dunnett's test for nonparametric analyses. Reprinted from *Pain*, Vol. 64, T.J. Brennan, E. Vandermeulen, and G.F. Gebhart, "Characterization of a rat model of incisional pain," p 493-501, 1996, with permission from Elsevier Press.

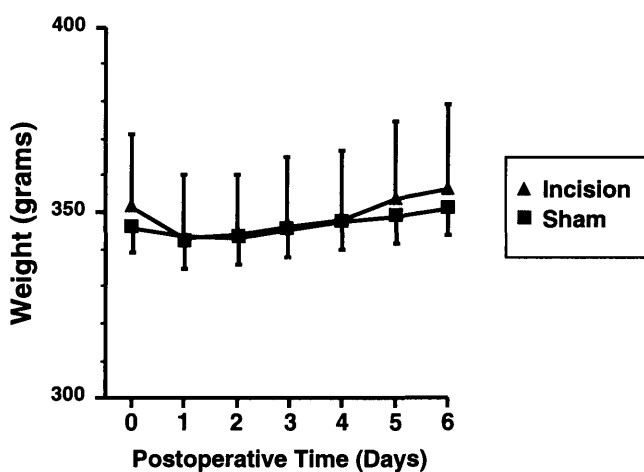


Figure 5 Weights of rats before surgery (day 0) and on postoperative days 1 through 6. There were no differences between the incision and the sham groups.

Significance

Pain research has progressed from the physiology of thermal and mechanical nociception in healthy humans and normal animals (hot plate and tail flick tests) to examining the pathophysiology of hyperalgesia and the neurophysiological correlate to hyperalgesia—sensitization—in the postinjury state. As we develop a better understanding of pain mechanisms and hyperalgesia, specific models for particular pain syndromes will be necessary. Animal models for postoperative pain will facilitate application of this pathophysiology to clinical postoperative pain.

Incision-induced Injury

A surgical incision produces tissue injury that is distinctly different from chemical irritation with formalin, inflammation by carrageenan injection, or nerve injury by ligation. First, few models utilize a surgical incision as the noxious event. An incision is a focal tissue injury of superficial and deep structures. The mechanisms for initiation and maintenance of pain after incision likely involve a combination of nerve injury, inflammation, pH changes, and central nervous system plasticity; however, it must be emphasized that the contribution of each of these components is not known. Perhaps a specific antiinflammatory agent may prevent the development of inflammation-induced hyperalgesia; little or no effect may be observed in an incision model. Because the etiology of incisional pain may be different from inflammatory pain, chemical irritation, or nerve injury, the responses to treatments inhibiting the development and maintenance of pain behaviors may also differ.

Hyperalgesia

The tests of pain-related behaviors studied in animals should be relevant to the measures of clinical pain in patients. Pain at rest and mechanical hyperalgesia are the most clinically relevant pain measures to examine in a postoperative pain model. Although reduced withdrawal latencies to radiant heat occur after plantar incision in the rat (Gonzalez and others 1998), the role of thermal hyperalgesia in postoperative pain is not clear. There is a disparity in how certain drugs and treatments inhibit responses to thermal and mechanical stimuli after particular injuries (Wegert and others 1997; Zahn and Brennan 1998; Zahn and others 1998). Therefore, even though a treatment may inhibit thermal hyperalgesia, reduction of enhanced responses to mechanical stimuli may not occur, and the likelihood of clinical benefit is probably less.

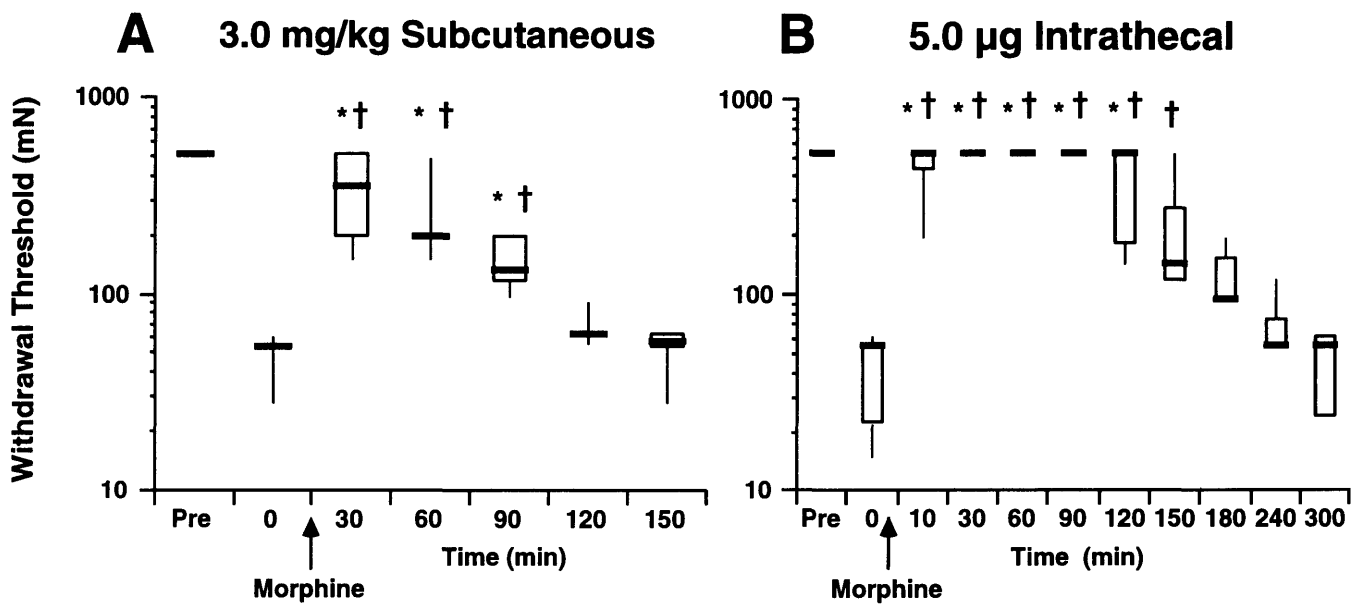


Figure 6 Effect of morphine on punctate withdrawal thresholds after plantar incision. (A) Withdrawal threshold after incision in rats treated with 3 mg/kg of subcutaneous morphine on the day of surgery. (B) Withdrawal threshold after incision in rats treated with 5 µg of intrathecal morphine on the day of surgery. Morphine was administered at the arrow. * $p < 0.05$ versus 0 min by Friedman and Dunnett's test. The box and whisker plots are described in the Figure 1 legend. Reprinted from *Anesthesiology*, Vol. 86, P.K. Zahn, D. Gysbers, and T.J. Brennan, "Effect of systemic and intrathecal morphine in a rat model of postoperative pain," 1997, p 1066-1077, with permission from Lippincott Williams & Wilkins.

Modulation of Pain Behaviors

The effect of intrathecal and subcutaneous morphine, effective for postoperative pain relief in patients, has been examined in this rat model for postoperative pain. Administration of either subcutaneous (0.3 to 3.0 mg/kg) or intrathecal (0.16 to 5.0 µg) morphine reversibly increased the withdrawal threshold to punctate mechanical stimulation (Figure 6). In addition, the response frequencies to the blunt mechanical stimulus and the pain scores were also decreased by subcutaneous and intrathecal morphine (Zahn and others 1997). We have also observed that local anesthetic infiltration of the incision with 0.5% bupivacaine decreased pain behaviors for at least 2 hr after incision (Vandermeulen and others 1994).

Time Scale

The onset, progression, and duration of persistent pain behaviors in animal models of chemical irritation, inflammation and nerve injury, and pain and hyperalgesia in postoperative patients are different. In the plantar incision model, the incision and closure require 15 min to perform. Hyperalgesia is profound immediately after surgery and on the first postoperative day, gradually decreases but persists for about 5 days, and then is similar to sham and preoperative levels. The onset of pain in other animal models varies from minutes after injection of formalin, to hours after injection of inflammatory agents, or to days after nerve injury. This

incisional model in the rat with quantifiable pain behaviors occurs on a time scale similar to most postoperative patients whose pain reports and mechanical hyperalgesia are usually the greatest immediately after surgery, severe for several days later, and then gradually diminish over 7 to 10 days (Moiniche and others 1997).

Advantages of the Rat Model

There are advantages to the use of this animal model of postoperative pain to clinical studies. Clinical studies utilize a particular intervention to modify two variables: pain score(s) and opioid utilization. Studies examining novel analgesic treatments can result in a mixed effect—a reduction in opioid utilization with the same pain scores or a reduced pain score and the same opioid requirement. In this rat model for postoperative pain, a behavioral assessment of surgical wound "mechanical sensitivity" is made, and confounding treatments and measures are avoided.

Other Postoperative Pain Models

Ovariohysterectomy has been used as an animal model for postoperative pain in rats (Lascelles and others 1995). After general anesthesia is administered, a midline ovariohysterectomy is performed. Thermal nociceptive thresholds (tail flick latency) and mechanical nociceptive thresholds (paw pres-

sure test) are examined at several times after surgery to study the development of central nervous system hypersensitivity. Only the paw pressure test was enhanced, indicating remote (secondary) mechanical hyperalgesia after ovariohysterectomy. This model has obvious relevance to a common clinical procedure, and the particular injury is different from a skin and muscle incision. Additional peritoneal and visceral injuries are likely associated with ovariohysterectomy and may intensify pain behaviors. The importance of remote hindlimb hyperalgesia after hysterectomy is not known; this does not occur in patients (Moinich and others 1997).

The same group of investigators has studied dogs electively scheduled for ovariohysterectomy (Lascelles and others 1997). A midline ovariohysterectomy is performed under general anesthesia. An algometer, a device used to deliver a quantifiable force, is applied to remote areas such as the tibia or pinna of the ear as well as directly to the surgical wound. The thresholds to withdrawal, vocalization, aggression, or guarding are measured. These results in dogs also indicate central nervous system hypersensitivity after ovariohysterectomy occurs and have the advantage of a measure of wound sensitivity. Pain measures are reduced by parenteral administration of pethidine, an opioid agonist. In addition, this dog model is unique because a visual assessment scoring system has been developed for pain measurement at rest. Pain at rest is commonly measured in postoperative patients.

Postoperative somatic injury may include not only skin and muscle trauma but also bone injury. An experimental model for investigating the mechanisms underlying bone pain has been described by Houghton and others (1997). In rats anesthetized with methohexital, a hole is drilled through the tibia or calcaneus. Nocifensive behaviors, a lifting and guarding of the injured hindlimb, are observed. Hyperalgesia to thermal and mechanical stimuli are also described. Drilling a hole through the tibia or calcaneus could be a useful experimental model for additional studies on postraumatic and postoperative bone pain.

Conclusion

Clinically, the severity of postoperative pain varies depending on the particular surgery performed, the activity during which the pain is assessed, and the analgesic regimen employed. It has been suggested that pain after particular surgeries is relatively insensitive to parenteral opioids compared with other surgeries (Kalso and Rosenberg 1995). At the time of this writing, the mechanisms for postoperative pain are not understood even after a simple cutaneous incision. We must develop a better understanding of the unique aspects of postoperative pain states caused by particular injuries because postoperative pain states after cutaneous incision, muscle injury, visceral surgery, and bone trauma have unique characteristics. Improved testing modalities to better understand the particular sensitization processes should be utilized so that better, more precise treatments for

specific clinical postoperative pain states can be advanced. In the future, treatment strategies that prevent the development of postoperative pain should be possible. A reduction in morbidity, mortality, and costs will follow.

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