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Effects of Laparotomy on Spontaneous Exploratory Activity and Conditioned Operant Responding in the Rat

A Model for Postoperative Pain

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Background: **Treatment of postsurgical pain is a major use of analgesics, particularly after abdominal surgery. Analgesics display a number of limiting side effects, including sedation, cognitive impairment, and ileus. Although several postoperative rodent models have been developed, these models do not address these concerns.**

Methods: **A model is presented in the rat in which a subcostal incision is performed, penetrating into the peritoneal cavity. The behavioral effects of this surgical procedure are assessed using exploratory locomotor activity and conditioned operant responding. The effects of morphine and ketorolac were assessed in both behavioral paradigms.**

Results: **Laparotomy decreased ambulation and rearing by approximately 50% 24 h after surgery, and stereotypy (small confined movements) was affected to a lesser degree. The effects of laparotomy on conditioned operant responding were more complex. Total number of sucrose pellets earned was decreased for 2–3 days after laparotomy; however, the amount of time required was increased for up to 2 weeks. Morphine reversed the effects of surgery on ambulation and stereotypy but not rearing, and the dose–effect curve for morphine was shifted to the left by 5 mg/kg ketorolac. Ketorolac produced significant improvement in operant responding after laparotomy, and coadministration of ineffective doses of morphine and ketorolac produced a positive response.**

Conclusion: **The current model is consistent with behavioral aspects of postoperative pain seen clinically. The effects of morphine and ketorolac alone and in combination were consistent with the reported analgesic efficacy and occurrence of side effects found with these agents clinically.**

THERE are several recent reviews that document the problems associated with major surgery in the clinic and the challenges that face pain management specialists.[1–3] Two major complications of major surgery include postoperative ileus, a slowing or paralysis of intestinal motility, and pulmonary complications.[3] Opioids, particularly when given systemically, have severe adverse consequences on these two important parameters, which are main determinants of adverse outcomes after surgery in the clinic.[3] For these reasons, multimodal pain control is advocated clinically, using combinations of cyclooxygenase inhibitors, local anesthetics, and other drugs such as α-adrenergic antagonists and gabapentin in conjunction with opioids.[4] The theory and guiding hypothesis is that analgesics of differing mechanisms do not share similar side effects and that separation can be achieved between dosages that produce pain relief and those that produce adverse effects. All multimodal recovery programs seek to improve outcome by early patient mobilization, adequate if not total perioperative analgesia, and early oral nutrition as quickly as possible.[5] Although systemic opioids produce a number of complications that interfere with such goals, these drugs remain the mainstay of postoperative therapy. Pain therapy is further recognized to have psychological implications that affect patient outcome as well.[6,7] For this reason, a considerable effort has been expended to develop novel treatment strategies that will replace or enhance opioid therapy in the postoperative setting. Local anesthetics and cyclooxygenase inhibitors have been used extensively to decrease opioid requirements after surgery with some success.[8] With the postoperative analgesic market at approximately $1 billion in the United States alone in 2000, there is considerable demand for more effective therapies.[9] A positive economic impact on recovery and outcome has been demonstrated with opioid-sparing therapies.[10] Combination therapy has also been shown to be particularly useful in a postoperative setting compared with pain control for more chronic conditions, and the strategies for optimizing drug combinations seem to be different for various conditions.[11] Clinicians recognize the need for increased research on mechanisms of surgical pain, the complications associated with surgery, and pharmacologic effects of analgesics and analgesic combinations in such a setting rather than relying on clinical impressions alone.[12] There is clearly a growing need for basic research on the physiologic effects of surgical intervention and the resulting effects on the pharmacology of commonly used analgesics. The psychological aspects of surgical procedures and analgesic administration also merit further investigation in the laboratory.

Several animal models have been developed to study the pathophysiology of surgical intervention. The development of such models represents a crucial advance in basic science research on analgesics and therapeutic adjuncts because previously most of the basic informa-

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tion on these agents was generated using normal laboratory animals. As documented above, a number of physiologic changes occur in a surgical setting, and it is important to investigate potential novel therapies in animal models that more closely mimic the clinical population. A paw incision model first described by Brennan *et al.*[13] (1996) has been studied extensively. This model involves performing an incision on the plantar surface of one hind paw and manipulating the underlying tendons, musculature, and fascia, thereby producing a hypersensitivity to mechanical and thermal stimuli. A number of analgesics have been studied in the paw incision model, and mechanisms by which surgical incisions induce hypersensitivity to external stimuli have been elucidated.[14] A comprehensive review of the pharmacology and physiology is beyond the scope of this review; however, basic mechanisms have been elucidated using this model. Nonsteroidal antiinflammatory drugs, such as flunixin, reverse the mechanical hypersensitivity using this procedure, as does buprenorphine.[15] However, discontinued administration of buprenorphine produced a rebound hypersensitivity. Intrathecal administration of a prostaglandin EP1 receptor antagonist reversed the mechanical but not the thermal hypersensitivity in this model.[16] Inhibition of cyclooxygenase 2 (COX-2) spinally potentiates the effects of morphine in reversing the mechanical hypersensitivity resulting from paw incision.[17] Administration of local anesthetics or opioids at the time of surgery does not produce analgesia beyond the expected duration of action of these compounds; however, administration of ketoprofen seems to have some preemptive analgesic effect.[18,19] There is an increase in the firing of wide-dynamic-range and high-threshold neurons in the dorsal horn of the spinal cord after paw incision.[20] α_2-Adrenergic antagonists are active in this model, as are gabapentin and natural killer 1 antagonists.[21–24] The effect of intrathecal clonidine in this model is mediated by both muscarinic and nicotinic cholinergic mechanisms.[25] There is some discrepancy regarding the ability of COX-2 inhibitors to reverse the mechanical hypersensitivity after paw incision.[24,26,27] Cyclooxygenase 1 (COX-1; SC-560)– but not COX-2 (NS-398)–selective agents reversed the hypersensitivity when given intrathecally, and the surgical procedure increases COX-1 expression in the ipsilateral dorsal horn with a time course correlated with the mechanical hypersensitivity.[26] Amino acid release is increased in the cord after this procedure, and non–*N*-methyl-D-aspartate antagonists attenuate the behavioral effects.[14,28,29] The availability of this model has clearly increased the basic knowledge of mechanisms mediating incisional pain related to orthopedic procedures and has led to an increase into the investigation of surgical manipulations on physiology and pharmacology in such a setting.

It is likely that other mechanisms are involved in other types of surgical procedures, particularly those that produce postoperative ileus and respiratory complications. Other investigators have studied the effects of abdominal incision (laparotomy) in rats on spontaneous descriptive behaviors, locomotion, immune function, or postoperative ileus.[30–34] Abnormal posturing and referred mechanical allodynia has also been observed after ovariohysterectomy in rats.[35] Analgesia has also been assessed in rats after implantation of artificial ureteric stone using nocifensive types of behavior and reduction in vocalization threshold to electrical stimulation of the left oblique muscle as the endpoints.[36] These studies have largely focused on nocifensive behavioral responses to surgery in the immediate postoperative period, immunologic changes resulting from tissue injury, pharmacologic treatments, and spontaneous behaviors.

The ability of abdominal incision to induce postoperative ileus in rats has been studied as well.[37] These investigators found that COX-2 inhibitors were more efficacious in reducing postoperative ileus after incision of the skin and musculature in the area of the peritoneal cavity in rats; however, COX-1 inhibitors were more efficacious after a similar procedure with vigorous manipulation of the intestines. Nonselective cyclooxygenase inhibitors were more effective than any of the selective compounds alone. This suggests that COX-2 may be primarily involved in mediating incisional pain but that COX-1 may have the primary role in mediating postoperative ileus after manipulation of the gut. The extent of postoperative ileus was greater when the intestines were manipulated compared with incision alone, consistent with the clinical observations summarized above. To date, there has been no documentation in the literature of effects of similar procedures on assessment of the effects of surgery or analgesic treatment on cognitive function and food-seeking behavior. As outlined above, these effects of surgery and the complications of pharmacotherapies that worsen these symptoms are major points of emphasis for improving postoperative outcomes, such as mobility and early oral nutrition after surgery.

To more completely assess the complex clinical manifestations of postoperative pain, we developed a model in which exploratory locomotor behavior and conditioned operant responding is assessed in rats after a subcostal laparotomy and manipulation of the underlying viscera. This type of incision was selected because abdominal wounds are particularly painful and result in postoperative ileus that may affect food-seeking behavior in addition to spontaneous locomotion. The two behavioral paradigms used in our model were designed to assess fundamentally different behaviors. The locomotor studies measure spontaneous, instinctive behaviors of rodents that are largely motivated by exploration of a novel environment for means of escape. The operant conditioning paradigm measures conditioned reinforcement that maintains a high rate of behavior in rats and

requires intact cognitive function as well as appetitive motivation, complex coordination of musculature, and stamina. The model is presented both as a means to address pharmacologic issues related to the treatment of postoperative pain and to understand the pathophysiology of surgical intervention and its behavioral consequences in the whole animal. We believe that a comprehensive approach will be beneficial in obtaining relevant basic science information that can be translated into improved clinical practice.

Materials and Methods

Subjects

Male Fisher 344 rats (Charles River Laboratories, Raleigh, NC; n = 429) were used for these studies. Animals were kept on a reversed light:dark cycle (dark 5:00 to 17:00) in a temperature- and humidity-controlled vivarium. Rats used for locomotion studies were given ad lib access to food and water except during experimental sessions. Rats used for operant conditioning studies were kept at 85% of their free-feeding weight and were given ad lib access to water except during experimental sessions. All experiments were conducted during the dark phase of the light:dark cycle. All procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals*[38] as adopted and promulgated by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences Center (Winston-Salem, North Carolina).

Surgical Procedure

After induction of anesthesia with 50 mg/kg sodium pentobarbital (Nembutal®; Abbott Laboratories, Chicago, IL) and 10 mg/kg atropine methyl nitrate given intraperitoneally, animals were shaved on the left lower quadrant of the abdomen. A diagonal 3-cm incision was placed 0.5 cm below and parallel to the lowest rib on the left side, penetrating into the peritoneal cavity. The viscera and musculature were vigorously manipulated by inserting 5 cm of the index finger into the peritoneal cavity and stretching the musculature. Approximately 10 cm of the small intestine was exteriorized and vigorously manipulated between the thumb and forefinger. The intestine was then placed inside the peritoneal cavity, and the wound was sutured in three layers consisting of the peritoneal lining, abdominal muscles, and skin using 4.0 chromic gut. Exterior wounds were dressed with antibiotic powder (Polysporin®; Glaxo-Wellcome, Research Triangle Park, NC), and animals were given 75,000 U penicillin G procaine (Butler Veterinary Supply, Columbus, OH) intramuscularly. Sham-treated animals were anesthetized, shaved, and given penicillin G procaine.

Measurement of Spontaneous Locomotion

Exploratory behavior was assessed beginning 24 h after laparotomy using commercially available equipment and software (Med Associates Inc., St. Albans, VT). Activity chambers consisted of acrylic enclosures measuring 42.5 × 42.5 cm that were 37.5 cm tall with an open top. Duplicate banks of 16 infrared transmitters spaced 2.5 cm apart were placed in both the X and Y directions, 2.5 cm above the floor surface, with aligned infrared detectors on the opposing sides of the chamber. A third bank of infrared transmitters and detectors was located in the X direction, 7 cm above the floor surface such that the rats used for these studies were required to rear on their hind limbs to interrupt these beams. Each activity chamber was housed within a light- and sound-attenuating enclosure. In one group of animals, sessions were conducted daily for 1 h on days 1, 2, 3, 4, and 7 after surgery. In two other groups, sessions were conducted only on days 2 or 3 after surgery. Measures collected included total distance traveled, total beam breaks in both the X and Y direction (ambulatory counts), repeated beam breaks within 3 cm of the animal in the absence of locomotion (stereotypy), total beam breaks in the upper X direction (rearing), time spent in ambulation, and time spent in stereotypy. All measures were collected in 6-min bins throughout the session as well as summed for the entirety of the session.

Measurement of Sucrose-maintained Responding

Lever presses were engendered and maintained by presentation of standard 45-mg sucrose pellets (Research Diets Inc., New Brunswick, NJ). Commercially available operant equipment was used consisting of an operant chamber containing a lever located 5 cm above a grid bar floor, a stimulus lamp located 2 cm above the lever, a house light located outside of the operant chamber, a pellet receptacle, a magazine-type pellet dispenser, and a tone generator (Med Associates Inc.). Each operant chamber was placed within a sound- and light-attenuating enclosure containing a ventilation fan. Initially, each lever press resulted in delivery of a sucrose pellet (fixed ratio 1 schedule) and the number of lever presses required to earn a pellet was gradually increased to a terminal value of 10 (fixed ratio 10 schedule) across several experimental sessions. Illumination of the stimulus light above the lever indicated pellet availability, and a time-out period of 5 s followed the delivery of each pellet during which the tone was activated, the stimulus light above the lever was turned off, and lever presses had no programmed consequences. Animals were allowed to earn a maximum of 200 pellets during each session, and sessions were limited to a maximum of 1 h in duration. The number of pellets delivered, the total time elapsed between the beginning of the session and the delivery of the last pellet, and the time elapsed between the delivery of each individual pellet (interre-

inforcement interval [IRI]) were recorded for each session. Sessions were conducted on weekdays only.

After stable responding was obtained, laparotomy or sham surgery was performed as described above. Stable responding was defined as five consecutive sessions during which the total number of pellets earned and the time elapsed between the beginning of the session and the delivery of the last pellet did not vary by more than 10% of the mean. Behavioral sessions were not conducted on the day of the surgery but began daily on weekdays starting 24 h after either laparotomy or sham treatment.

Administration of Morphine and Ketorolac

Morphine sulfate (National Institute on Drug Abuse drug supply program; National Institutes of Health, Bethesda, MD) or ketorolac (Sigma Chemical, St. Louis, MO) were administered intraperitoneally in 0.9% NaCl (pH 7.4) in a volume of 1 ml/kg 10 min before assessment of locomotor activity or sucrose-maintained responding beginning 24 h after laparotomy or sham surgery. All doses are given in terms of the free base of the drug.

Data Analysis

Exploratory behavioral data were analyzed using a two-way analysis of variance (ANOVA) with surgical treatment (incision or sham) and postoperative day as the independent variables and ambulation, stereotypy, or rearing as the dependent measures. Data from the operant conditioning paradigm were analyzed using a two-way ANOVA with the same independent variables and using the number of sucrose pellets earned and the time required from the start of the session to earn the last sucrose pellet as the dependent measures. IRIs were converted to a frequency distribution using Microsoft Excel (Microsoft Inc., Redmond, WA) and were analyzed by ANOVA similarly as for the other two variables for this paradigm. *Post hoc* analyses were performed using the Dunnett *t* test for multiple comparisons to a control with postoperative day 1 serving as the control day for measuring effects on exploratory activity over time and baseline data used as control for measuring effects on conditioned sucrose reinforcement over time. Morphine and ketorolac data were analyzed using a three-way ANOVA with surgical treatment, morphine dose, and ketorolac coadministration serving as the independent variables and the other measures listed above for the spontaneous locomotor studies serving as the dependent measures. For sucrose reinforcement studies, morphine and ketorolac data were analyzed using a two-way ANOVA with surgical treatment and dose of either morphine or ketorolac serving as the independent variables and the other measures listed above serving as the dependent measures. $P \leq 0.05$ was considered statistically significant.

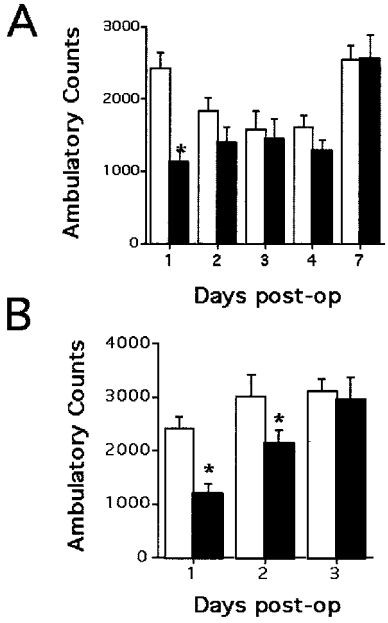

Fig. 1. Effects of laparotomy on ambulatory activity. Ambulatory counts were determined after sham surgery (*open bars*) or laparotomy (*dark bars*) (mean ± SEM) either in the same groups of animals each day after surgery (*A*) or in separate groups of animals that were exposed to the chamber only once on the indicated postoperative day (*B*). * Significantly different from sham, $P \leq 0.05$.

Results

Effects of Laparotomy on Exploratory Locomotor Activity

Ambulation. *Ambulation* was defined as movements consisting of 3 cm or greater. Laparotomy significantly decreased ambulation compared with sham-treated animals ($F_{1,23} = 8.86$, $P = 0.0036$), and this effect was dependent on the time elapsed after laparotomy ($F_{4,23} = 8.03$, $P < 0.0001$) (fig. 1A). Ambulation was decreased 24 h after laparotomy by 50.6 ± 7% relative to that observed in sham-treated animals. Evaluating the time course of these effects was confounded by adaptation to the environment that occurred in the sham-treated subjects; however, there was a trend toward an interaction between surgical treatment and time after surgery for the effects on ambulatory activity ($F_{1,23} = 2.3$, $P = 0.063$). The ambulatory activity of the incision group was not significantly different from day 1 for days 2, 3, and 4 but was significantly increased on day 7 after the incision. Adaptation to the environment occurred in the sham-treated animals, however, demonstrated by a decrease in exploratory activity on days 2, 3, and 4 relative to days 1 or 7 in these animals. Therefore, ambulatory behavior was significantly different between sham and incision groups only on day 1 after the procedure.

The time course of the effect of laparotomy on ambulation was also compared in separate groups of animals that were exposed to the locomotor chamber only on postoperative day 1, 2, or 3 (fig. 1B). With these data,

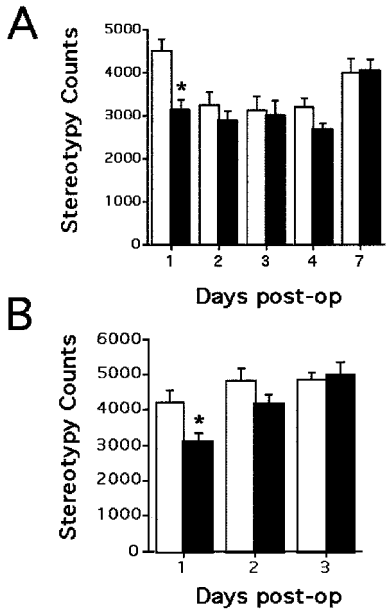

Fig. 2. Effects of laparotomy on stereotypy. Stereotypic counts were determined after sham surgery (*open bars*) or laparotomy (*dark bars*) (mean ± SEM) either in the same groups of animals each day after surgery (*A*) or in separate groups of animals that were exposed to the chamber only once on the indicated postoperative day (*B*). * Significantly different from sham, $P \leq 0.05$.

laparotomy likewise significantly decreased ambulatory activity compared with sham treatment ($F_{1,66} = 10.1$, $P = 0.002$), and the effect was dependent on the time elapsed after surgery ($F_{2,66} = 9.04$, $P = 0.003$). The ambulatory activity was not significantly different between the sham groups on postoperative day 1, 2, or 3, indicating that the decrease in behavior observed in figure 1A was likely due to adaptation to the environment. Laparotomy had a significant effect on ambulation for 2 days when comparisons were made between these groups.

Stereotypic Behavior. *Stereotypic behavior* was defined as movements that consisted of less than 3 cm in length and usually reflected small head movements and grooming. These movements therefore required less movement of the abdomen and the area of the incision. This behavior was significantly affected by the laparotomy compared with sham treatment ($F_{1,23} = 7.8$, $P = 0.006$), and this effect was dependent on the time elapsed after surgery ($F_{4,23} = 6.7$, $P < 0.0001$) (fig. 2A). As with ambulation, adaptation to the environment occurred to a greater degree after sham treatment than in the incision group. There was a trend toward an interaction between surgical treatment and time after surgery ($F_{1,23} = 2.11$, $P = 0.084$) similar to that observed with ambulation. This type of behavior was altered to a lesser extent after surgery than ambulation, being decreased by 30.8 ± 5% relative to sham-treated animals. As with ambulation, sham-treated animals displayed an adaptation to the locomotor chamber resulting in less movement during days 2, 3, and 4 after sham-treatment compared with postoperative day 1 or 7. This type of behavior recovered with a time course similar to that of ambulation after the laparotomy.

As with ambulation, stereotypic behavior was similar across postoperative days when separate groups of animals were used to determine the time course of the effects of surgery (fig. 2B). Laparotomy significantly decreased stereotypic behavior ($F_{1,66} = 4.8$, $P = 0.03$) and was significantly influenced by postoperative day ($F_{2,66} = 8.8$, $P = 0.004$). As with ambulatory activity, exposing animals to the environment only once produced similar stereotypic counts across postoperative days in sham-treated subjects. There was only a difference between the laparotomy and sham surgery groups on postoperative day 1.

Rearing. Vertical counts, or rearing, were decreased by 47.1 ± 7% after laparotomy relative to that observed in sham-treated subjects (fig. 3A). There was a significant main effect of surgical treatment on rearing ($F_{1,23} = 10.9$, $P = 0.0003$), and this effect was dependent on the time after treatment ($F_{4,23} = 14.9$, $P < 0.0001$). There was a significant interaction between surgical treatment and postoperative day ($F_{1,23} = 3.05$, $P = 0.02$), and the sham-treated animals displayed an adaptation to the environment across days as was found for the other behavioral measures. Rearing was significantly different between the incision and sham-treated groups on both postoperative days 1 and 2. The number of rearing counts in the laparotomy group was not significantly

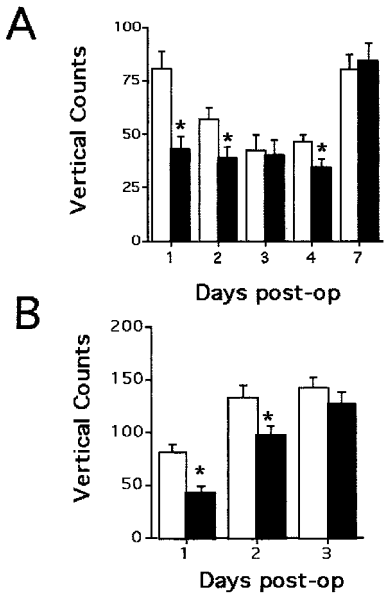

Fig. 3. Effects of laparotomy on rearing. Vertical counts were determined after sham surgery (*open bars*) or laparotomy (*dark bars*) (mean ± SEM) either in the same groups of animals each day after surgery (*A*) or in separate groups of animals that were exposed to the chamber only once on the indicated postoperative day (*B*). * Significantly different from sham, $P \leq 0.05$.

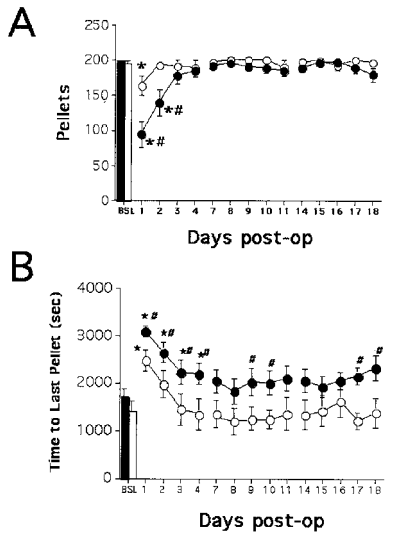

Fig. 4. Effects of laparotomy on conditioned operant responding. The number of sucrose pellets earned (*A*) was determined after sham surgery (*open bar and circles*) or laparotomy (*dark bar and circles*) (mean ± SEM). Data are also presented indicating the amount of time required for these animals to earn the pellets (*B*). BSL = baseline data that was obtained by averaging the data from the five sessions immediately before surgery. * Significantly different from baseline, $P \leq 0.05$. # Significantly different from sham, $P \leq 0.05$.

different from days 1, 2, 3, and 4 but was significantly increased on day 7.

The effect of laparotomy on rearing behavior was similar to the effect on ambulation when separate groups of animals were used to determine the time course (fig. 3B). Laparotomy decreased rearing compared with sham surgery ($F_{1,66} = 15.8$, $P = 0.0002$), and this effect was dependent on the postoperative day ($F_{2,66} = 32.7$, $P < 0.0001$). Unlike the other two measures of exploratory behavior, however, rearing was significantly different between animals that were exposed to the chamber only on postoperative days 2 and 3 relative to day 1 in sham-treated subjects. This may be due to an anesthetic effect on postoperative day 1 that has subsided by postoperative day 2 and thereafter. The time course of laparotomy on rearing was similar regardless of whether the data were obtained in the same group of animals across postoperative days or in separate groups of animals.

Effects of Laparotomy on Operant Conditioned Responding

The baseline data did not differ between the two groups of animals used for laparotomy (n = 7) or sham surgery (n = 6) for the mean number of pellets earned ($F_{1,11} = 1.25$, $P = 0.29$) (199 ± 0.7 and 195 ± 5 for the incision and sham groups, respectively) (fig. 4A). The time required to earn the last pellet before surgery was likewise not significantly different between these groups ($F_{1,11} = 1.25$, $P = 0.29$) (fig. 4B). There was a significant main effect of surgery on both the number of pellets earned ($F_{1,194} = 21.2$, $P < 0.0001$) and the time required to earn the last pellet ($F_{1,194} = 55.1$, $P < 0.0001$). There was a significant main effect of postoperative day on number of pellets earned ($F_{14,194} = 9.58$, $P < 0.0001$) and the time required ($F_{14,194} = 3.25$, $P = 0.001$) as well. There was a significant interaction between surgical treatment and postoperative day for the number of pellets earned ($F_{14,194} = 3.09$, $P = 0.0003$). Sham treatment affected both the number of pellets delivered and the time required to earn these pellets on postoperative day 1 relative to baseline values for both measures. The effect of laparotomy persisted for up to 3 days on number of pellets earned and up to 4 days on the time required to earn the pellets relative to baseline values in this group. There were significant, although intermittent differences between the laparotomy and sham-treated groups in the time required to earn the total pellets delivered for up to 15 days after the surgical procedure.

The temporal pattern for the delivery of the sucrose pellets was significantly different for these two groups as well. Figure 5 depicts the frequency distribution of IRIs of 20 s or less before or after surgery for both sham and operated groups. IRIs of 20 s or less comprise the majority of the IRIs in both groups of animals before surgery, with a large percentage of IRIs being between 3 and 7 s. The probability of observing a given IRI is dependent on the IRI ($F_{19,600} = 33.12$, $P < 0.0001$), and the probability of observing IRIs of 2–9 s was significantly greater than the probability of observing an IRI of 1 s ($P \leq 0.05$). Sham surgery moderately reduced the number of IRIs of 2 or 3 s but had no effect on the frequency of other IRIs in this range, and there was no significant interaction between postoperative days and IRI frequency ($F_{95,600} = 0.9$, $P = 0.73$). Laparotomy, however, had a dramatic effect on the frequency distribution of IRIs of less than 10 s, reducing the occurrence of IRIs between 2 and 9 s. As with the sham group, the frequency of the occurrence of individual IRIs was dependent on IRI value ($F_{19,\ 6,000} = 30$, $P < 0.0001$). There was a significant interaction between postoperative day and IRI value ($F_{95,600} = 3.5$, $P < 0.0001$). The time course of the effect was dependent on the IRI, with IRIs of 3, 4, or 5 s being affected for the longest period of time (3–4 days). Therefore, surgery significantly reduces the delivery of sucrose pellets with IRIs of 3–5 s for up to 4 days after laparotomy.

The occurrence of IRIs of greater than 20 s was a relatively rare event in both groups of animals before surgery, comprising approximately 5% of the total number of IRIs (fig. 6, top). The probability of observing IRIs of greater than 20 s was significantly higher than that of observing IRIs greater than 50 or 100 s ($F_{2,72} = 23.9$, $P < 0.0001$). There was a significant main effect of sham treatment across days on occurrence of IRIs greater than 20, 50, or 100 s ($F_{5,72} = 2.47$, $P = 0.04$), with the only effect occurring with IRIs greater than 20 s on postoperative day 1 ($P \leq 0.05$). Sham treatment approximately

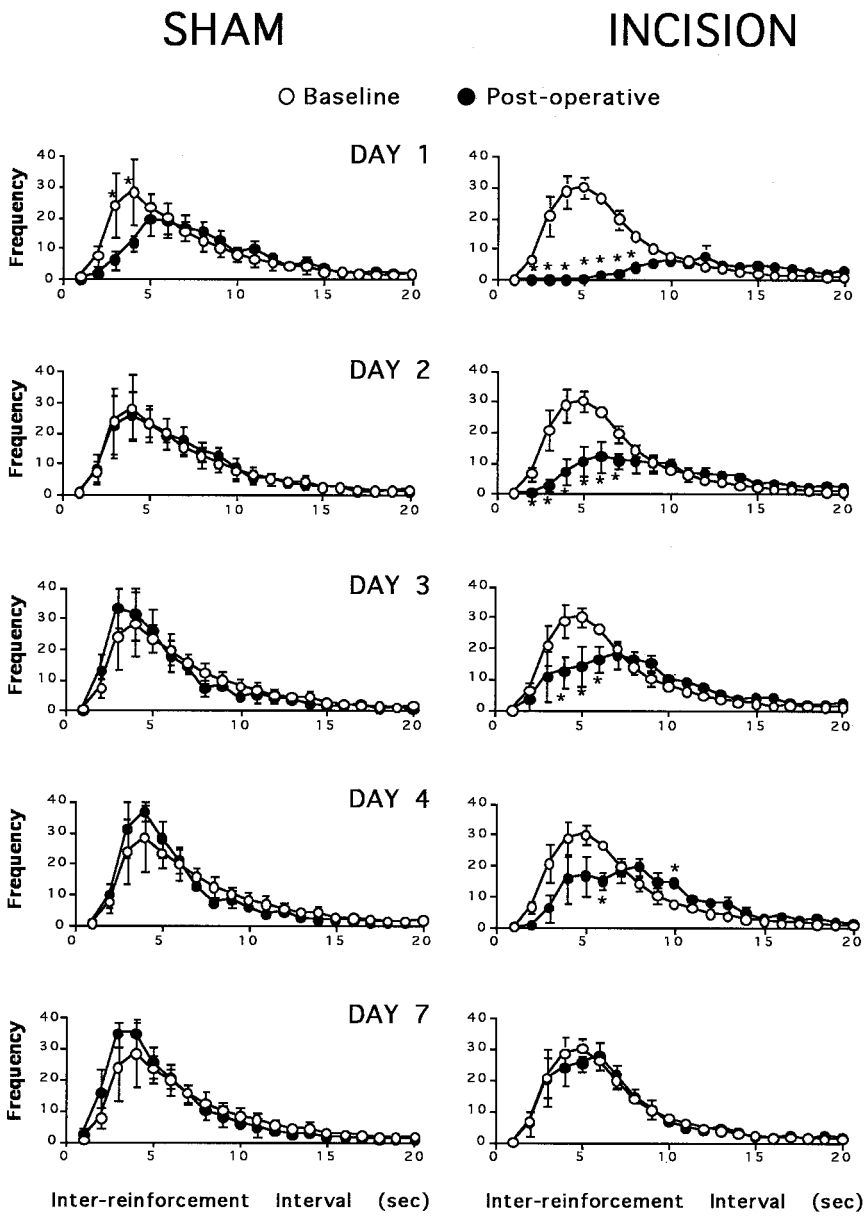

Fig. 5. Effect of laparotomy on frequency distribution for interreinforcement intervals less than 20 s for sucrose-maintained responding. The frequency of the occurrence of interreinforcement intervals from 1 to 20 s are shown for sham (*left*) or incision (*right*) groups on postoperative days 1, 2, 3, 4, or 7. Baseline data are shown in the graphs for each postoperative day for comparison but are the same data as defined in the Materials and Methods (mean ± SEM for the 5 days immediately before surgery). * Significantly different from baseline, $P \leq 0.05$. # Significantly different from sham, $P \leq 0.05$.

doubled the occurrence of IRIs of greater than 20 s at the 24-h postoperative time point. There was a significant main effect of postoperative day after laparotomy on frequency of IRIs greater than 20, 50, or 100 s ($F_{5,80} = 9.23$, $P < 0.0001$) and a significant interaction between the probability and postoperative day in this group ($F_{10,80} = 3.71$, $P = 0.0004$). Laparotomy increased the probability of the occurrence of IRIs of greater than 20 s by sixfold 24 h later and by threefold after 48 h. The probability of observing IRIs of greater than 50 or 100 s is low in both groups of animals before surgery, occurring on average approximately 1% or 0.5% of the time, respectively (fig. 6, middle and bottom). Sham surgery did not significantly affect the occurrence of IRIs in this range; however, laparotomy increased the probability of observing IRIs of greater than 50 s by sevenfold and of greater than 100 s by ninefold for 2–3 days after surgery (fig. 6).

Effects of Morphine and Ketorolac on Locomotor Activity after Surgery

Ambulatory Activity. Both morphine and ketorolac produced significant effects on ambulation after laparotomy ($F_{19,207} = 5.43$, $P < 0.0001$), with there being a significant main effect of surgical treatment ($F_{1,207} = 7.1$, $P = 0.009$) and a significant three-way interaction between surgical treatment, morphine dose, and ketorolac administration ($F_{19,207}$) = 2.7, $P = 0.04$). For the sham treatment group, there was no significant effect of morphine ($F_{3,930} = 0.22$, $P = 0.63$) or ketorolac ($F_{1,93} = 0.24$, $P = 0.63$) and no morphine–ketorolac interaction ($F_{1,93} = 0.01$, $P = 0.92$). However, for the incision

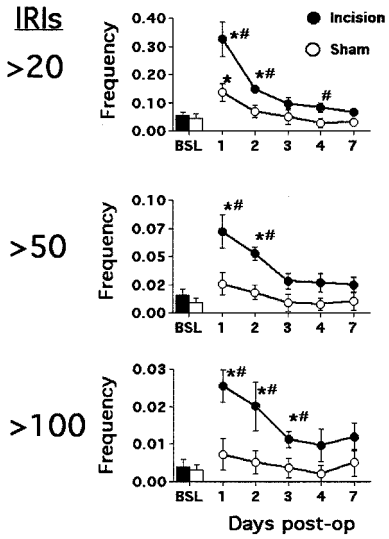

Fig. 6. Effect of laparotomy on frequency of interreinforcement intervals (IRIs) greater than 20, 50, or 100 s for sucrose-maintained responding. The percentage of IRIs exceeding 20, 50, or 100 s of the total number of IRIs throughout the session is shown after sham surgery or abdominal incision. BSL = baseline data that were obtained by averaging the data from the five sessions immediately before surgery. * Significantly different from baseline, $P \leq 0.05$. # Significantly different from sham, $P \leq 0.05$.

group, there were significant main effects of morphine ($F_{3,113} = 35.1$, $P < 0.0001$) and ketorolac ($F_{1,113} = 14.1$, $P = 0.0003$) and a significant interaction between morphine dose and ketorolac administration ($F_{1,113} = 4.6$, $P = 0.03$). *Post hoc* analyses demonstrated a significant increase in ambulation after administration of 1 or 3 mg/kg morphine alone and for 0.3 and 1 mg/kg morphine in the presence of 5 mg/kg ketorolac (fig. 7).

Stereotypic Activity. The effect of morphine and ketorolac administration after laparotomy was similar on stereotypic behavior. There was a significant main effect of surgical treatment on stereotypy ($F_{1,207} = 4.6$, $P = 0.03$) and a significant three-way interaction between

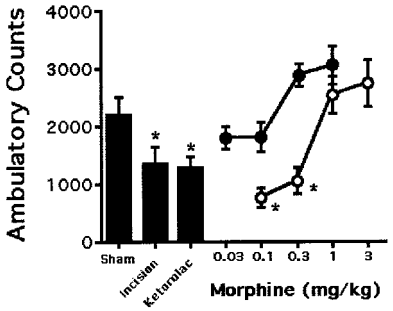

Fig. 7. Reversal of effects of abdominal surgery on ambulatory behavior by morphine and ketorolac. The ambulatory counts are shown 24 h after sham (sham) or abdominal (incision) surgery after injection of saline or the given doses of morphine in the absence (*open circles*) or presence (*filled circles*) of 5 mg/kg ketorolac. The bar labeled *Ketorolac* represents data after abdominal surgery from animals given ketorolac (5 mg/kg) alone. * Significantly different from animals given saline after sham surgery, $P \leq 0.05$.

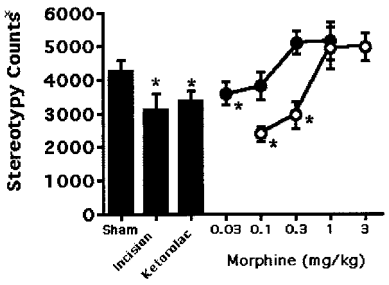

Fig. 8. Reversal of effects of abdominal surgery on stereotypic behavior by morphine and ketorolac. The stereotypy counts are shown 24 h after sham (sham) or abdominal (incision) surgery after injection of saline or the given doses of morphine in the absence (*open circles*) or presence (*filled circles*) of 5 mg/kg ketorolac. The bar labeled *Ketorolac* represents data after abdominal surgery from animals given ketorolac (5 mg/kg) alone. * Significantly different from animals given saline after sham surgery, $P \leq 0.05$.

surgical treatment, morphine administration, and ketorolac administration ($F_{19,207} = 3.8$, $P = 0.01$). As with ambulation, there was no significant main effect in sham-treated animals of morphine ($F_{3,93} = 1.9$, $P = 0.13$) or ketorolac ($F_{1,93} = 0.22$, $P = 0.64$) and no morphine–ketorolac interaction ($F_{1,93} = 2.7$, $P = 0.1$). After laparotomy, both morphine ($F_{3,113} = 26.4$, $P < 0.0001$) and ketorolac ($F_{1,113} = 8.0$, $P = 0.006$) produced significant effects, but there was no significant morphine–ketorolac interaction ($F_{1,113} = 3.1$, $P = 0.08$). *Post hoc* comparisons demonstrated a significant increase in stereotypic behavior by all doses of morphine 0.3 mg/kg or greater, and this effect was not increased significantly by coadministration of 5 mg/kg ketorolac (fig. 8).

Rearing. The effects of morphine and ketorolac on rearing were similar to the effects on the other two parameters of exploratory activity after laparotomy (fig. 9). There was a significant main effect of surgical treatment ($F_{1,207} = 9.4$, $P = 0.003$) and a significant three-way interaction between surgical treatment, morphine dose, and ketorolac administration ($F_{19,207} = 5.7$, $P < 0.0001$). As with the other two measures of locomotion,

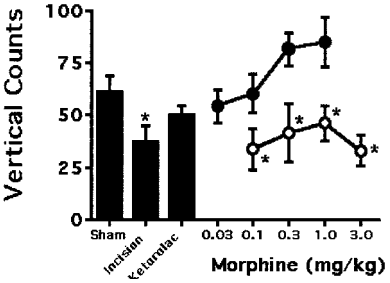

Fig. 9. Reversal of effects of abdominal surgery on rearing behavior by morphine and ketorolac. The rearing counts are shown 24 h after sham (sham) or abdominal (incision) surgery after injection of saline or the given doses of morphine in the absence (*open circles*) or presence (*filled circles*) of 5 mg/kg ketorolac. The bar labeled *Ketorolac* represents data after abdominal surgery from animals given ketorolac (5 mg/kg) alone. * Significantly different from animals given saline after sham surgery, $P \leq 0.05$.

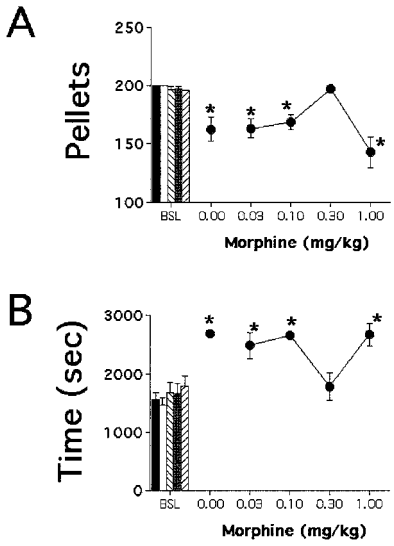

Fig. 10. Reversal of the effects of abdominal surgery on sucrose reinforcement by morphine. The number of sucrose pellets earned (*A*) and time required to earn the final pellet (*B*) are shown 24 h after laparotomy in animals given saline or morphine (mean ± SEM). Baseline data (BSL) are indicated for each group by the *vertical bars*, with the *first bar* representing the saline group, the *second bar* representing the group given the lowest dose of morphine, and so forth. * Significantly different from baseline values, $P \leq 0.05$.

neither morphine ($F_{3,93} = 0.49$, $P = 0.48$) nor ketorolac ($F_{1,93} = 2.9$, $P = 0.1$) produced significant effects, and there was no morphine–ketorolac interaction ($F_{1,93} = 1.5$, $P = 0.22$). After abdominal incision, morphine produced significant increases in rearing ($F_{3,113} = 6.4$, $P = 0.01$), as did ketorolac ($F_{1,113} = 21.7$, $P < 0.001$). There was a significant interaction between morphine and ketorolac administration after laparotomy as well ($F_{1,113} = 11.4$, $P = 0.001$). *Post hoc* analyses demonstrated a significant increase in rearing by only the 1-mg/kg dose of morphine after laparotomy but an increase after all doses of morphine of 0.3 mg/kg or higher with coadministration of 5 mg/kg ketorolac.

Effects of Morphine and Ketorolac on Conditioned Operant Responding after Surgery

Both morphine and ketorolac produced dose-related attenuation of the effects of abdominal surgery on sucrose-maintained responding in animals after laparotomy but not sham surgery. Morphine produced a significant, dose-related reversal on the number of sucrose pellets earned ($F_{4,67} = 3.1$, $P = 0.02$), and there were a significant main effect of surgical treatment ($F_{1,97} = 10.6$, $P = 0.002$) and a significant interaction between treatment and morphine dose ($F_{4,97} = 2.8$, $P = 0.03$) (fig. 10). *Post hoc* comparisons demonstrated that there was no difference between animals receiving 0.3 mg/kg morphine 24 h after surgery from their presurgery baseline values. The effects of morphine on the time required to earn the last pellet of the session was also dose dependent ($F_{4,97} = 3.0$, $P = 0.03$), with a significant main effect of surgical treatment ($F_{1,97} = 23.8$, $P < 0.0001$) and a significant interaction between surgical treatment and morphine dose ($F_{4,97} = 2.7$, $P = 0.04$) (fig. 10). Only the dose of 0.3 mg/kg morphine attenuated the effects of laparotomy on the time required to earn the last sucrose pellet. Administration of morphine to sham-treated animals had no significant effect on the number of sucrose pellets earned ($F_{4,97} = 0.27$, $P = 0.89$), no significant dose-related effect on the time required to earn the last sucrose pellet ($F_{4,97} = 2.0$, $P = 0.11$), and no significant interaction between sham treatment and morphine dose for either measure [$F_{4,97} = 0.14$, $P = 0.97$ and $F_{4,97} = 0.4$, $P = 0.81$ for pellets earned and time required, respectively]. For ketorolac, there was a significant main effect of dose on the number of sucrose pellets earned after laparotomy, with a significant interaction between ketorolac and surgical treatment ($F_{3,55} = 3.9$, $P < 0.002$) and similarly for time required ($F_{3,55} = 9.7$, $P < 0.0001$) (fig. 11). Doses of both 1 and 5 mg/kg but not 0.1 mg/kg ketorolac produced a significant attenuation of the effects of laparotomy on both parameters of sucrose reinforcement. There were no significant effects of ketorolac in sham-treated subjects for either parameter ($F_{3,51} = 0.04$, $P = 0.99$ for pellets earned and $F_{3,51} = 0.31$, $P = 0.82$ for time required) of sucrose-maintained responding.

A combination of an ineffective dose of morphine (0.03 mg/kg) with and ineffective dose of ketorolac (0.1 mg/kg) produced a significant attenuation of the effects of abdominal surgery on sucrose reinforcement without affecting behavior in sham-treated subjects. The

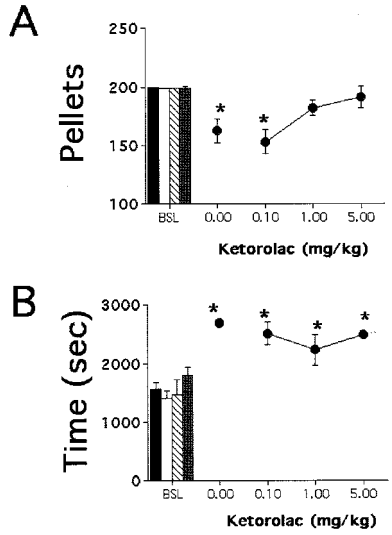

Fig. 11. Reversal of the effects of abdominal surgery on sucrose reinforcement by ketorolac. The number of sucrose pellets earned (*A*) and time required to earn the final pellet (*B*) are shown 24 h after laparotomy in animals given saline or ketorolac (mean ± SEM). Baseline data (BSL) are indicated for each group by the *vertical bars*, with the *first bar* representing the saline group, the *second bar* representing the group given the lowest dose of morphine, and so forth. * Significantly different from baseline values, $P \leq 0.05$.

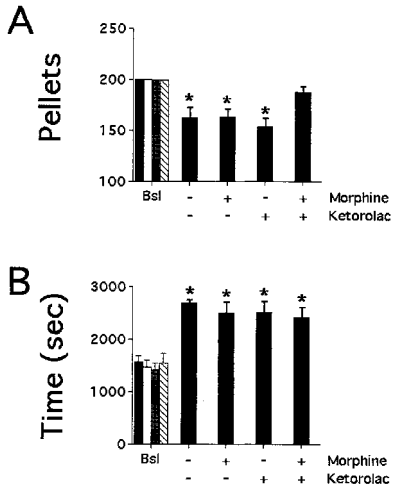

Fig. 12. Interaction between morphine and ketorolac on the effects of abdominal surgery on sucrose reinforcement. The number of sucrose pellets earned (*A*) and time required to earn the final pellet (*B*) are shown 24 h after laparotomy in animals given saline, morphine, ketorolac, or a combination of both drugs (mean ± SEM). Baseline data (Bsl) are indicated for each group by the *vertical bars*, with the *first bar* representing the saline group, the *second bar* representing the group given the lowest dose of morphine, and so forth. * Significantly different from baseline values, $P \leq 0.05$.

combination of morphine and ketorolac reversed the effects of surgery on number of pellets earned ($F_{1,27} = 4.3$, $P = 0.049$) but not in the time required to earn the last pellet ($F_{1,27} = 0.9$, $P = 0.35$) (fig. 12). There were no significant effects of this drug combination in sham-treated animals ($F_{1,25} = 2.2$, $P = 0.15$ for pellets earned and $F_{1,25} = 0.1$, $P = 0.94$ for time required).

Discussion

The major findings of these studies are that an abdominal incision in the subcostal region selectively suppresses exploratory locomotor activity for 1–2 days while affecting operant responding for sucrose reinforcement substantially longer. The types of exploratory behavior were differentially affected, with ambulatory and rearing activity being the most sensitive to disruption by abdominal surgery, and small, confined movements such as grooming being less affected and affected for a shorter duration after surgery. Behavioral indices were also differentially affected by surgery in the sucrose pellet reinforcement paradigm. Although the total number of sucrose pellets earned returned to baseline values within 3 days after laparotomy, the efficiency with which these animals were able to earn the pellets was significantly affected for much longer when compared with sham-treated subjects. The temporal pattern of responding and the frequency distribution of the occurrence of short IRIs suggests that these animals were incapable of or unwilling to engage in high rates of responding for long periods of time during this recovery period and is consistent with the effects of this type of surgery in the clinic. These data indicate that it is important to discern different types of activity in assessing the behavioral effects of surgery on laboratory animals and that the paradigms presented may be useful in assessing both beneficial and harmful effects of analgesics in the postoperative state.

The pharmacologic effects of both morphine and ketorolac are consistent with analgesia in both the exploratory locomotor activity and operant conditioning paradigms. Morphine increased all three types of locomotion in a dose-related manner, returning the activity of animals after laparotomy to that of sham-treated animals given saline while having no dose–response effects in sham subjects. The enhancement of the actions of morphine by ketorolac is also consistent with the use of ketorolac in addition to morphine clinically. This enhancement was most pronounced for reversal of the effects of surgery on rearing behavior, and the administration of ketorolac greatly enhanced the dose–response profile and efficacy of morphine in reversing the effects of surgery on rearing. The pharmacologic profiles of morphine and ketorolac were somewhat different in the sucrose reinforcement paradigm. Morphine reversed the effects of surgery on operant responding only at a single dose but reversed the effects on both number of pellets and the efficiency of responding. Ketorolac was effective over a broader range of doses at increasing the number of sucrose pellets earned but did not reverse the effects of surgery on the time required to earn these pellets. As with the exploratory locomotion paradigm, combination of morphine and ketorolac produced a greater effect than either drug given alone at a single dose. The steep dose–response curve for morphine relative to ketorolac is likely due to two disparate effects, namely cognitive impairment and effects on postoperative ileus. The lack of effect of morphine after surgery at 1 mg/kg could not be due to sedative effects because this dose increases exploratory activity after surgery. More likely, it is due to cognitive impairment because this dose of morphine decreases operant behavior in normal subjects and decreased behavior slightly in sham-treated subjects in the current study.[39–44] Ketorolac has little if any effect on cognition even at high doses and produced no effects at any dose in sham subjects in this study. Morphine also decreases gastrointestinal motility, which is already compromised after abdominal surgery.[37] Administration of cyclooxygenase inhibitors has been found to partially restore gastrointestinal motility after laparotomy in rats.[37] The morphine and ketorolac data in the operant conditioning paradigm are therefore consistent with postoperative ileus having a major role in postoperative pain and diminution of early oral nutrition. These data support the notion that morphine, while providing analgesia and improving some symptoms of postoperative pain, also possesses dose-limiting side effects such as

cognitive impairment and postoperative gastrointestinal disturbances. The exact role of these two disparate pharmacologic effects of morphine in limiting positive behavioral outcome after abdominal surgery can be explored in the operant paradigm, as well as exploring pharmacologic candidates for adjuncts to improve opioid therapy after surgery.

Morphine has behavioral effects in normal animals that are relevant to these paradigms as well. Notably, morphine stimulates locomotor activity and disrupts operant responding in rats.[39–47] A comprehensive review of the literature on both of these effects of morphine is well beyond the scope of this discussion; however, several important general findings are noteworthy. Increased locomotion has been studied after morphine administration to rats; however, these studies acclimated the animals to the test environment for periods of at least 1 h and sometimes used repeated exposures to decrease exploratory locomotion.[45–47] Stimulation of activity by morphine is more robust when exploratory activity is diminished in this manner. Even under such conditions, the dose range of the stimulant effects of morphine is very narrow, generally in the 1- to 2-mg/kg range.[45–47] Comparing data across time among groups that were repeatedly exposed to the locomotor chambers to those that were exposed only once after surgery demonstrated that studying locomotion without previous acclimation to the chamber is better for assessment of deficits after surgery. This procedure also likely reduces the psychomotor stimulation in sham subjects that would confound the current data, at least at the 1-mg/kg dose of morphine. The data for ketorolac, which is not a psychomotor stimulant under any conditions, as well as the potentiation of the actions of morphine by ketorolac lend confidence that this paradigm can be used to assess analgesia in a postoperative state.

The literature regarding disruption of operant responding by opioids is equally vast. Morphine and related opioids consistently disrupt operant responding for a variety of reinforcers, including food pellets and sucrose.[39–44] The effective dose range for morphine is typically higher than that used in the current study, although these effects are dependent on the operant requirements for reinforcement, and disruption was found in sham-treated animals at the highest dose tested (1 mg/kg). Disruption of operant responding by ketorolac has not been documented, and no such effect was found in the current study. The positive behavioral effects of ketorolac and the interaction between ketorolac and morphine after abdominal surgery again suggest that this paradigm is an effective assessment of postoperative analgesia. The strength of this procedure is that intact cognition is required for a positive effect, and therefore, the negative, unwanted effects of drugs such as opioids can be assessed unlike other more simple, reflexive types of behaviors.

The motivation of the animal to engage in a particular type of behavior may have a significant role in the effect of surgery on that behavior, as well influence the potency and efficacy of analgesics in reversing the behavioral effects of surgery. In the exploratory locomotor activity paradigm, this was seen across experimental sessions in the same animal. As the animal becomes more familiar with the locomotor chamber, the motivation to explore the chamber decreases, and it became difficult to detect differences between sham and laparotomy groups with such low rates of behavior. Using separate groups of animals in which the animals at each time point after surgery were exposed to the locomotor chamber only once, the rate of behavior for ambulation and stereotypy in sham groups was more consistent and gives more confidence that the surgery influenced these two types of behavior differently across the postoperative recovery period. The increases in rearing activity on days 2 and 3 relative to day 1 in the separate sham groups suggests that the anesthetic had some residual effect up to 24 h after administration, consistent with the disruptive effects that were found in the operant-conditioning paradigm. The data from both paradigms taken together suggest that laparotomy affects relatively low rates of behavior for 1–2 days, but higher rates of behavior for much longer.

One of the interesting features of this model is that analgesia will be associated with increased rates of behavior. Two of the major unwanted effects of a variety of analgesics, including opiates, are sedation and cognitive impairment. For these reasons, behavioral measures that measure latency to reflexive responses, such as tail or paw movements in rodents, can be confounded by sedative effects or cognitive impairment. Such effects would worsen the behavioral outcome after laparotomy in the current paradigms, as was found with both rearing behavior and operant reinforcement after administration of morphine. The current paradigm is also the first example of a procedure that can measure effects of analgesics on cognition in a postoperative pain setting. A study by Cain *et al.*[48] (1997) used performance on a more complicated operant task to assess behavioral deficits after adjuvant-induced arthritis. These investigators found that morphine treatment improved conditionally reinforced operant behavior only in arthritic animals but consistently impaired performance in normal animals. Such findings are similar to the current data in animals after laparotomy. As the pharmacology of the current model is explored, a cogent picture should emerge that can be related to what is known clinically and can be used to explore novel strategies for restoring functionality after abdominal surgery.

There are examples in the literature of laparotomy models in the rat that have been used to explore behavioral changes that occur after surgical intervention into the peritoneal cavity. A study by Roughan and Fleck-

nell[32] surveyed complex spontaneous behaviors elicited after a midline incision in rats. Using videotaped sessions in a home cage environment, these investigators categorized a number of normal and nocifensive behaviors, examining their frequency and duration. Unfortunately, the complexity of these behaviors resulted in less than robust measures, and numerous handling and control procedures produced a number of confounding effects, particularly after treatment with buprenorphine. In a second study, these investigators found that three specific behaviors of 150 classified behaviors were most reliable for predicting the analgesic efficacy of ketoprofen and carprofen.[31] These behaviors were abnormal posturing, writhing, and twitching while in an inactive state. The main effect of the antiinflammatory agents was to decrease the frequency of these behaviors, which were assessed within a 6-h time period after surgery. Although these measures may prove useful for assessing potential antiinflammatory drugs, measuring decreases in behavior in the presence of opioids could be confounded by sedation.

A series of studies has examined the effects of a similar type of incision on the ability of rats to mount an immune response to implanted tumor cells and on rearing behavior. Similar to the current results, midline laparotomy and manipulation of the small intestine decreased rearing activity in rats.[34] These assessments were obtained in the first 4 h after the surgery. Morphine and fentanyl given systemically were both effective in increasing rearing activity after surgery; however, neither compound increased this behavior to a level similar of that in control subjects.[49] Interestingly, both morphine and fentanyl decreased the retention of metastatic tumor cells in the lungs and increased natural killer cell activity in rats exposed to abdominal surgery, suggesting that relief of postoperative pain improves immune function.[49] These studies indicate the need to improve pain therapy after surgery, although they do not necessarily address cognitive impairment or motor performance after surgery. The current data extend these findings to include more complex behaviors and provide a longer time course for potentially examining analgesics with an extended duration of action.

In summary, the two paradigms presented here for examining behavioral effects of abdominal surgery are capable of discerning different types of effects with different recovery periods. These paradigms together should be able to differentiate between analgesia and sedative effects as well as provide some measure of both cognitive and motor impairment. The current model together with the other models reviewed above should provide a framework for studying the myriad of behavioral effects that result from surgical intervention as well as addressing relevant pharmacologic issues related to the treatment of postoperative pain.

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