

# Abdominal Surgery Decreases Food-reinforced Operant Responding in Rats

## Relevance of Incisional Pain

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**Background:** Establishment of early oral nutrition after surgery is associated with a decrease in morbidity and mortality. The following studies were undertaken to determine how surgery influences food-reinforced behavior in rats and to determine the relevance of afferent input from the incision site on this behavior.

**Methods:** Rats were trained to press a lever for food pellets to assess the effects of various abdominal surgical manipulations. Operant requirements and food availability were also manipulated. The effects of wound infiltration with bupivacaine and denervation of the abdominal musculature in the area of the incision were similarly examined.

**Results:** Incision of the skin and abdominal musculature produced significant behavioral effects. Food pellets earned were significantly decreased, with gut manipulation producing effects of greater magnitude and duration than incision alone. Operant requirements or different schedules of food availability did not influence the effect of surgery on behavior. Infiltration of the wound with bupivacaine produced a reversal of the effects of surgery on behavior after skin and muscle incision but had minimal effects when the viscera were manipulated. Similarly, denervation of the abdominal musculature reversed the effects of abdominal incision on behavior.

**Conclusions:** Food maintained behavior is disrupted after laparotomy in rats. The time course and magnitude of this disruption, as well as its reversal by bupivacaine or denervation, are consistent with postoperative incisional pain. Manipulation of the viscera produces a greater effect than laparotomy alone, and additional mechanisms unrelated to incisional pain affect food reinforcement and feeding after surgery.

PAIN arising from abdominal surgery is a common clinical problem, and current treatment poses some dilemmas. Several factors have been identified that are predictive of postoperative morbidity and mortality. Of these, early ambulation and normalization of oral nutrition and bowel function are predictive of improved outcome.<sup>1,2</sup> Abdominal surgery induces temporary paralysis of the gastrointestinal tract, resulting in decreased appetite and pain.<sup>3–5</sup> Countering these postsurgical complications is essential to achieving early oral nutrition, which is associated with a decrease in a number of complications arising from surgery, including infection.<sup>2</sup> In many cases,

these treatment objectives are thwarted by side effects of postoperative analgesics, including opioids, which are the mainstay of postoperative pain therapy.<sup>6,7</sup> Abdominal surgery in rats induces ileus in the immediate postoperative period, and the extent of the intestinal paralysis is correlated with the degree of manipulation of the viscera.<sup>8</sup> The effect of such surgical manipulations on establishment of early food-seeking behavior in laboratory animals has not been documented.

Numerous postoperative pain studies have focused on stimulus-evoked pain responses and have elucidated mechanisms of incisional pain after paw incision.<sup>9,10</sup> We recently developed behavioral models that are designed to detect spontaneous responses in rats after surgery, and we found that the potency and efficacy of morphine and ketorolac are dependent on the behavioral measure, the route of drug administration, and the motivation of the animal to engage in respective behaviors.<sup>11,12</sup> Operant responding for sucrose reinforcement is especially diminished by laparotomy in rats, and morphine is more potent and efficacious in reversing some of the effects of abdominal surgery than others.<sup>12</sup> Comparing the effects of surgery on spontaneous or elicited responses in rats, ketorolac seems to be much more efficacious in reversing the effects of surgery on spontaneous behavior compared to elicited or stimulus-evoked nocifensive responses.<sup>11</sup> In the current study, a behavioral model is used to measure effects of abdominal surgery on operant behavior maintained by food reinforcement. This model allows continuous measurement of the behavioral effects of surgery beginning in the immediate postoperative period, while measuring an important variable that has been associated with decreased morbidity and mortality after surgery in a clinical setting, namely the establishment of early oral nutrition after surgery. The goal is to develop a behavioral model that will be a meaningful addition to the armamentarium of techniques that can be used to assess mechanisms of the pathology associated with surgery that limit or diminish effective postoperative pain treatment and prolong inpatient care.

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## Materials and Methods

### Subjects

Male, Fisher 344 rats (Charles River, Raleigh, NC) weighing 300–350 g were used for all experiments (n = 84). Rats were housed in a temperature- and humidity-controlled environment under a reversed 12:12-h light:

dark cycle (dark 05:00–17:00) and given *ad libitum* access to water. Food was available *ad libitum* before operant training procedures. All procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals*<sup>13</sup> 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).

#### *Food-reinforced Operant Behavior*

**Apparatus.** Operant behavior was assessed by using commercially available equipment (Med Associates, St. Albans, VT). Standard operant conditioning chambers were contained within sound- and light-attenuating enclosures that contained a light and a ventilation fan. Operant conditioning chambers contained a response lever located 2 cm from the rear of the chamber and 6.5 cm above a grid bar floor. A stimulus light was located 2 cm above the lever, and a standard food pellet receptacle was located 2 cm to the right of the lever. The conditioning chambers also contained a standard magazine pellet dispenser for 45-mg pellets. Operant equipment was controlled using a PC-compatible computer and interface and a proprietary programming language (MED-PC; Med Associates).

**Behavioral Procedure.** Following at least 5 days of acclimation to the laboratory, after arrival at the institution from the vendor, rats were placed in operant chambers, and lever presses were reinforced by delivery of a single 45-mg rat chow pellet (Research Diets Inc., New Brunswick, NJ). Food pellets were available *ad libitum* on a continuous basis for most of the rats, and water was available *ad libitum* for all animals at all times during the study. No supplemental food was offered, and all food had to be obtained through operant responding for each of the animals studied. The number of lever presses required to earn each food pellet was gradually increased across five to seven sessions to a terminal value of 10 (fixed ratio [FR] 10). For two groups of rats ( $n = 6$ /group), the FR value remained at 1 throughout the experiment. Food availability was restricted to three discrete components in two groups of animals ( $n = 6$ /group). For these subjects, when the FR value was increased to 10 and behavior was stable (the number of pellets earned daily did not vary by more than 5% of the mean for 5 successive days), food access was restricted such that the lever was extended into the operant chamber only 11 h/day (05:00–09:00, 12:00–16:00, 18:00–21:00). Pilot studies revealed that the number of food pellets earned under these restricted-access conditions was not significantly different than under free-access conditions. The 12:12-h light:dark cycle (dark 05:00–17:00) was maintained by operation of a white stimulus light located in the ceiling of the sound- and light-attenuating enclosure for all rats.

#### *Surgical Procedures*

**Subcostal Laparotomy.** After behavior was stable as defined in the previous paragraph, rats were removed from the operant chamber and placed in a separate surgical facility. After 30 min, rats were anesthetized with isoflurane, their upper abdomen was shaved, and a subcostal laparotomy was performed as described previously.<sup>12</sup> Briefly, an abdominal incision (3.0 cm) was placed 0.5 cm below and parallel to the lowest right rib, penetrating into the peritoneal cavity. In some of the animals, 10 cm of small intestine was exteriorized and manipulated vigorously with the thumb and forefinger, and the incised muscle was manipulated by inserting approximately 1 cm of the index finger. The incision was closed in three layers, and exterior wounds were dressed with antibiotic powder (Polysporin; Pfizer Consumer Healthcare, Morris Plains, NJ). Animals were allowed to recover for 1 h in the surgical facility before being returned to their operant chamber. Sham-treated rats were anesthetized and shaved and remained under isoflurane anesthesia for the same duration as a paired laparotomy subject (20–30 min).

**Thoracic Spinal Afferent Denervation.** Twenty-four rats underwent spinal afferent denervation of T8–T12 on the right side before initiation of operant conditioning studies. Rats were anesthetized with isoflurane, and the upper back region was shaved and prepared with povidone iodine solution and isopropyl alcohol. An incision was made on the right side that extended from the midscapular region to just below the lowest rib (T13). Muscle and fascia were bluntly dissected to reveal the two lowest ribs (T12 and T13). The spinal nerve traversing between these ribs (T12) was gently lifted from the underlying muscle using a small stainless steel probe, with care to not penetrate the thoracic cavity. The 12th thoracic spinal nerve was grasped with tissue forceps and severed. Each end of the nerve was cauterized using a pen-type cautery (Aaron Medical Industries, St. Petersburg, FL). In a similar manner, the four spinal nerves rostral to T12 were isolated, transected, and cauterized. The muscle and skin layers were closed separately, and the exterior wound was dressed with antibiotic powder. Sham denervation surgery consisted of isoflurane anesthesia and shaving the back region. Rats were allowed to recover from surgery for 10 days before operant training was initiated as described above (see “Behavioral Procedure” section). Before behavioral training, anesthesia of the right abdominal region was verified using a blunted 20-gauge needle. Each rat was placed in a restraining tube with access ports in the abdominal area (Braintree Scientific, Braintree, MA). After the animal was calm, the blunted needle was applied to the subcostal area with sufficient force to perturb the abdomen by at least 1 cm and was held in place for 3 s. A response was defined as struggling or flinching of the abdominal site, and each test consisted of 10 applica-

tions of this stimulus along the future abdominal incision site. The test was performed on both the right and the left sides for all denervated and sham-denervated subjects. Only denervated animals that did not respond to any of the 10 applications of the stimulus on the right and that responded to at least 8 of the 10 stimuli applied to the left side were used for subsequent operant training and laparotomy studies. Only sham-denervated subjects that responded to at least 8 stimuli on both the right and left sides of the abdomen were used for subsequent experiments. Eleven of the 12 denervated rats and all 12 of the sham-denervated subjects were found to meet these respective criteria.

#### Bupivacaine Administration

In four groups of rats ( $n = 6/\text{group}$ ), bupivacaine was administered perioperatively. Half of these rats received abdominal incision only, and half received abdominal incision with gut manipulation. After closure of the peritoneal lining, 3.0 ml of either 0.25% (wt/vol) or 0.75% bupivacaine hydrochloride (Sensorcaine-MPF; Astra Pharmaceutical, Westborough, MA) was instilled into the open wound site. After 5 min, the outer abdominal muscle layer and skin layer were closed, and the animals were treated in a similar manner as described above (see "Subcostal Laparotomy" section).

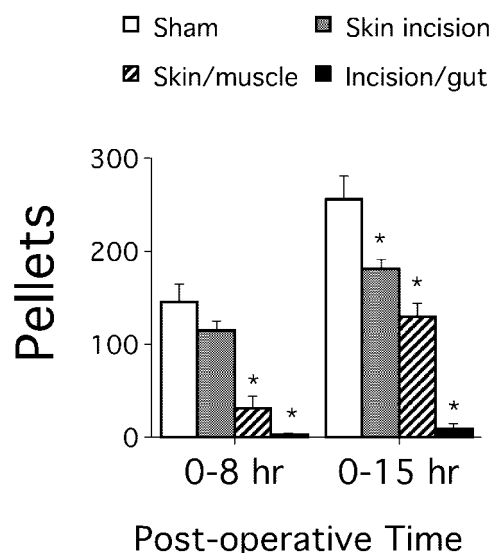
#### Data Analysis

Food-reinforcement data were analyzed using a two-way analysis of variance, with number of food pellets earned serving as the dependent measure and surgical intervention and postoperative time serving as the independent variables. Data were analyzed in a similar manner for examining the effects of FR value and food-access schedules on food reinforcement subsequent to surgery. For denervated animals, analyses of variance were conducted for denervated and nondenervated subjects separately and with surgical intervention and postoperative time serving as the independent variables and number of food pellets as the dependent measure. *Post hoc* analyses were performed using the Fisher least significant difference test, and  $P \leq 0.05$  was considered statistically significant.

## Results

#### Relevance of Extent of Surgical Manipulation on Food-reinforced Responding

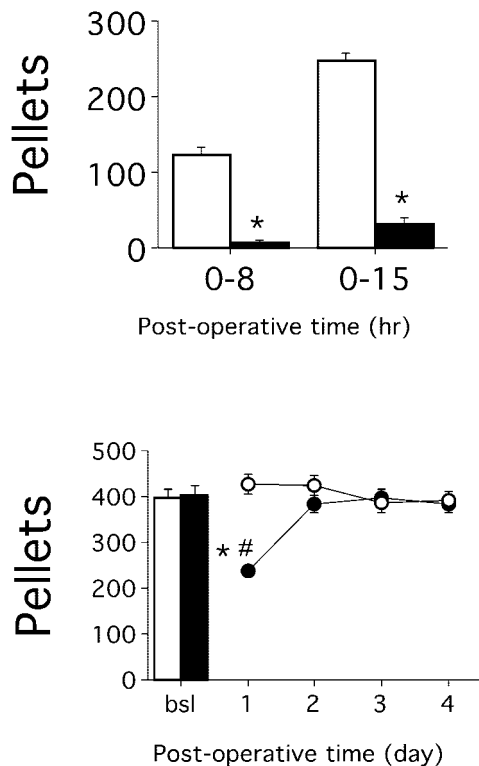
**Total Food Pellets Earned.** Food reinforcement was compared between groups of rats after sham surgery, incision of the skin only, incision of skin and underlying abdominal musculature, or incision of skin and musculature with extensive manipulation of the gut. There was a significant main effect of the extent of surgical manipulation ( $F_{3,161} = 39.3, P < 0.0001$ ) and time after surgery



**Fig. 1.** Relevance of the extent of abdominal surgery on food-reinforced behavior. The mean (SEM) food pellets earned in the first 8 or 15 h of the postoperative period is shown after sham surgery (anesthesia only), incision of the skin only, incision of the skin and underlying musculature, or incision of the skin and musculature with exteriorization and manipulation of 10 cm of intestine (incision/gut). \* Significantly different from sham surgery ( $P < 0.05$ ).

( $F_{6,161} = 220, P < 0.0001$ ) on the total number of food pellets earned. There was also a significant interaction between surgical intervention and postoperative time period on food reinforcement ( $F_{18,161} = 7.1, P < 0.0001$ ; figs. 1 and 2). Baseline food reinforcement was not significantly different between the groups of animals before surgery, and there was no significant effect of sham surgery on food reinforcement at any postoperative time point ( $P > 0.05$ ). In the first 8 h after surgery, incision of the skin alone did not produce a significant effect compared with sham treatment (fig. 1). However, incision of the skin and abdominal muscle with or without gut manipulation produced a  $100 \pm 0$  or  $79 \pm 9\%$  decrease, respectively, in food reinforcement compared with sham surgery in the first 8-h postoperative period ( $P < 0.05$ ; fig. 1). Examination of food reinforcement in the first 15 h after surgery showed that incision of the skin alone produced a modest effect ( $29 \pm 4\%$  decrease), whereas the effect of incision of the skin and musculature produced a robust effect ( $49 \pm 5\%$  decrease), as did incision with subsequent manipulation of the intestines ( $96 \pm 2\%$  decrease) (fig. 1).

The time courses of the effects of surgery were similar after skin and muscle incision with or without gut manipulation. Sham surgery did not produce any significant effects on food reinforcement when postoperative days 1-4 were compared to the presurgery baseline values ( $F_{4,29} = 0.05, P = 0.99$ ). Placing an incision in the skin only likewise was without significant effect over this time period ( $F_{4,29} = 2.1, P = 0.12$ ). However, placing an incision in the skin and underlying abdominal musculature



**Fig. 2.** Effects of abdominal surgery on food reinforcement at minimal response requirement. The mean (SEM) food pellets earned in the immediate postoperative period (*upper*) or at later time points (*lower*) are shown after sham surgery (*open bars and circles*) or abdominal surgery with gut manipulation (*dark bars and circles*) at fixed ratio 1. \* Significantly different from sham surgery ( $P < 0.05$ ). # Significantly different from baseline ( $P < 0.05$ ).

ture produced a significant time-dependent effect ( $F_{4,29} = 7.5$ ,  $P = 0.001$ ), with the number of food pellets earned on postoperative day 1 being significantly different from baseline data for these subjects. The numbers of food pellets earned on days 2–4 were not significantly different from the control data. Abdominal incision with gut manipulation decreased food-reinforced responding with a similar time course as incision only ( $F_{4,29} = 24.1$ ,  $P < 0.0001$ ), and the data obtained on postoperative day 1 were significantly different from the baseline values for this group of animals ( $P \leq 0.05$ ).

**Circadian Patterns of Responding.** Before surgery, all groups of animals responded on the lever for food significantly more during the dark compared with the light phase of the circadian cycle. The total numbers of food pellets earned during the dark phase before surgery were  $317 \pm 21$ ,  $278 \pm 22$ ,  $267 \pm 21$ , and  $290 \pm 14$  for the groups used for sham surgery, skin incision only, skin and muscle incision, or incision with gut manipulation, respectively. Surgical manipulation produced a significant main effect on food pellets earned during the dark cycle ( $F_{3,107} = 23.3$ ,  $P < 0.0001$ ), and there was a significant interaction between the type of surgical intervention and time after surgery ( $F_{12,107} = 3.9$ ,  $P < 0.0001$ ). Comparing baseline data with that obtained for

each postoperative day, there was no significant effect of sham surgery ( $F_{4,29} = 0.14$ ,  $P = 0.97$ ) or skin incision ( $F_{4,29} = 0.3$ ,  $P = 0.91$ ) on the number of food pellets delivered during the dark cycle. There was a significant effect of skin and muscle incision alone ( $F_{4,29} = 3.4$ ,  $P = 0.05$ ) or incision with gut manipulation ( $F_{4,29} = 23.7$ ,  $P < 0.0001$ ). For both of these groups, the number of food pellets earned during the dark cycle was decreased on the first postoperative day only ( $P \leq 0.05$ ) and was decreased to  $90 \pm 3\%$  of baseline control for animals after skin and muscle incision and to  $35 \pm 5\%$  of baseline control for animals after incision with gut manipulation.

Responding during the light cycle was significantly lower for all groups of animals compared with the dark cycle, and surgical intervention had no effect on the total number of food pellets delivered during the light phase. The numbers of food pellets earned during the light phase before surgery were  $100 \pm 14$ ,  $68 \pm 11$ ,  $63 \pm 9$ , and  $114 \pm 14$  for the groups used for sham surgery, skin incision only, skin and muscle incision, or incision with gut manipulation, respectively. There was no significant interaction between surgical intervention and time after surgery on the number of food pellets delivered during the light phase ( $F_{12,107} = 0.5$ ,  $P = 0.9$ ), and comparison of the number of food pellets earned during the light phase after surgery to presurgical baseline values showed no significant effects for any group (sham:  $F_{4,29} = 0.5$ ,  $P = 0.7$ ; skin incision:  $F_{4,29} = 1.5$ ,  $P = 0.24$ ; skin and muscle incision:  $F_{4,29} = 2.1$ ,  $P = 0.12$ ; incision with gut manipulation:  $F_{4,29} = 1.6$ ,  $P = 0.2$ ).

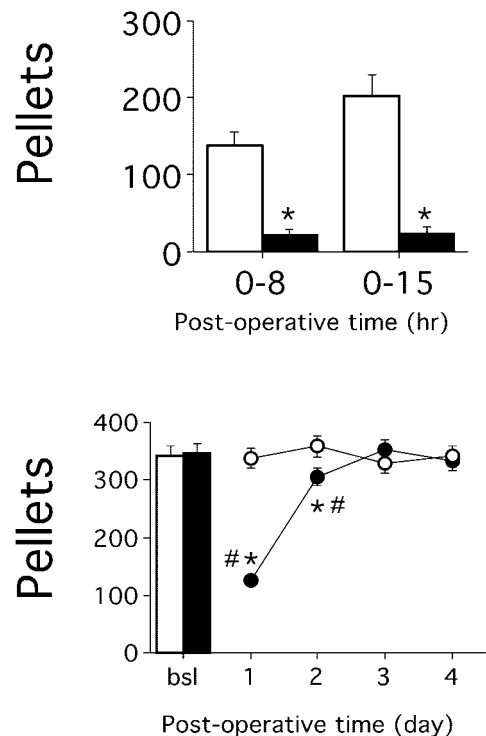
The number of hours during which the animals did not receive a single food pellet was also recorded (hours off). The number of hours off was significantly greater during the light cycle compared with the dark cycle for all groups before surgery. Surgical manipulation produced a significant main effect on the number of hours off in the dark cycle ( $F_{3,107} = 2.6$ ,  $P = 0.05$ ), and there was a significant interaction between surgical manipulation and time after surgery ( $F_{12,107} = 2.2$ ,  $P = 0.02$ ), with the skin and muscle incision groups with or without gut manipulation being significantly different than the sham surgery or skin incision-only groups ( $P \leq 0.05$ ). Sham surgery or skin incision did not affect the hours off during either the light cycle (sham:  $F_{4,29} = 0.2$ ,  $P = 0.9$ ; skin incision:  $F_{4,29} = 0.8$ ,  $P = 0.5$ ) or the dark cycle (sham:  $F_{4,29} = 0.9$ ,  $P = 0.5$ ; skin incision:  $F_{4,29} = 2.1$ ,  $P = 0.12$ ) for these groups. However, skin and muscle incision increased the dark hours off for 2 days after surgery ( $F_{4,29} = 7.0$ ,  $P = 0.001$ ), and skin and muscle incision with gut manipulation increased the dark hours off on the first postoperative day after surgery ( $F_{4,29} = 5.7$ ,  $P = 0.002$ ). Neither surgical manipulation affected the hours off during the light cycle on any postoperative day compared with baseline control data (skin and muscle incision:  $F_{4,29} = 0.9$ ,  $P = 0.5$ ; incision with gut manipulation:  $F_{4,29} = 0.4$ ,  $P = 0.8$ ).

*Relevance of Response Requirement on Food Reinforcement after Surgery*

In separate groups of animals, the effect of abdominal incision with gut manipulation on responding for food under an FR1 schedule was compared to sham surgery, and the results were compared to those reported above under the more strenuous FR10 schedule (see all previous text in "Results" section). As with the FR10 schedule, responding for food under an FR1 schedule was reduced by abdominal incision with gut manipulation in the first 8 or 15 h after surgery compared with sham treatment ( $F_{3,23} = 163.3, P < 0.0001$ ), and there was a significant interaction between time and surgical treatment ( $F_{1,23} = 34.2, P < 0.0001$ ; fig. 2). There was a significant main effect on the number of food pellets earned after abdominal incision with gut manipulation when compared with the baseline data ( $F_{9,59} = 9.6, P < 0.001$ ). There was a significant interaction between time and surgical manipulation ( $F_{4,59} = 11.9, P < 0.0001$ ), with incision producing a significant decrease in the number of food pellets earned on postoperative days 1 and 2 and sham surgery having no significant effect (fig. 2). There was no significant difference between baseline responding in sham or abdominal incision animals under the FR1 schedule of reinforcement ( $P > 0.05$ ). Comparing the FR1 *versus* FR10 groups, there was no significant three-way interaction among FR value, surgical treatment, and days after surgery comparing baseline data with data on postoperative days 1–4 ( $F_{4,119} = 0.1, P = 0.99$ ). There was also no significant interaction between FR schedule and surgical treatment for either the first 8 or 15 h after sham or abdominal incision ( $F_{1,47} = 0.0005, P = 1.0$ ).

*Effect of Abdominal Surgery on Operant Behavior during Restricted Food Access*

Restricting food access to three discrete segments throughout the day did not affect total daily food intake, nor did it affect the circadian patterns of behavior. As with unrestricted-access conditions, responding maintained by food was significantly affected by abdominal surgery ( $F_{1,83} = 34.4, P < 0.0001$ ) and time after surgery ( $F_{6,83} = 48.7, P < 0.0001$ ), and there was a significant interaction between postsurgical time point and sham surgery *versus* abdominal incision ( $F_{6,83} = 7.9, P < 0.0001$ ; fig. 3). The time course of recovery from surgery under the restricted food access conditions was slightly longer than that found with unrestricted access. For the incision group, operant responding maintained by food was significantly less than that found in the sham animals in the first 8 or 15 h after surgery ( $P < 0.05$ ). Total food intake was decreased for up to 48 h after surgery under restricted-access conditions compared with the baseline data from these subjects as well as compared with data from sham-treated rats at this postsurgical time point ( $P < 0.05$ ) (fig. 3). Responding on the lever was not



**Fig. 3.** Effects of abdominal surgery on food reinforcement under restricted-access conditions. The mean (SEM) food pellets earned are shown when food was available only for 11 h/day (see Materials and Methods for access conditions). The mean (SEM) food pellets earned in the immediate postoperative period (upper) or at later time points (lower) are shown after sham surgery (open bars and circles) or abdominal surgery with gut manipulation (dark bars and circles). \* Significantly different from sham surgery ( $P < 0.05$ ). # Significantly different from baseline ( $P < 0.05$ ).

significantly different on days 3 or 4 after laparotomy when the comparisons were made between baseline data for these subjects or data obtained from sham-treated rats at this time point.

*Effect of Local Wound Infiltration with Bupivacaine on Operant Behavior after Laparotomy: Relevance of Gut Manipulation*

To assess the contribution of sensation in the incised area after laparotomy on the decrease in operant responding, bupivacaine was applied in the wound area before closure of the outer musculature either with or without gut manipulation. After laparotomy with gut manipulation, infiltration of the abdominal musculature with 3.0 ml of either 0.25% or 0.75% bupivacaine had no significant effect on responding maintained by food pellets at any time point compared with incision animals without bupivacaine administration ( $P > 0.05$ ). However, when a skin and muscle incision was performed without manipulating the intestines, local wound infiltration with bupivacaine partially reversed the effects of surgery. The effects of bupivacaine were dependent on the bupivacaine dose ( $F_{3,167} = 57.7, P < 0.0001$ ) and time after surgery ( $F_{6,167} = 192.8, P < 0.0001$ ). Respond-

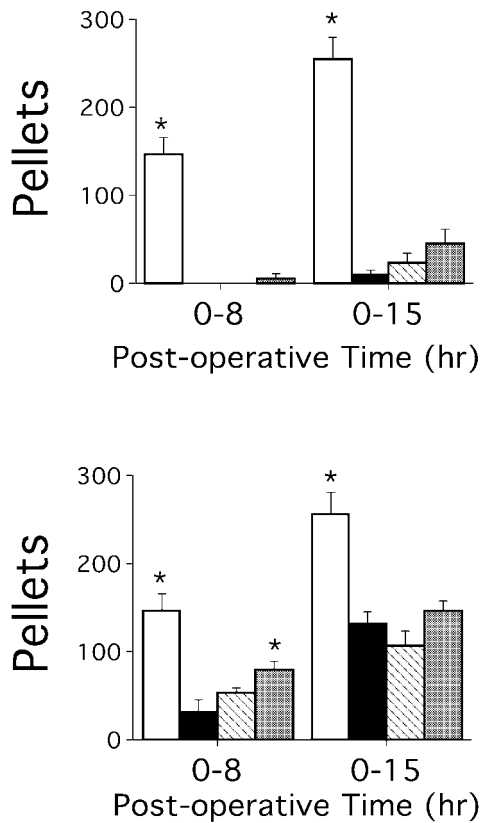


Fig. 4. Effects of bupivacaine infiltration on food reinforcement following abdominal surgery. The mean (SEM) food pellets earned in the first 8 or 15 h after abdominal incision with (upper) or without (lower) gut manipulation is shown. The groups include sham surgery control (open bars), surgery with saline infiltration (dark bars), surgery and perioperative infiltration with 3 ml bupivacaine, 0.25% (hatched bars), and surgery and perioperative infiltration with 3 ml bupivacaine, 0.75%. \* Significantly different from saline infiltration ( $P < 0.05$ ).

ing for food was significantly higher in the first 8 h after surgery following infiltration of the wound with 0.75% but not 0.25% bupivacaine compared with saline ( $P > 0.05$ ; fig. 4). All groups were significantly different from the sham control, however. The effect of bupivacaine diminished with time, such that by the 15-h postoperative time point, responding for food was not different between the groups of rats given 0.75% bupivacaine or saline into the wound site. Similar to the findings reported above (see all previous text in "Results" section), by 24 h after incision of the skin and musculature without gut manipulation, operant responding had returned to baseline values for all groups, and the number of food pellets earned was not significantly different between groups given saline or bupivacaine and the group of rats after sham treatment (data not shown).

#### Identification of Spinal Nerves Innervating Incised Abdominal Musculature

Visualization of fluorogold uptake by spinal afferents and retrograde labeling of dorsal root ganglion revealed

that the spinal nerves T8 through T12 innervate the muscles that were incised in the surgical procedure used for these studies. Injection of fluorogold along the incision site was accompanied by the appearance of fluorescent cell bodies in the dorsal root ganglion harvested from the ipsilateral side. The most rostral dorsal root ganglion containing fluorogold was T8, and no fluorescence was observed caudal to T12 in any of the animals studied. No fluorescence was observed in the dorsal root ganglion that were harvested contralateral to the injected muscle.

#### Effect of T8-T12 Transection on Food Reinforcement after Abdominal Surgery

To extend the findings of the bupivacaine experiment, spinal nerves T8-T12 were severed and cauterized to denervate the abdominal musculature before food training and subsequent abdominal surgery. After recovery from spinal denervation or sham surgery, the baseline behavior was not different for these groups of rats compared with sham-treated animals or rats subjected to laparotomy with or without gut manipulation ( $P > 0.05$ ). Similar to the data obtained with bupivacaine infiltration, transection and cauterization of the right T8-T12 spinal nerves did not significantly inhibit the effect of laparotomy with gut manipulation on food reinforced responding at any time point (fig. 5). The number of food pellets earned was significantly diminished in the first 8, 15, and 24 h after surgery compared with sham-treated subjects and was not significantly different between denervated and nondenervated rats (fig. 6). In the absence of gut manipulation, transection and cauterization of the right T8-T12 spinal nerves reversed the effects of abdominal incision on food reinforced responding at all time points (fig. 6). There was a significant main effect of spinal denervation ( $F_{2,89} = 33.7, P < 0.0001$ ) and a significant main effect of postsurgical time ( $F_{4,89} = 143.2, P < 0.0001$ ) on food pellets earned. The number of food pellets delivered was not significantly different between sham-operated rats and rats subjected to laparotomy without gut manipulation after denervation of the abdominal musculature at all time points, and the numbers of food pellets earned in denervated subjects did not differ from their baseline values at any time after surgery (figs. 5 and 6). The number of food pellets earned by both sham laparotomy control and T8-T12 denervated rats was significantly higher than nondenervated subjects in the first 8, 15, or 24 h after laparotomy without gut manipulation ( $P < 0.05$ ). Therefore, denervation of the incised musculature prevents the incision from decreasing operant responding maintained by food only if the intestines are not manipulated during the surgery.

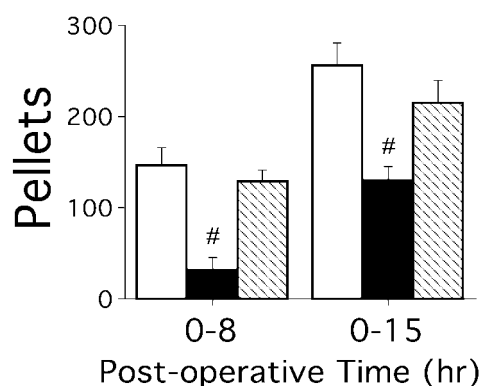
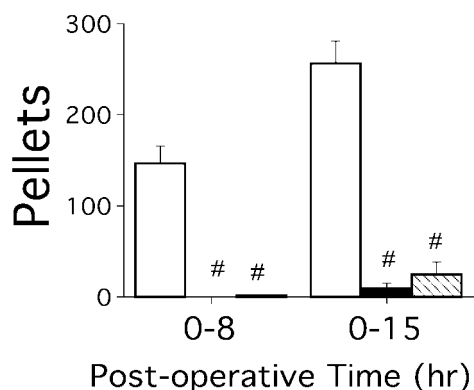


Fig. 5. Effects of T8-T12 denervation on food reinforcement after abdominal surgery. The mean (SEM) food pellets earned in the first 8 or 15 h after abdominal incision with (upper) or without (lower) gut manipulation is shown. The groups include sham surgery control (open bars), surgery after sham denervation (dark bars), and surgery after T8-T12 denervation (hatched bars). # Significantly different from sham surgery ( $P < 0.05$ ).

Discussion

The major findings of this study are that upper abdominal surgery decreases operant responding in rats that is maintained by food presentation, and that afferent input from the incision site combined with gut manipulation seems to contribute to disrupted feeding behavior. The effect is relatively short-lived, lasting less than 72 h and being most dramatic within the first 15 h after surgery. The bupivacaine and T8-T12 denervation studies suggest that the decrement in food reinforcement after abdominal incision alone is likely mediated almost exclusively by incisional pain within the affected musculature. In contrast, when the intestines are disturbed during the surgical intervention, alleviation of incisional pain by these methods produces little to no benefit behaviorally, suggesting that there is a strong visceral pain component likely arising from postoperative ileus that has a predominant behavioral consequence compared to pain arising from the incision itself. Assuming the stimulus from gut manipulation is neural to the spinal cord rather than humeral or on the intrinsic nervous system of the gut,

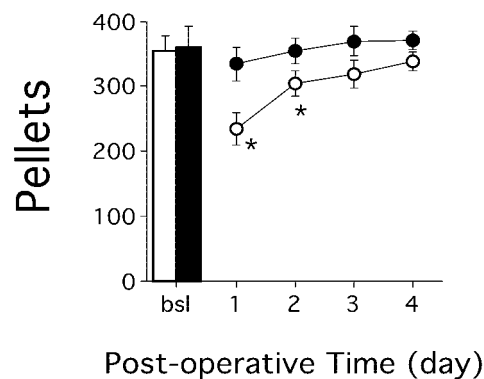


Fig. 6. Effect of T8-T12 denervation on recovery from abdominal surgery. The mean (SEM) food pellets earned for 5 days before abdominal surgery (bsl) and at indicated postoperative days with (open bars and circles) and without (dark bars and circles) gut manipulation is shown for rats that had T8-T12 denervation before food training. \* Significantly different from baseline ( $P < 0.05$ ).

one would not have anticipated disrupting this input by spinal nerve transection, because transection was performed distal to the site where visceral afferents diverge and course with sympathetic efferents along the sympathetic chain.

The current study underscores several advantages of using operant behavior compared with more simple behaviors in rodents. For one, a longitudinal, relatively continuous time course of the behavioral effect is obtained in each animal. Other behavioral endpoints are difficult to measure in such a manner. Repeated stimulation of the paw or other regions using von Frey filaments can lead to behavioral sensitization, particularly in the presence of inflammation or after nerve injury.<sup>12,14</sup> In previous work using a simple exploratory locomotion paradigm, the novelty of the test environment is a relevant variable when examining the effects of surgery on behavior, and repeated testing of the same subject at various time points after laparotomy is problematic.<sup>15</sup> One other advantage of the operant procedure is that analgesia is associated with an increase in the behavioral endpoints, unlike simple reflexive nocifensive responses in which it can be difficult to discriminate between sedation and analgesia. Because extended loss of appetite and diminished food intake is a major determinant of postoperative morbidity and is a major factor in extending hospital stays, another advantage of the current design is that this important variable is measured directly in a model that necessitates that postoperative treatment restore both the ability and motivation to engage in a simple task to obtain oral nutrition.

Inflammatory pain induced by formalin injection into the hind paw diminishes food-reinforced operant behavior in rats.<sup>16</sup> These investigators assessed food reinforcement and nociception in food-deprived rats using the formalin test and 1-h operant test sessions. Food-reinforced lever pressing was diminished in the first 10 min after formalin injection into the hind paw by approxi-

mately 40%, and the pain score was increased approximately fourfold in animals self-administering food pellets. The pain score was similar between animals responding for food and animals that were not trained in the operant paradigm. However, during the delayed phase of formalin-induced nociception, food-reinforced responding returned to normal, and pain score was significantly diminished in operant-trained rats relative to rats that were not trained in the operant task. These investigators concluded that the rats given access to food display fewer signs of nociception in the late-phase formalin response because of increased attention to appetitive motivation relative to nociception and that different homeostatic control mechanisms may take precedent depending on the context of stimulus presentation.

The effect of laparotomy on food-reinforced behavior was consistent across a number of experimental paradigms. The number of lever presses required to earn a single food pellet was not a relevant variable in examining either the magnitude or the duration of the effect of surgery on food reinforced behavior. The postoperative effect and time course were virtually identical when a single lever press or 10 lever presses were required. It may be that FR values higher than 10 would result in an extended duration of the effect of surgery, however. Restricting food access to three discrete components throughout the day also resulted in little to no significant difference between the magnitude or duration of the effect of surgery on responding when compared with unrestricted, continuous access.

The current data would suggest that postoperative ileus produced by the incision alone, if it exists, has less of an impact on behavior than incisional pain because both wound infiltration with bupivacaine and spinal denervation of the incision site abolished the effects of this surgery on food reinforcement. Conversely, when the intestines are manipulated vigorously, the predominant stimulus that results in diminished behavior seems to be something other than afferent input from the incision, because neither bupivacaine administration nor spinal denervation significantly reversed the behavioral consequences of this surgical manipulation. This stimulus is possibly related to a strong visceral component arising from postoperative ileus subsequent to intestinal manipulation that predominates over the incisional component. That the T8-T12 denervation did not result in behavioral improvement in such animals suggests that this stimulus occurs bilaterally, that this stimulus is transmitted across dermatomes outside of the denervated region, or both. The current study suggests that examining food reinforcement after abdominal surgery may provide a tool with which to examine central and peripheral mechanisms that are relevant to complex behavioral effects regarding oral nutrition after surgery.

Postoperative ileus has been studied extensively in rats, and several important findings are noteworthy. Il-

eus occurs in two phases after surgery and gut manipulation, with the first phase lasting approximately 3 h followed by a rapid recovery.<sup>17</sup> The second phase peaks at 24 h and is correlated with leukocyte infiltration of the intestinal muscularis layer and increased release of a number of inflammatory cytokines.<sup>17-19</sup> Prominent mediators include monocyte chemoattractant protein 1 that is released by resident macrophages, leading to recruitment of leukocytes and increased expression of cyclooxygenase (COX) 2 and interleukin 6.<sup>18-20</sup> The decrease in gut motility of the small intestine is primarily mediated by prostaglandins generated by COX-2, which is up-regulated in resident macrophages, neurons, and recruited leukocytes and by nitric oxide generated from inducible nitric oxide synthase.<sup>21</sup> The effect in the lower intestine seems to be due exclusively to inducible nitric oxide synthase activation after intestinal manipulation however.<sup>21</sup> Others have found that COX-2 is primarily involved in ileus induced by incision alone, whereas both COX-1 and COX-2 inhibitors are more effective in reversing ileus after both incision and gut manipulation.<sup>8</sup> In addition to inhibitors of COX isoforms and the various inflammatory cytokines mentioned, agonists of serotonin receptor subtype 4 receptors have been found to reverse the effects of gut manipulation on intestinal motility, both *in vivo* and in tissue organ bath studies.<sup>22,23</sup> Therefore, a number of targets are available for pharmacologic manipulation to address the role of postoperative ileus and both inflammatory and neural mediators in the effects of abdominal surgery on food-reinforced operant behavior using the current model. It is hoped that such studies can provide therapeutic targets to improve postoperative therapy and decrease morbidity and mortality after abdominal surgery.

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