Efficacy of Common Analgesics for Postsurgical Pain in Rats

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Each year, millions of rats undergo surgery for research purposes and receive analgesics to alleviate pain. We sought to evaluate the efficacy of common analgesics in tests of hot-plate nociception and postsurgical pain by using the Rat Grimace Scale. Rats received a single dose of one of several drug–dose combinations and were tested by using the hot-plate test (acute pain) or after laparotomy (with either prophylactic or intraoperative analgesic). The efficacy of analgesics for hot-plate pain was generally not predictive of efficacy for surgical pain. Carprofen and ketoprofen were rarely effective in any of the conditions tested. With the exception of the opioid buprenorphine, several of the drugs we tested required higher-than-recommended doses to alleviate pain. Taken together, our data suggest that current analgesic use frequently is insufficient, and many rats may experience significant postsurgical pain even when analgesics are used in commonly recommended doses.

Abbreviation: RGS, Rat Grimace Scale.

Studies using animal models allow an understanding of the underlying basis of human conditions at a cellular and molecular level, validation of future human research, and identification of specific targets for pharmaceutical interventions.[12] Given that millions of rats are used in research,[20] practices that minimize both pain and distress to the greatest degree possible are crucial.[5] However, in one survey,[15] only 20% of researchers reported administering analgesics to rodents after surgery, suggesting that many animals potentially experience pain.

A lack of pain indicators is often the reason for insufficient analgesia,[15] possibly due to a lack of consensus regarding a determination method. To assess pain, researchers have used telemetry,[4] extensive videorecording,[16,17] and ultrasonic vocalizations,[7,13,22] but such methods can be time-consuming, overly technical, or expensive to implement, resulting in their limited use by the research community. In addition, each method can be confounded by the fact that rodents actively conceal pain behaviors,[1] especially when they sense a predator.[18]

The treatment of pain itself varies as well. Drug and dosage recommendations vary from institution to institution. Buprenorphine, carprofen, and ketoprofen in addition to other opioids frequently are suggested for moderate to severe pain, but few relevant supporting data are available for all recommended drugs. Assessing the effectiveness of these drugs has traditionally been limited to acute pain testing, which assesses a type of pain vastly different from postsurgical pain, making the acute tests poor models.[12]

The prophylactic versus intraoperative timing of the analgesic administration can also influence efficacy. Prophylactic treatment may prevent sensitization that leads to postsurgical hypersensitivity; for example, ketoprofen, a NSAID, is more effective when given prophylactically than intraoperatively.[14] However, the opioids buprenorphine and morphine are most effective when given before and during surgery.[2,4]

In the present study, we used the Rat Grimace Scale (as described in reference 19) to determine effective drug dose and schedule combinations for minimizing postsurgical pain in laboratory rats. Previous work has shown that commonly recommended drugs and doses often are insufficient for postsurgical pain in mice,[11] but whether these findings similarly apply to rats has been unknown. We also assessed various drugs for their potential to reduce acute pain, to determine whether acute analgesia is predictive of postsurgical efficacy.

Materials and Methods

All experiments complied with animal care and use guidelines and were approved by the University of Alabama IACUC. Rats of both sexes (*n* = 246, 123 male and 123 female) were included in all testing, but because no sex-associated differences emerged, all data were combined for reporting. All drugs and vehicle injections were injected at a volume of 1 mL/kg. Buprenorphine and carprofen were diluted in saline; acetaminophen, ibuprofen, and ketoprofen were diluted in 30% polyethylene glycol.

Animals. To extend previous work that focused on mice,[19] all subjects were Wistar rats (weight, 250 to 300 g) obtained from Charles River Laboratories (Hartford, CT). Rats were housed in groups of 4, under a 12:12-h light:dark cycle (lights on, 2130) and provided with food (7017 NIH31, Harlan Laboratories, Indianapolis, IN) and sterile water (Hydropac, Seaford, DE) free choice. Animals were housed in acrylic cages with aspen woodchip bedding in a pathogen-free room with food and water checked daily, and cages were changed twice a week. Cotton squares were added for enrichment purposes. Air handlers controlled and filtered the air in the housing room separately from the rest of the building. Cohorts of rats were tested in the hot-plate test, given a week for drug washout, and then were tested in the laparotomy assay. To avoid sensitization or tolerance effects, no rat received the same drug class twice. All drugs were administered at a volume of 1 ml/kg. All studies were approved by the University of Alabama at Birmingham IACUC. The University of Alabama at Birmingham is AAALAC-accredited.

Hot-plate assay—acute pain. The hot plate (IITC Life Science, Woodland Hills, CA) was set to 53 °C. All subjects were tested at baseline and at 30 and 60 min after injection. To prevent tissue damage, a cutoff of 60 s was used. The analgesics used were buprenorphine (0.01 to 0.05 mg/kg SC; Sigma, St Louis, MO; n = 4 to 7 per group), carprofen (5 to 25 mg/kg SC; Pfizer, ; n = 4 to 6), saline (Hospira, SC, n = 7), acetaminophen (25 to 100 mg/kg SC; MP Biomedicals, ; n = 3 to 7), ibuprofen (15 to 30 mg/kg SC; MP Biomedicals; n = 5 or 6), ketoprofen (10 to 25 mg/kg SC; MP Biomedicals, n = 4 to 7), and 30% polyethylene glycol (SC; EMD Millipore, Billerica, MA; n = 7). For this assay, common doses were chosen to assess the utility to predict efficacy; we typically used additional doses in other tests to determine the threshold for analgesia.

Laparotomy—spontaneous pain. A laparotomy, used as a representative surgery that produces spontaneous pain, was performed under isoflurane–oxygen (2%) anesthesia. After shaving and disinfection (alternating povidone–iodine and alcohol, repeated 3 times), a 2-cm incision was made through the skin, fascia, and muscle by using a scalpel. Wounds (muscle wall and skin) were closed by using surgical glue (Vetbond, 3M, Maplewood, MN).

Prophylactic analgesia. For all prophylactic treatments with analgesics, rats were injected 15 min prior to laparotomy. Analgesics used were buprenorphine (0.01 to 0.025 mg/kg SC; n = 4 or 5 per group), carprofen (5 to 25 mg/kg SC; n = 5 to 7), saline (SC; n = 7), acetaminophen (50 to 100 mg/kg SC, n = 4 to 8/group), ibuprofen (5 to 30 mg/kg SC, n = 3 or 4/group), ketoprofen (10 to 25 mg/kg SC, n = 2 to 5/group), and 30% polyethylene glycol (SC; n = 10).

Intraoperative analgesia. Intraoperative analgesics were administered while the surgery was being performed and the rat was anesthetized. Analgesics used were buprenorphine (0.01 to 0.05 mg/kg SC, n = 6 per group), carprofen (5 to 25 mg/kg SC, n = 5 to 12), saline (SC, n = 13), acetaminophen (25 to 100 mg/kg SC, n = 6 to 8), ibuprofen (5 to 30 mg/kg SC, n = 6 to 8), ketoprofen (5 to 25 mg/kg SC, n = 4 to 9), and 30% polyethylene glycol (SC, n = 10).

Digital videorecording. Rats (4 at a time) were placed on a tabletop and allocated between 4 isolated cubicles. The cubicles (21.25 × 8.75 × 10.00 cm) were made of clear acrylic with an opaque, stainless steel, separating wall and perforated, stainless steel floor. Digital high-definition video cameras (HMX-QF20 Full HD, Samsung) were placed on both sides of the cubicles to maximize the opportunity to record clear images of rats' heads. Rats were recorded in 30-min sessions in a closed room, with no experimenters present.[18] Baseline recordings were collected on a day prior to the day of surgery. On the test day, recording began 45 min after surgery.

Analysis of videos. To determine pain scores for each rat, representative images were taken from each 3-min time bin and randomized into a PowerPoint (Microsoft, Redmond, WA) presentation. Each image was scored independently by 2 raters who were blinded to the experimental condition. For both scoring experiments, intraclass correlations were computed for the baseline (prophylactic, 0.68; intraoperative, 0.67) and test (prophylactic, 0.73; intraoperative, 0.77) scores. Each rater was responsible for rating each image on all 4 action units (orbit, nose–cheek, whiskers, and ears). For each of the 2 raters, a mean was calculated for each action unit within a single test session (baseline or after surgery) and entered into SPSS (IBM, Armonk, NY). Mean scores were calculated for the baseline and postsurgery conditions (for each rater and then as a combined mean), and a difference score was calculated. Additional details

Table 1. Main effects of drug treatment for all ANOVA from all assays, separated according to drug vehicle

Vehicle	Test	F	df	P
Saline	Hot plate	4.393	6, 33	0.002
30% polyethylene glycol	Hot plate	2.740	8, 40	0.016
Saline	Intraoperative	3.314	7, 53	0.005
30% polyethylene glycol	Intraoperative	2.606	10, 66	0.010
Saline	Prophylactic	2.587	5, 27	0.049
30% polyethylene glycol	Prophylactic	5.227	8, 34	0.001

regarding scoring pain by using the Rat Grimace Scale are found in reference 19.

Statistical analysis. All statistical analyses were performed by using SPSS version 22 (IBM). Data from each assay were analyzed by using univariate ANOVA, followed by the Dunnett posthoc test to compare each drug with its vehicle. An α level of 0.05 was applied to designate significance.

Results

All treatments showed a significant ($P < 0.05$) effect of drug (Table 1). For all statistical analyses, data were separated according to the vehicle used and assay—that is, all drugs with the same vehicle (saline or 30% polyethylene glycol) were analyzed together in each of the 3 assays (Table 1). Significant effects of drug are as compared with their vehicle. Figures for laparotomy data represent the change in scores from baseline to postsurgery. All data for the hot-plate assay represent the maximal percentage change in response. We define an effective drug or dose as one that significantly ($P < 0.05$) reduces pain (that is, increases latency in the hot-plate assay, decreases the behavior score after laparotomy) when compared with the control condition.

Drugs diluted in saline. *Buprenorphine.* Three doses of buprenorphine were used: 0.01, 0.025, and 0.05 mg/kg. Of these, only doses of 0.025 and 0.05 mg/kg significantly ($P < 0.01$) increased the threshold of the animals in the hot-plate assay (Figure 1 A). When used as an analgesic for postsurgical pain, buprenorphine was effective at 0.01 and 0.025 mg/kg ($P < 0.05$) when injected prophylactically (Figure 1 B) and at 0.025 and 0.05 mg/kg ($P < 0.01$) when administered intraoperatively (Figure 1 C).

Carprofen. Carprofen was ineffective as an analgesic in all tests (Figure 2).

Drugs diluted in 30% polyethylene glycol. *Acetaminophen.* Three doses of acetaminophen were used in these assays: 25, 50, and 100 mg/kg. In the hot-plate assay (Figure 3 A), acetaminophen was effective ($P < 0.05$) at the 50-mg/kg dose only. When used as an analgesic for postsurgical pain, the drug was significantly effective at 50 mg/kg ($P < 0.05$) when administered intraoperatively (Figure 3 C) and at 100 mg/kg ($P < 0.01$) prophylactically (Figure 3 B).

Ibuprofen. Three doses of ibuprofen were used in these assays: 5, 15, and 30 mg/kg. In the hot-plate assay (Figure 4 A), ibuprofen significantly ($P < 0.01$) increased the percentage change at the 30-mg/kg dose. When administered intraoperatively (Figure 4 C), all doses were effective ($P < 0.05$) in reducing postsurgical pain. However when ibuprofen was administered prophylactically (Figure 4 B), only the 15-mg/kg dose was effective ($P < 0.01$).

Ketoprofen. Four doses of ketoprofen were used in these assays: 5, 10, 15, and 25 mg/kg. Ketoprofen had no effect
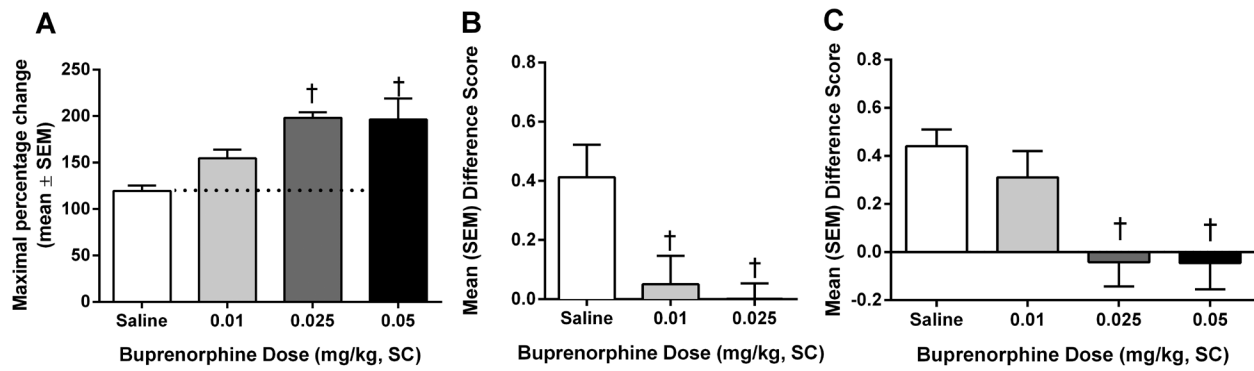
Figure 1. Analgesic effects of buprenorphine on (A) acute pain when tested by using the hot plate at 53 °C, (B) spontaneous pain when administered prophylactically (15 min prior to surgery), and (C) spontaneous pain when administered intraoperatively (during surgery). †, Value is significantly ($P < 0.01$) different from that for saline.
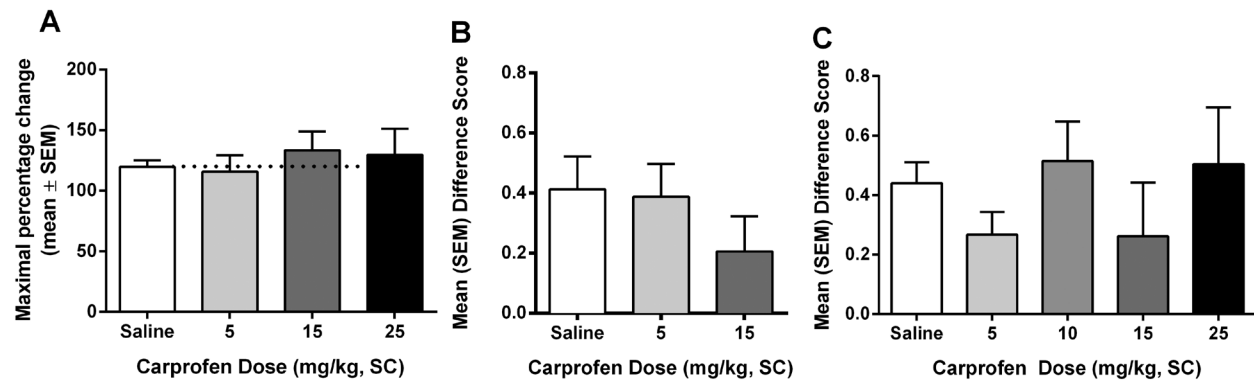

Figure 2. Analgesic effects of carprofen on (A) acute pain when tested by using the hot plate at 53 °C, (B) spontaneous pain when administered prophylactically (15 min prior to surgery), and (C) spontaneous pain when administered intraoperatively (during surgery). All differences were nonsignificant.
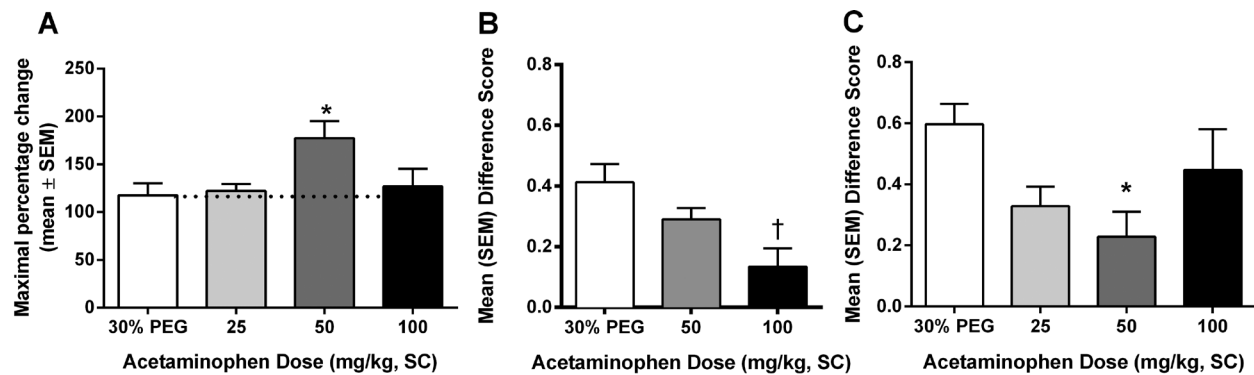

Figure 3. Analgesic effect of acetaminophen on (A) acute pain when tested by using the hot plate at 53 °C, (B) spontaneous pain when administered prophylactically (15 min prior to surgery), and (C) spontaneous pain when administered intraoperatively (during surgery). Value is significantly (*, $P < 0.05$; †, $P < 0.01$) different from that for 30% PEG.

in the hot-plate assay (Figure 5 A) at any dose. Similarly, all doses of ketoprofen were ineffective when the drug was administered intraoperatively (Figure 5 C). For postsurgical pain, ketoprofen was effective when given prophylactically (Figure 5 B) at the 15-mg/kg ($P < 0.01$) and 25-mg/kg ($P < 0.05$) doses.

Discussion

To determine the efficacy of common analgesics for reducing pain after laparotomy, we administered common analgesic drugs intraoperatively or prophylactically. Our data indicate that the timing of injections relative to surgery can alter whether a drug significantly reduces postsurgical pain and that many common analgesics are ineffective altogether or at various points within their recommended dose ranges. The data also suggest that the effectiveness of analgesics in acute pain is not necessarily indicative of their efficacy regarding postsurgical pain.

In addition to humane concerns, pain must be minimized to avoid confounding experimental outcomes. Spontaneous pain is the most common complication after surgery and may seri-

Figure 4. Analgesic effect of ibuprofen on (A) acute pain when tested by using the hot plate at 53 °C, (B) spontaneous pain when administered prophylactically (15 min prior to surgery), and (C) spontaneous pain when administered intraoperatively (during surgery). †, Value is significantly ($P < 0.01$) different from that for 30% PEG.
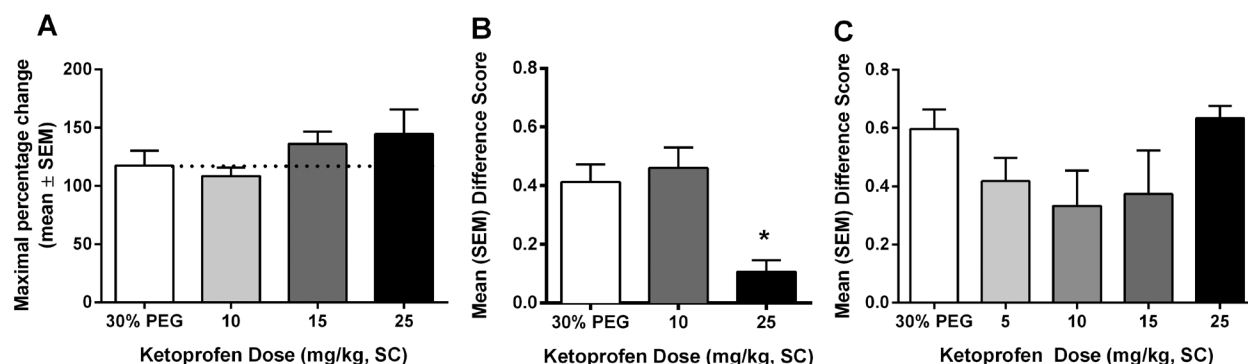

Figure 5. Analgesic effect of ketoprofen on (A) acute pain when tested by using the hot plate at 53 °C, (B) spontaneous pain when administered prophylactically (15 min prior to surgery), and (C) spontaneous pain when administered intraoperatively (during surgery). *, Value is significantly ($P < 0.05$) different from that for 30% PEG.

ously affect experimental results in animals when not treated sufficiently. Currently available means to assess spontaneous pain in animals include complex behavioral assessment,[16,17] ultrasonic vocalizations,[7,13,22] and surgically implanted telemetry,[4] but all of these methods have drawbacks.

One study[16] evaluated the use of behavioral indicators in an approach that was intended to be less subjective than other methods for measuring pain. Although the authors assert that their measures are more effective and less subjective than most, rats were filmed for 8 h, behavioral analysis software was used for data collection, and the researchers reviewed 150 behaviors before establishing a list of the most representative. By using the pared list of behaviors, rats were filmed to determine the frequency and duration of specific behaviors associated with pain.[17] Software was used to collect data, and all footage was reviewed by a single treatment-blinded observer, thus preventing measures of interrater reliability and allowing for objectivity. Despite its reported validity, this method is complicated, time-consuming, and impractical for common use. Our review of these behavioral assessments suggests that at least 22 *different* behaviors appear in these reports,[9,16,17] with only 5 being consistently reported (arch, fall, stagger, twitch, and writhe). In addition, a recent report[9] suggested that the 5 common behaviors occur "so infrequently" that they should be combined into a composite score. These comments lend credence to our claim that the method, although seemingly

valid and comparable to facial expression scoring,[9] is more complicated.

The measurement of ultrasonic vocalizations has been used as another method to assess both evoked and chronic pain, with mixed results. After using both partial sciatic nerve ligations and formalin to induce a chronic pain state, one study[22] recorded no vocalizations. In contrast, others have found that ultrasonic vocalizations correlate with testing of mechanical hypersensitivity.[7] Furthermore, ultrasonic vocalizations have been recorded during the formalin test and can be suppressed by administration of morphine.[13] Unfortunately, recording ultrasonic vocalizations requires special chambers, recording equipment, and software to collect and analyze the data, making this method an unlikely means to rapidly and routinely assess pain in laboratory animals.

Armed with the knowledge that rodents often suppress their pain in the presence of an experimenter,[1,18] animal care staff and veterinarians must be able to assess pain rapidly (within seconds). With the publication of the grimace scales for assessing spontaneous pain in mice[8] and rats,[19] pain can be assessed reliably, quickly, and after minimal training. Additional analysis can be done by using recorded videos. These methods have led to the determination that common NSAID are ineffective at their recommended doses for postsurgical pain in mice.[11] Here, we show similar findings for carprofen and ketoprofen, which respectively were completely and

moderately ineffective for postsurgical pain in rats. We further found that buprenorphine was effective in rats, as it is in mice.[11] Acetaminophen, although ineffective in mice,[11] was effective in rats, whereas ibuprofen was effective in rats but has not been tested in mice.

In terms of studies examining postoperative analgesia in rats after laparotomy, our results are consistent with previous explorations in only one case: buprenorphine is an effective analgesic. Early seminal work examining body weight change, food and water intake, and locomotor activity showed that buprenorphine (0.05 mg/kg) was more effective than saline as an analgesic.[10] This effect was replicated (for the most part) several years later by the same group.[6] Whereas we found no analgesic effect for carprofen and moderate analgesia for ketoprofen, others have found these drugs to be significantly better than saline in treating post-laparotomy pain.[16] Subcutaneous carprofen and ketoprofen (at identical doses to those we used [0.5 mg/kg]) were effective in reducing weight loss after surgery.[3] With the publication of pain-related behaviors as outcome measures, buprenorphine, carprofen, and ketoprofen have been shown to be efficacious in reducing several of these behaviors.[16,17] Another group examined locomotor activity and food and water intake and found carprofen to be effective in reducing pain.[23] Why we were unable to document analgesia due to carprofen is unknown, but the reason may reflect the specificity of the Rat Grimace Scale for pain expression. Whereas pain clearly affects locomotor activity and food intake, several other factors, such as time of day, relative hunger, and the presence of other conspecifics can affect these measures as well. Some of these factors also affect facial expressions of pain in rats,[18] but we controlled for these effects in the current study.

Our current data support the reevaluation of commonly recommended analgesics for postsurgical pain in laboratory rats. Regarding each of the tested drugs, the opioid buprenorphine was effective in all conditions, but higher doses were needed for acute pain and intraoperative injection than for preemptive injection. Unfortunately single exposures to opioid drugs can have long-lasting effects[21] and, as controlled substances, have limited usefulness. From the NSAID class of drugs, acetaminophen and ibuprofen performed similarly, with moderate to high doses showing analgesia. In contrast, the most commonly recommended NSAID, carprofen and ketoprofen, were never or only rarely, respectively, effective as analgesics. Ketoprofen was more effective when given prophylactically, suggesting that its onset of action may be slower than other utilized drugs or that using ketoprofen before surgery to reduce the inflammatory process has some benefits for subsequent pain. Our data indicate that the recommended drugs and doses of NSAID should be reconsidered.

The guidelines set forth in the United States and United Kingdom for addressing pain and distress in laboratory animals are intended for general use for various surgical treatments, but research suggests they are falling short of full adoption.[15] This discrepancy may be the result of conflicting or variable recommendations between institutions or due to limited empirical research on which to base recommendations. Until recently, measures of spontaneous pain in laboratory rats have been highly subjective, required expensive equipment, or were inconsistent and unreliable. The publication of the Rat Grimace Scale[19] as an alternative to more complicated and expensive measures provides a simpler and reliable method for assessing spontaneous pain in laboratory rats that is available to all researchers and animal care staff. We assert that institutions should reevaluate their recommended drugs and doses with reference to the appropriate surgical model, as illustrated in the current study.

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