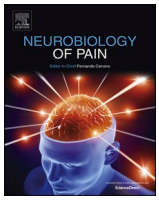
Neurobiology of Pain 12 (2022) 100103

Contents lists available at ScienceDirect

Neurobiology of Pain

journal homepage: www.sciencedirect.com/journal/neurobiology-of-pain



Comparison of Pain-Like behaviors in two surgical incision animal models in C57BL/6J mice

Esad Ulker [a], Martial Caillaud [a], Eda Koseli [a], Katherine Contreras [a], Yasmin Alkhlaif [a], Eric Lindley [a], Mitali Barik [a], Sofia Ghani [a], Camron D. Bryant [c], M. Imad Damaj [a,b,*]

[a] Department of Pharmacology and Toxicology, Virginia Commonwealth University, USA
[b] Translational Research Initiative for Pain and Neuropathy, Virginia Commonwealth University, USA
[c] Department of Pharmacology and Experimental Therapeutics and Psychiatry, Boston University School of Medicine, USA

ARTICLE INFO

Keywords:
Plantar incision
Laparotomy
Spontaneous Behavior
Evoked Behavior
Acute Pain

ABSTRACT

Background: Management of pain post-surgery is crucial for tissue healing in both veterinary and human medicine. Overuse of some analgesics such as opioids may lead to addictions and worsen pain syndromes (opioid-induced hyperalgesia), while underuse of it may affect the welfare of the patient. Therefore, the importance of using surgery models in laboratory animals is increasing, with the goal of improving our understanding of pain neurobiology and developing safer analgesics.

Methods: We compared the widely used plantar incision model with the laparotomy surgery model and measured pain-related behaviors using both spontaneous and evoked responses in female and male C57BL/6J mice. Additionally, we assessed conditioned place preference (CPP) and sucrose preference tests to measure pain-induced motivation for the analgesic ketoprofen and anhedonia-like behavior.

Results: Laparotomized mice showed increased abdominal sensitivity while paw-incised mice showed increased paw thermal and mechanical sensitivity up to seven days post-surgery. Laparotomy surgery reduced all spontaneous behaviors in our study however this effect dissipated by 24 h post-laparotomy. On the other hand, paw incision only reduced the percentage of cage hanging in a sex-dependent manner at 6 h post-incision. We also showed that both surgery models increased conditioned place preference for ketoprofen while preference for sucrose was only reduced at 24 h post-laparotomy. Laporatomy, but not paw incision, induced a decrease in body weight at 24 h post-surgery. Neither surgery model affected fluid intake.

Conclusion: Our results indicate that post-surgery hypersensitivity and behavioral deficits may differ by the incision site. Furthermore, factors associated with the surgery including length of the incision, duration of the anesthesia, and the layers that received stitches may affect subsequent spontaneous behaviors. These findings may help to improve drug development or the choice of the effective analgesic, depending on the surgery type.

Introduction

Acute postsurgical pain (PSP) is a highly prevalent consequence of surgery, with an incidence of 5–85 % depending on the type of surgery. Inadequately treated acute PSP is a strong predictor for development of chronic post-surgical pain, a known risk factor for prescription opioid misuse. Currently, the most common indication for opioid initiation is surgery (Ladha et al., 2019) and unfortunately, opioids remain to be the cornerstone treatment of postsurgical pain despite their unreliable efficacy and adverse effects (Gan, 2017). In addition, ineffective PSP control is associated with poor outcomes including increased length-of-stay and sleep disturbance. Furthermore, as stated in the Federal Pain Research Strategy Recommendations, "acute pain assessment and management are commonly inadequate, pharmacologic treatments are imprecise and associated with adverse effects". Considering that >320 million surgeries are performed each year worldwide (Weiser et al., 2016), more effective and safer analgesic drugs are needed. Basic science studies in rodent models of surgical incision are critical to identify potential targets and enable translation of these findings into clinical practice more rapidly.

The widely used postsurgical plantar incision pain model was developed by Brennan et al. in rats in the 90 s (Brennan et al., 1996) and

* Corresponding authors at: 1217 East Marshall Street Richmond, VA 23298, USA.
E-mail address: m.damaj@vcuhealth.org (M. Imad Damaj).

https://doi.org/10.1016/j.ynpai.2022.100103
Received 22 August 2022; Received in revised form 5 September 2022; Accepted 5 September 2022
Available online 7 September 2022

this model was brought to the mice by Pogatzki and Raja (Pogatzki & Raja, 2003). Other pain models related to surgical injuries have been developed, in particular abdominal surgery, such as the laparotomy mouse model. The primary aim of this study is to compare these two widely used mouse models in terms of pain-related behaviors using the same experimental conditions. Measuring pain-related behaviors in rodents is challenging because as prey animals, this species does not voluntarily exhibit obvious signs of pain. The majority of behavioral tests currently used to measure pain in animals are evoked nociceptive mechanical, thermal and cold sensitivity tests. These tests have the advantage of being simple to set up and quite reproducible. Nevertheless, they have the disadvantage of being experimenter dependent. Moreover, since pain is an unpleasant sensory and emotional experience, more and more studies are using non-evoked tests for the evaluation of pain in mice and rats. Recently, we have proposed the use of wheel running, the burrowing and nesting tests to evaluate behaviors supposed to be measure "activities of daily living" in humans (Toma et al., 2017; Contreras et al., 2021). In addition, the conditioned place preference (CPP) test coupled with the use of analgesic represents a valuable tool to assess pain relief in animals.

Using a battery of evoked nociceptive testing and spontaneous behavioral measures, we compared assessment of the two PSP models in adult female and male C57BL/6J mice, the most widely used inbred mouse strain in biomedical research. In addition, we assessed the analgesic-like effect of ketoprofen in the two models using the conditioned place preference (CPP) test.

Material and methods

Animals

The experiments were performed on C57BL/6J adult female and male (50 % female and 50 % male for each group) mice 8–10 weeks years old purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were housed four per cages with an enriched environment and maintained on a 12 h light/dark cycle (lights on at 07:00 h), with a room temperature of 22 °C, with ad libitum access to food (global 18 % protein chow diet; Envigo Teklad, Indianapolis, IN, USA) and water. All experiments were performed during the light cycle. The study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. All studies were carried out following the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. All mice were observed daily for general well-being and their weight was measured daily.

Paw incision procedure

The paw incision procedure was performed as described by Cowie & Stucky, (2019). Mice were anesthetized with 5.0 % isoflurane and maintained using a constant stream of 2.5 % isoflurane in oxygen using a face mask and vaporizer. The surgical site was disinfected with beta-iodine. All surgeries were performed by the same surgeon (EU) to ensure consistency, and all surgeries performed between 08:00 AM and 11:00 AM. A 0.5- to 1.0-cm incision was made through the skin and fascia of the right hind paw. After the incision flexor muscle was elevated and cut longitudinally with a scalpel, keeping the origin and insertion intact. Two sutures (5–0 nylon, discontinuous) were used to close the wound and only the skin was sutured and not the muscle. The total duration of surgery was between 7 and 10 min. Once recovered from anesthesia, mice were placed back into their home cages instead of new cages to avoid novelty-induced anxiety. Every surgical group's corresponding sham group received the same duration of isoflurane at the same time and the surgical site was disinfected but no incisions were made. Animals that removed the suture were not included in the studies and sutures were not removed by the experimenter to avoid extra anesthesia and manipulation of the paw. The thickness of the right paw was measured both before and after paw incision at the end of mechanical and thermal sensitivity assays using a digital caliper (Traceable Calipers, Friendswood, TX, USA). Data were recorded to the nearest ± 0.01 mm and expressed as a percent change from baseline paw thickness.

Laparotomy procedure

Laparotomy surgeries were performed as described by Oliver et al. (2018) with slight modification. Mice were anesthetized as described above. Abdominal hair was clipped with nose hair clippers and the surgical site was disinfected with beta-iodine. All surgeries were performed by the same surgeon (EU) to ensure consistency, and all surgeries performed between 08:00 AM and 11:00 AM. A 1.0- to 1.5-cm midline abdominal incision was made through the skin and then extended through the linea alba. To mimic visceral manipulation associated with various surgeries, a sterile cotton swab was inserted and moved throughout the abdomen for 30 s. The abdominal muscle layers and skin was closed by using 5–0 nylon suture in a discontinuous pattern. The total duration of surgery was between 7 and 10 min. Once recovered from anesthesia, mice were housed back to their cages instead of new cages to avoid novelty-induced anxiety. Every surgical group's corresponding sham group received the same duration of isoflurane at the same time and their hair was clipped and surgical site disinfected but no incisions were made.

Behavioral testing

Testing protocol. For each test, a different cohort of mice was used, except for mechanical and thermal sensitivity after paw incision in which these two tests were performed in the same cohort. For reflexive tests in paw incision, baseline measurements were taken one day before the surgery. Paw sensitivity was assessed daily, and abdominal sensitivity was assessed every other day based on our preliminary experiments. For non-reflexive tests, baseline tests were performed one day before the surgery. After surgery, the same mouse was subjected to the same test at 2 h, 6 h, 24 h, and 48 h post-surgery except for wheel running. Because the wheel running test was 2 h long, to prevent fatigue of the animals, a separate cohort was used for postop 6 h. Mice were randomizing to treatment and behavioral observations were blinded to treatments and sex of mice.

Paw mechanical sensitivity. Mechanical withdrawal thresholds were determined according to the method of Chaplan et al. (1994) with slight modifications. Mice were placed in clear plastic cylinders (9 × 11 cm) with mesh metal flooring and allowed to acclimate for 15 min before testing. A series of calibrated von Frey filaments (Stoelting, Inc., Wood Dale, IL, USA) with logarithmically incremental stiffness ranging from 2.83 to 4.56 units expressed log10 of (10 × force in (mg)) were applied to the paw using a modified up-down method. Each hair was presented perpendicularly against the paw, with sufficient force to cause slight bending, and held 2 to 3 *sec*. The stimulation of the same intensity was applied two times to the hind paw at intervals of a few seconds. The paw withdrawal threshold (PWT) was expressed in grams (g), indicating the force of the von Frey hair to which the animal reacted (paw withdrawn, licking, or shaking). The data are expressed with the results from the ipsilateral paw of each mouse.

Abdominal mechanical sensitivity. Abdominal sensitivity was assessed with a scoring system, adapted from Schiene et al. (2019), on a scale of 0–3 the withdrawal reactions to 5 abdominal stimulations with a 0.4 g von Frey filament (one stimulation every 2 min), where a score of 0 indicated no response; 1 indicated lifting of the abdomen, licking or movement; 2 indicated hind-paw extension or flinching, slight jumping or strong licking; and 3 indicated strong jumping or running inside the chamber. The pain score was the sum of scores for all stimulations. Based on our preliminary studies, animals were tested every other day with only one filament to avoid sensitization. The filament of 0.4 g was

chosen again based on our preliminary studies as 0.16 g was not enough to elicit a substantial response and 0.6 g was over-stimulating as sham and surgery reacted in the same manner.

Thermal Sensitivity. Thermal hyperalgesia was measured via the Hargreaves test. Mice were placed in clear plastic chambers (7 × 9 × 10 cm) on an elevated surface and allowed to acclimate to their environment before testing. The radiant heat source was directed to the plantar surface of each hind paw in the area immediately proximal to the toes. The paw withdrawal latency (PWL) was defined as the time from the onset of radiant heat to withdrawal of the animal's hind paw. 20-s cut-off time was used. Three measures of PWL were taken and averaged for each hind-paw using the Hargreaves test. Withdrawal latencies were measured for each hind paw. Results are expressed as withdrawal latency for each paw.

Rearing Behavior. Rearing is a spontaneous behavior, reflecting the search phase of exploratory behavior. Animals were taken from their home cages and place into clear cages (same dimension as their home cage but without bedding). Animals spent 5 min in the test cage before recording their behavior to avoid novelty-induced anxiety. After the initial 5 min, the number of rears performed over 5 min was recorded by an observer. Rearing was defined as both forelimbs off the ground and touch the sides of the testing cage and hindlimbs preforming plantar flexion.

Nesting Consolidation Test. The nesting procedure was adapted as previously described by Oliver et al. (2018). For the nesting assay, all mice were single housed two days before surgeries. All previous nesting material was removed from the home cage at the time of surgery and four half-pieces of cotton were placed into each corner of the cage. After surgery/anesthesia, mice were placed back to their prepared home cages. At various timepoints, their nesting was scored based on this scoring system: 1, no nestlet piece grouped; 2, nestlet pieces grouped in one or two pair; 3, 3 nestlet pieces grouped; 4, all nestlet pieces grouped; 5, all nestlet pieces grouped and completely shredded).

Wheel Running. Voluntary wheel running was assessed in polycarbonate activity wheels (diameter 21.6 cm: width 6 cm) with a steel rod axle. Mice were placed in the inside of the wheel (mice did not have free access to the wheel). The wheel could only be turned in a single direction. Six activity cages were contained within a testing room. The number of rotations completed was measured over 2 h. Baseline measurements were reference points for each mouse and data were expressed as a percentage of rotations completed compared to baseline session.

Burrowing Behavior. Custom-made tubes as described in Deacon's burrowing protocol were used for burrowing assay (Deacon, 2006). Empty gray tubes (20 cm long × 7 cm in diameter with ~ 10-degree upwards tilt) were placed in 300 mm × 234 mm × 412 mm cages (Allentown NexGen Rat 900) filled with up to 175–180 g of fresh corncob bedding (Teklad 7097 ¼" corncob bedding; ENVIGO). Animals first had a training session and a subsequent baseline session. They were exposed to the tube and the cage for 1 h. After the training session, tubes were filled with bedding material. Animals were placed into the testing boxes again for 30 min. The latency to burrow was recorded and at the end of 30 min, the amount of bedding taken out was also calculated.

Home cage hanging. The hanging procedure was adapted as previously described by Zhang et al. (2021). Mice were placed in individual cages (Allentown Micro-Vent cage) with no bedding or nesting material in front of a mirror. Cages were covered with a stainless-steel cage lid that did not contain food or a water bottle. Mice were observed for 30 min. A camcorder (Aluratek AWC01F) was set up beside the cage to allow for an unobstructed side view. Videos were recorded in MPEG format and then converted to MP4 format (320 × 240) for analysis. Cage hanging was marked when the mice had at least one paw gripping the cage. Each individual hang was counted and timed.

Conditioned Place Preference (CPP). The CPP protocol was adapted from our previous work in pain models (Neddenriep et al., 2019; Caillaud et al., 2021). Mice were handled for 5 days before the start of the

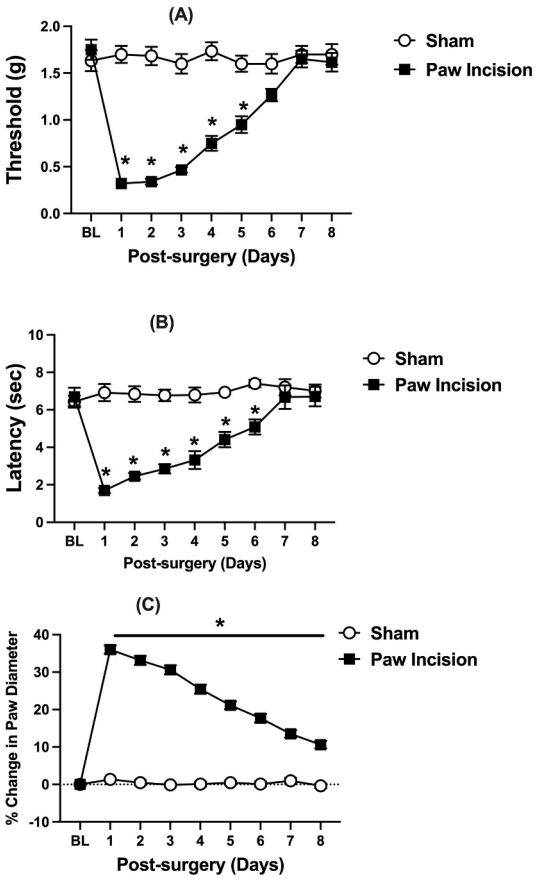


Fig. 1. Effect of Paw Incision Surgery on Evoked Behaviors. We observed evoked behaviors for eight days post-surgery **(A)** Mechanical sensitivity was measured by the von Frey test and the results are expressed as the threshold (g) of response. **(B)** Thermal sensitivity was measured by the Hargreaves test and the results are expressed as paw withdrawal latency (s). **(C)** We also presented a % change in paw diameter after the surgery for eight days. Values are expressed as mean ± SEM. n = 8/group. Results were compared using two-way ANOVA with time and treatment as a factor and post-hoc Sidak's test (*p < 0.05 vs vehicle).

experiment. Mice were first tested for baseline preference for each of the sides in a 3-chamber design where mice were confined to the middle compartment (where no conditioning occurs) to acclimate for 5 min then they were provided free access to all compartments for 15 min. Mice were then randomized assigned to the different groups. Immediately after assessment of baseline preference, surgeries were performed. Twenty-four h after surgery, conditioning session began where the mice were first administered a vehicle injection (10 ml/kg, s.c.), placed into their home cage for 30 min, and then placed into the vehicle-paired compartment where they were confined for 20 min. Four h later, mice were administered an injection of either vehicle (10 ml/kg, s.c.) or ketorprofen (20 mg/kg, s.c.) and were confined to the drug-paired compartment for 20 min. On test day (day 2), mice were placed into the middle chamber and were allowed access to all three chambers for 15 min in a drug-free state. The preference score was calculated as the difference (s) in time spent in the drug paired side between day 1 and day 2. In another cohort of mice, another two-day CPP study was conducted with ketoprofen at 7 to 8 days post-laparotomy using the same procedure described above.

Sucrose Preference Test. Mice were housed individually for a week with ad libitum access to food. Mice were presented with two sipper tubes, one containing normal drinking water and the other containing 2 % sucrose solution (w/v, Sucrose ≥99.5 % (GC) Sigma, United States, cat# S7903). The total volume consumed from each tube was measured

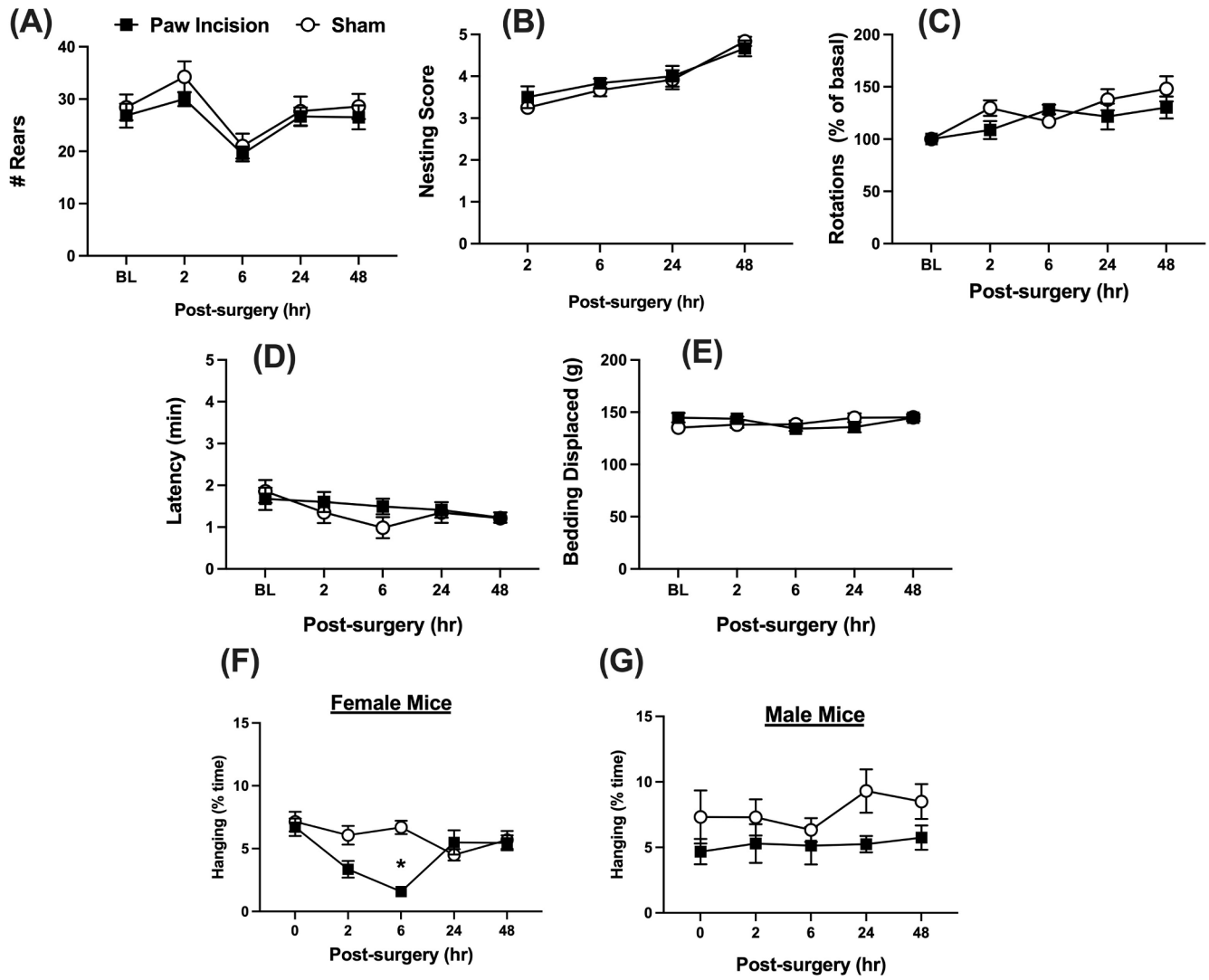


Fig. 2. Effect of Paw Incision Surgery on non– Evoked Behaviors. The following non-evoked behaviors were measured post-surgery 2, 6, 24, and 48 h: **(A)** The number of rears, **(B)** nesting score, **(C)** wheel running, **(D)** time to start digging in min, **(E)** amount of removed bedding in grams, percentage of cage hanging time in **(F)** female, and **(G)** male. Values are expressed as mean ± SEM. n = 8/group. Results were compared using two-way ANOVA with treatment as the factor and post-hoc Dunnett's test (*$p < 0.05$ vs vehicle).

after 24 h. The position of both sipper tubes was switched for each drinking session to avoid place preference. Sucrose preference was calculated as a percentage of 2 % sucrose volume consumed over the total fluid intake, multiplied by 100 %. Preference is reported as the average per group.

Statistical analysis

Data were analyzed using the GraphPad software, version 9.3 (GraphPad Software, Inc., La Jolla, CA), and are expressed as the mean ± S.E.M. Analysis of the results did not show any significant effect of sex on most post-surgical outcome measures. Therefore, females and males were combined for analysis. Normality and equality of variances of all data sets except nesting were confirmed by using the Shapiro–Wilk and Levene tests, respectively. All other tests were analyzed with 2-way ANOVA [post hoc analysis (Sidak test)]. Data are expressed as the mean ± S.E.M. of mice/per sex/per group for all tests. P values <0.05 were considered significant.

Results

Paw incision studies

The effect of paw incision on mechanical and thermal sensitivity was measured by two reflexive tests, including the von Frey filaments test and the Hargreaves test, respectively (Fig. 1A and B). We observed a significant decrease [$F(\text{Time} \times \text{Surgery})\ (8, 176) = 27.26$; $P < 0.0001$); $F\text{surgery}\ (1, 22) = 95.88$, $p < 0.0001$] in the paw withdrawal threshold from day 1 through 5 (D1 to D4: $p < 0.05$; D5 $p < 0.01$) in the paw incision group in comparison to the sham mice. Similar results were observed in the thermal hypersensitivity test with a significant decrease [$F(\text{Time} \times \text{Surgery})\ (8, 198) = 28.98$; $P < 0.0001$); $F\text{surgery}\ (1, 22) = 51.18$, $p < 0.0001$] in the paw removal time in the surgery group at day 1 through day 6 (D1 to D3: $p < 0.05$; D4 to D6: $p < 0.01$). These results clearly show that paw incision induces a mechanical and thermal hypersensitivity in C57BL/6J mice that lasts for nearly-one week.

In addition, our paw diameter data shows that paw incision caused significant edema in animals [$F(\text{Time} \times \text{Surgery})\ (8, 198) = 85.11$; $P < 0.0001$); $F\text{surgery}\ (1, 22) = 461.1$; $p < 0.0001$], being most in post-surgery day 1 and it decreased gradually over time but did not reach

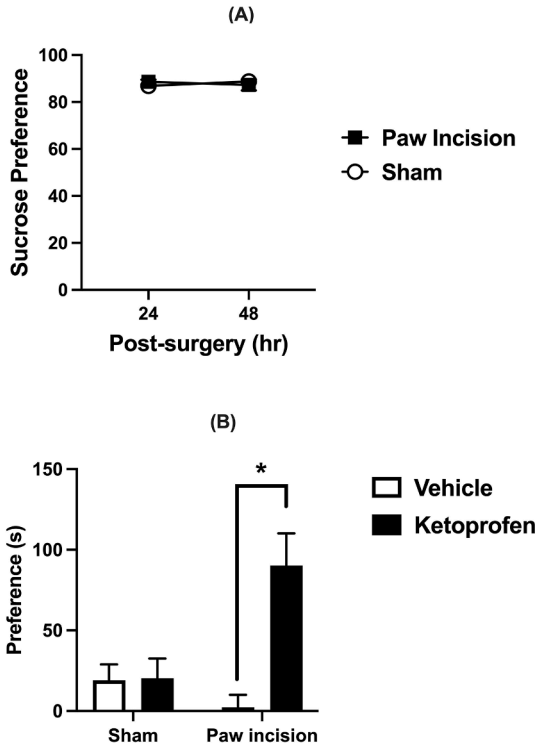


Fig. 3. Paw Incision Increased the Ketoprofen Preference but did not Affect Sucrose Preference. (A) Sucrose preference was measured post-surgery at 24 h and 48 h. Results are presented as the percentage of sucrose preference. Conditioned place preference (CPP) in mice treated with ketoprofen (s.c. 20 mg/kg). **(B)** Results represent data at 24 h post-surgery. Values are expressed as mean ± SEM. n = 8/group. Results were compared using two-way ANOVA with treatment as the factor and post-hoc Dunnett's tes (*p < 0.05 vs vehicle).

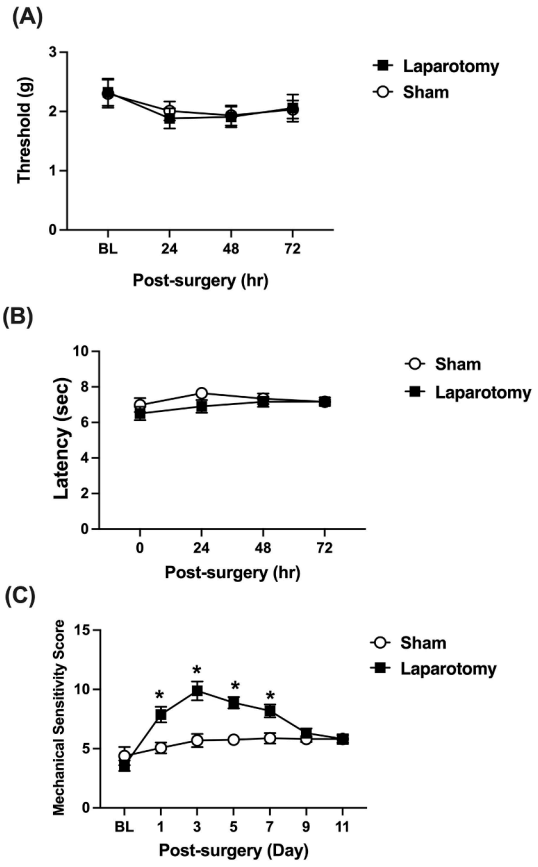


Fig. 4. Effect of Laparotomy on Evoked Behaviors. We observed evoked behaviors for eight days post-surgery **(A)** Mechanical sensitivity was measured by the von Frey test and the results are expressed as the threshold (g) of response. **(B)** Thermal sensitivity was measured by the Hargreaves test and the results are expressed as paw withdrawal latency (s). **(C)** Mechanical abdominal sensitivity was expressed as a sensitivity score**.** Values are expressed as mean ± SEM. n = 8/group. Results were compared using two-way ANOVA with time and treatment as a factor and post-hoc Sidak's test (*p < 0.05 vs vehicle).

its baseline levels at the last day of measurement day 8 (Fig. 1C).

Paw incision did not alter mice rearing behavior after the surgery [Fsurgery (1,22) = 0.5852; P = 0.4524] (Fig. 2A) or their nesting behavior [Fsurgery (1,22) = 0.6381; P = 0.4330] (Fig. 2B). Even though animals' paw were hypersensitive and edematous, their running activity was not changed during the time course [Fsurgery (1,22) = 1.130; P = 0.2993] (Fig. 2C). Burrowing is also commonly seen in the wild across different species and its function serves to create shelters and storage. Paw incision did not affect the latency of burrowing [Fsurgery (1, 22) = 0.0864; P = 0.3547] (Fig. 2D) nor the amount of bedding displaced [Fsurgery (1, 22) = 0.01310; P = 0.9099] (Fig. 2E). However, we found that paw incision had a significant effect on hanging behavior in female mice. The 3-way ANOVA analysis confirms sex as a significant factor in paw incision inducing decrease in hanging behavior [Fsex (1, 144) = 6.646; P = 0.0109] and interaction between sex, surgery, and time [Finteraction (1, 144) = 2.287; P = 0.0491]. We observed a decrease in hanging behavior in female [Fsurgery (1, 12) = 4.750; P = 0.049] (Fig. 2F) but not in male mice [Fsurgery (1, 12) = 2.647; P = 0.1297] (Fig. 2G) 2 h, 6 h and 24 h after surgery [Fsurgery × Time (4, 48) = 7.44; P < 0.0001].

The affective components of post-surgical pain-like behavior were also assessed using the sucrose preference assay (anhedonia-like behavior) and CPP Test (pain relieving-like behavior). Sucrose preference was reported at 24 h and 48 h after surgery. Paw incision did not affect mice sucrose preference compared to sham mice [Fsurgery (1,22) = 0.008443, P = 0.9276] (Fig. 3A).

The CPP test was used in our model of paw incision to evaluate the ability of ketoprofen to induce preference in injured mice. Preference for the drug-paired side could be interpreted as pain-induced motivation to seek ketoprofen (e.g., for pain relief) in mice experiencing ongoing, spontaneous pain. In injured mice and 24 h after incision, injection of ketoprofen (20 mg/kg, s.c) induced a significant preference (p < 0.05) for the ketoprofen-paired side of the CPP chamber [F(surgery × treatment) (1, 76) = 10.68; P = 0.0016; Fsurgery (1, 76) = 4.012; p = 0.0487] (Fig. 3B). Treatment with the vehicle did not show a significant preference in mice. In sham mice, ketoprofen at the same dose (20 mg/kg, s.c.) did not induce a significant preference compared to vehicle-treated mice (Fig. 3B).

Finally, paw incision did not alter animals' body weight [Fsurgery (1, 30) = 0.02113, P = 0.8854] (Supp. Fig. 1A) nor fluid consumption [Fsurgery (1, 22) = 0.06560, P = 0.8002] (Supp. Fig. 1B) after the surgery.

Laparotomy studies

To compare our two incision models, similar tests for mechanical sensitivity and other behavioral assessments were conducted in the laparotomy model. In addition, we measured abdominal mechanical sensitivity in this model. Mechanical and thermal sensitivities of the paws were evaluated after laparotomy surgery to assess if there was any referred evoked nociception in the hindlimbs. The assays showed that laparotomy did not affect hindpaws' mechanical [Fsurgery (1, 18) = 0.7393, P = 0.4012] (Fig. 4A) nor thermal sensitivity [Fsurgery (1, 18) = 0.9460, P = 0.3436] (Fig. 4B). However, laparotomy increased abdominal mechanical sensitivity as compared the sham group till day 7 after surgery [F(Time × Surgery) (6, 180) = 9.963; P < 0.0001);

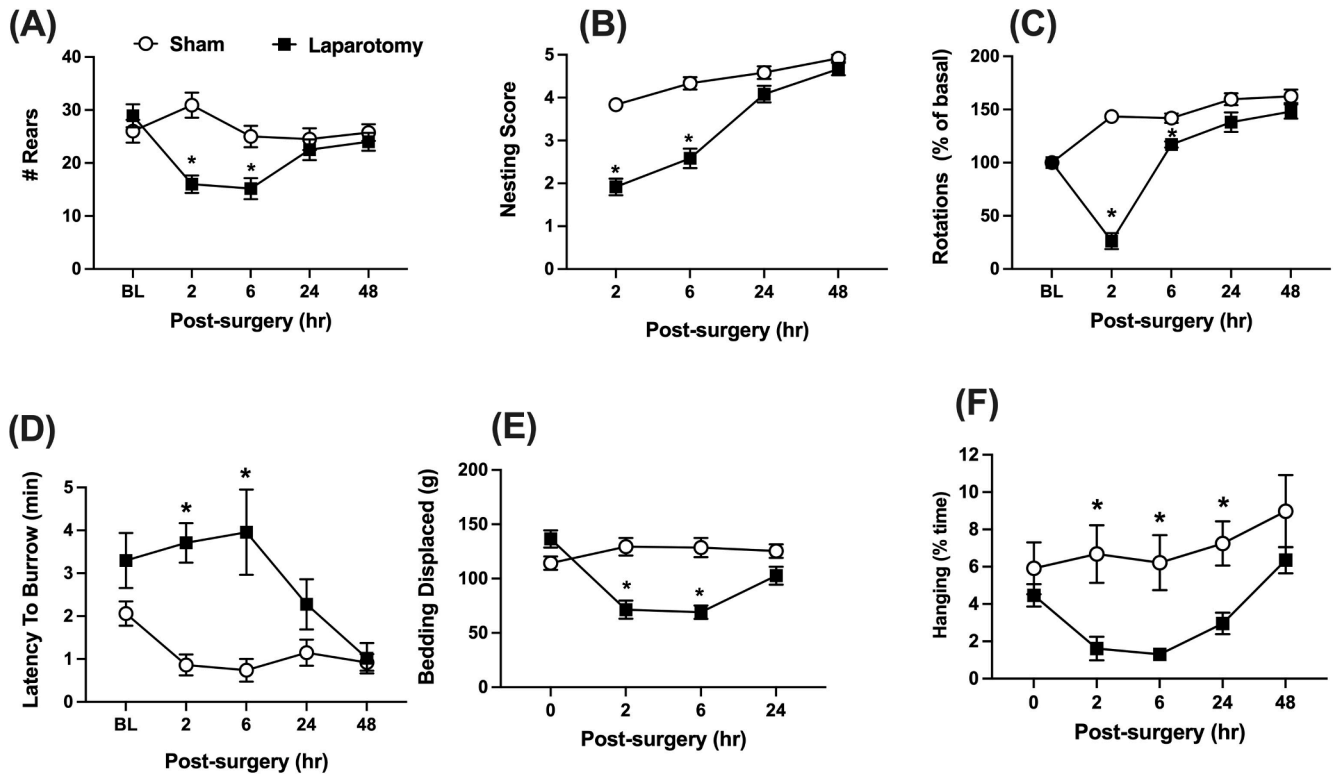


Fig. 5. Effect of Laparotomy on non– Evoked Behaviors. The following non-evoked behaviors were measured post-surgery 2, 6, 24, and 48 h. **(A)** The number of rears, **(B)** nesting score, **(C)** wheel running, **(D)** time to start digging in min, **(E)** amount of removed bedding in grams, and **(F)** percentage of cage hanging time. Values are expressed as mean ± SEM. n = 8/group. Results were compared using two-way ANOVA with treatment as the factor and post-hoc Dunnett's test (*p < 0.05 vs vehicle).

Fsurgery (1, 210) = 39.86; P < 0.0001] (Fig. 4C).

Innate behaviors of mice were affected differently by the laparotomy surgery (Fig. 5). Surgery did have a significant effect on rearing behavior [F(Time × Surgery) (4, 88) = 12.13; P < 0.0001); Fsurgery (1, 22) = 5.9, P = 0.0238] and posthoc analysis revealed animals at 2 h and 6 h post-surgery showed significantly less rearing than the sham group (p < 0.0001) (Fig. 5A). This difference was not observed at other time points (p > 0.05). Nesting behavior was significantly affected by the laparotomy [F(Time × Surgery) (3, 66) = 23.21; P < 0.0001); Fsurgery (1, 22) = 42.27, P < 0.0001] at 2 h and 6 h after post-surgery (p < 0.0001), but its effect was resolved after 24 h (P > 0.05) (Fig. 5B). Laparotomy significantly decreased wheel running behavior [F(Time × Surgery) (4, 88) = 48.89; P < 0.0001); Fsurgery (1, 22) = 29.54, P < 0.0001]. The number of rotations was significantly lower at 2 h (p < 0.0001) post-laparotomy in wheel running but these effects were resolved after 24 h (P > 0.05) (Fig. 5C). Laparotomy increased the latency to burrow [F (Time × Surgery) (4, 110) = 3.47; P = 0.01); Fsurgery (1, 110) = 30.07; P < 0.0001] (Fig. 5D) and decreased the amount of bedding displaced [F (Time × Surgery) (3, 66) = 15.32; p < 0.0001); Fsurgery (1, 22) = 20.26; P = 0.0002] at 2 h and 6 h after post-surgery (p < 0.0001), but its effect was resolved after 24 h (P > 0.05) (Fig. 5E). In addition, we found that laparotomy significantly decreased hanging behavior for up to 24 h post-incision [F(Time × Surgery) (4, 105) = 3.47; P = 0.0072); Fsurgery (1, 105) = 27.00; P < 0.0001] (Fig. 5F).

Laparotomy also induced anhedonia-like behavior as measured in the sucrose preference test [F(Time × Surgery) (1, 22) = 5.80; P = 0.024); Fsurgery (1, 22) = 8.809, P = 0.0071] 24 h after surgery (P = 0.0008) (Fig. 6A).

In injured mice and 24 h after incision, injection of ketoprofen (20 mg/kg, s.c) induced a significant preference (p < 0.05) for the ketoprofen-paired side of the CPP chamber test [F(Time × Surgery) (1, 44) = 18.44; P < 0.0001); FSurgery (1, 44) = 14.86; P = 0.0004] (Fig. 6B). Treatment with the vehicle did not show a significant preference in mice. In sham mice, ketoprofen at the same dose (20 mg/kg, s.c.) did not induce a significant preference compared to vehicle-treated mice (Fig. 6B). However, ketoprofen failed to induce preference in the CPP test in injured mice 7 days after surgery [F(Time × Surgery) (1, 44) = 3.18; P = 0.08); F Surgery(1, 44) = 0.1652; P = 0.6864] (Fig. 6C).

Laparotomy caused a significant decrease in animals' bodyweight [F (Time × Surgery (2, 60) = 9.72; P = 0.002); Fsurgery (1, 30) = 6.265, P = 0.0180] 24 h after surgery (P < 0.0001) but this effect was not observed 48 h after (p > 0.05) (Supp. Fig. 2A). Animals' fluid consumption [F(Time × Surgery) 1, 22) = 0.029; P = 0.866; Fsurgery (1, 22) = 0.1131, P = 0.7398] was not affected by laparotomy (Supp. Fig. 2B).

Discussion

Understanding the various mechanisms responsible for acute post-surgical pain is important and may lead to the development of more effective and safer analgesics to improve the quality of life after surgery. In that regard, preclinical models provide valuable insights into acute pain mechanisms after incisional surgery. Thus, in this study, we compared the two most widely used and relevant surgical models in C57BL/6J female and male mice. Our results show that the effect of incision in both models lasts up to a week on evoked behaviors and the percentage change in paw diameter correlated with hypersensitivity in the paw incision surgery model. The effect of surgery on spontaneous behaviors in both models did not last >24 h. Surgerized mice from both experimental models showed an increased preference for ketoprofen in this time period which indicates that the spontaneous behavior models we chose are effective in measuring pain-related behaviors in laboratory mice.

In this study, the use of evoked behavior tests showed differences in

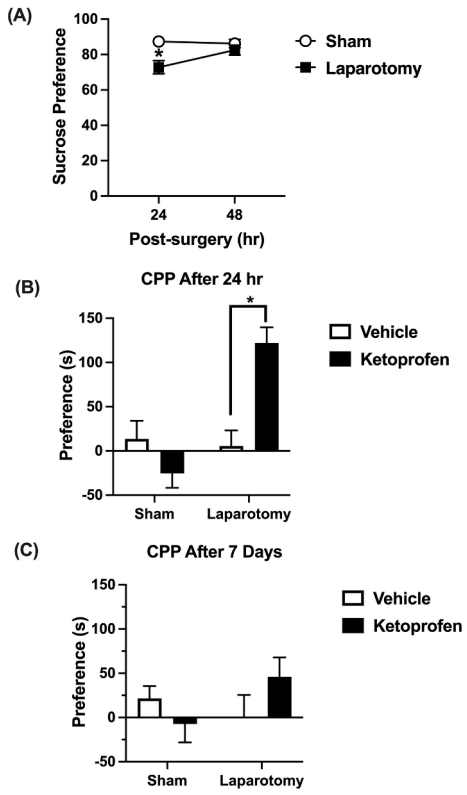


Fig. 6. Laparotomy Increased the Ketoprofen Preference and Decreased Sucrose Preference post-surgery 24 h. (A) Sucrose preference was measured post-surgery 24 and 48 h. Results are presented as the percentage of sucrose preference. Conditioned place preference test (CPP) on mice treated with ketoprofen (s.c. 20 mg/kg). CPP results are given postoperative **(B)** twenty-four hours, and **(C)** seven days. Values are expressed as mean ± SEM. n = 8/group. Results were compared using two-way ANOVA with treatment as the factor and post-hoc Dunnett's test (*$p < 0.05$ vs vehicle).

response between the two surgical models studied. In our paw incision model, we saw an increase in mechanical and thermal hypersensitivity as described by others previously (De Rantere et al., 2016; Pogatzki & Raja, 2003). The time course of thermal and mechanical hypersensitivity showed a correlation with the percentage change in paw diameter which indicates that hypersensitivity might arise from inflammation of the paw [Mechanical sensitivity: $r = -0.9635 \pm (-0.9925$ to -0.8314; $p < 0.0001$) and Thermal sensitivity: $r = 0.9507 \pm (-0.9899$ to -0.7775; $p < 0.0001$)]. Previously, mechanical hypersensitivity was reported followed the laparotomy surgery model on female rats (Dolan & Nolan, 2007; Oliver et al., 2018). However, mice subjected to laparotomy surgery did not show paw thermal or mechanical hypersensitivity. On the other hand, abdominal mechanical hypersensitivity at the region of the incision lasted for 7 days post-surgery. Cata et al. (2021) previously reported abdominal hypersensitivity in rats after laparotomy surgery. However, rats start showing recovery post-surgery day one while mice were hypersensitive up to seven days in our study (Cata et al., 2021). These findings indicate that the nociceptive hypersensitivity was limited to the site of the incision. Finally, we found no sex differences in evoked behaviors in both surgery models.

For spontaneous behavioral changes, we used a battery of innate behaviors to assess the general well-being of rodents, including the latency to burrow and amount of substrate voluntarily burrowed, nesting behavior, and % time spent in a cage hanging and rearing. These behaviors have been suggested to represent the "activities of daily living" in humans. Functional impairment behaviors were assessed using the voluntary wheel running test. Among all the behaviors measured, paw incision only affected the % time spent in the cage hanging in a sex-dependent manner. Female mice hanged significantly less than males at 6 h post-paw incision. We are not aware of any prior studies in mice that assessed spontaneous behaviors after paw incision. However, one study in rats assessed grimacing and mechanical hypersensitivity in the paw incision surgery model. Grimace Scale (GS) is a non-invasive model designed to measure pain via facial expressions and has been widely employed over the past decade (Mogil et al., 2020). In the study, the authors reported that the effect of paw incision on grimacing lasted 9 h post-surgery while hypersensitivity lasts for up to 72 h (De Rantere et al., 2016).

Our laparotomy surgery model significantly decreased all non-evoked behaviors up to 24 h post-surgery in both sexes. Similarly, laparotomy surgery in mice was reported to decrease burrowing (Jirkof et al., 2010), nesting (Oliver et al., 2018), wheel running (Kendall et al., 2016) and rearing (Jirkof et al., 2012; Kendall et al., 2016), but there is no data reported on cage hanging. The difference in spontaneous behaviors between the two surgery models in the present study might have been influenced by the degree of the injury due to the sutured tissue layers. In the paw incision surgery model, the surgery suture side is limited to the skin while in the laparotomy model both abdominal muscles and skin are sutured.

It has been reported previously that laparotomy surgery can induce anhedonia-like behaviors in rats (Cata et al., 2021; Martin et al., 2004). In our study in addition to behavior data, we tested if anhedonia-like behavior would differ between the two surgery models by using the sucrose preference test in mice. Paw incision did not show a significant difference in sucrose preference while there was a significant reduction in the laparotomy group compared to their sham groups. Our sucrose preference data showed a correlation with our non-evoked behavior results as it was reported by other researchers (Cata et al., 2021; Martin et al., 2004).

Conditioned Place Preference (CPP) is a is a Pavlovian conditioning test of reward (Bardo & Bevins, 2000) and is commonly used to evaluate the reinforcing and aversive properties of drugs (Sufka, 1994). CPP had been used for decades by researchers to assess the motivational aspect of inflammatory pain (Sufka, 1994), nerve injury (Neddenriep et al., 2019), and chemotherapy-induced pain models (Caillaud et al., 2021). Nonsteroidal anti-inflammatory drugs (NSAIDs) and mu-opioid receptor agonists are the most commonly used analgesics to treat post-surgery acute pain by clinicians (Gan, 2017). Research shows that injection of NSAIDs (meloxicam) after laparotomy surgery reduces COX-2-mediated inflammation but does not affect GS or behavioral activity in mice (Roughan et al., 2016). In contrast, some studies reported that ketoprofen, an NSAID analgesic, reduces the nesting depression in acute inflammatory pain models (Negus et al., 2015). However, the literature on CPP in surgery models is limited.

As a final experiment, we used CPP to evaluate the preference to ketoprofen, a NSAID, in our surgery models. Mice showed increased preference for ketoprofen in both surgery models 24 h post-surgery. These results indicate that both surgery methods can induce pain aversion, even though paw-incision had no effect on non-evoked behaviors. When we repeated CPP on day seven for the laparotomy group, mice did not show a significant pain-induced preference for ketoprofen even though abdominal mechanical sensitivity was significantly higher compared to the sham group. These results are consistent with previous studies that show a disconnect between the time-course of evoked and non-evoked measures in pain models (Contreras et al., 2021).

Behavioral assessment in rodents may not be consistent across studies since many variables may affect the results such as experimenter, time, and animal handling prior to the test. The technique and the experience of the experimenter are also crucial factors for evoked behaviors while non-evoked behaviors can be affected by stress levels and the general well-being of the mice. All these variables could affect the assessment of pain-related behaviors (Tappe-Theodor & Kuner, 2014). A strength of our study is that we took great caution to minimize the impact of these variables. Surgeries were conducted mostly by the same

person. All behavior tests were done in the same laboratory. Female and male mice were assessed in different test rooms. We used same strain of mice in all of our experiments and kept the same experimental procedures.

Our CPP data shows that preference for ketoprofen is increased post-surgery 24 h in both surgery models. However, we did not assess the effect of ketoprofen in any of our evoked or spontaneous behavior data with our surgery models.

Overall, our results confirm and extend previous reports that in mice that paw incision and laparotomy induce pain-related nociceptive measures. Our evoked behavior data suggest that hypersensitivity of the incision site can last longer than the pain-related depression of spontaneous behaviors. This study therefore improves our knowledge on the relevance of the behavioral tests to be used according to the model studied.

CRediT authorship contribution statement

Esad Ulker: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Martial Caillaud:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Eda Koseli:** Formal analysis, Investigation, Writing – review & editing. **Katherine Contreras:** Formal analysis, Investigation, Writing – review & editing. **Yasmin Alkhlaif:** Investigation, Writing – review & editing. **Eric Lindley:** Investigation, Writing – review & editing. **Mitali Barik:** Investigation, Writing – review & editing. **Sofia Ghani:** Investigation, Methodology, Writing – review & editing. **Camron D. Bryant:** Conceptualization, Writing – review & editing. **M. Imad Damaj:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by funds from VCU School of Medicine and National Institute of Health grant T32 DA007027 to EU.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynpai.2022.100103.

References

Bardo, M.T., Bevins, R.A., 2000. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 153 (1), 31–43. https://doi.org/10.1007/s002130000569.

Brennan, T.J., Vandermeulen, E.P., Gebhart, G.F., 1996. Characterization of a rat model of incisional pain. Pain 64 (3), 493–502. https://doi.org/10.1016/0304-3959(95)01441-1.

Caillaud, M., Patel, N.H., White, A., Wood, M., Contreras, K.M., Toma, W., Alkhlaif, Y., Roberts, J.L., Tran, T.H., Jackson, A.B., Poklis, J., Gewirtz, D.A., Damaj, M.I., 2021. Targeting peroxisome proliferator-activated receptor-α (PPAR- α) to reduce paclitaxel-induced peripheral neuropathy. Brain Behav. Immun. 93, 172–185.

Cata, J.P., Patiño, M., Lacagnina, M.J., Li, J., Gorur, A., Agudelo-Jimenez, R., Wei, B., Hagberg, C.A., Dougherty, P.M., Shureiqi, I., Yang, P., Grace, P.M., 2021. A rat model to investigate quality of recovery after abdominal surgery. PAIN Rep. 6 (2), e943. https://doi.org/10.1097/PR9.0000000000000943.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L., 1994. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods 53 (1), 55–63. https://doi.org/10.1016/0165-0270(94)90144-9.

Contreras, K.M., Caillaud, M., Neddenriep, B., Bagdas, D., Roberts, J.L., Ulker, E., White, A.B., Aboulhosn, R., Toma, W., Khalefa, T., Adel, A., Mann, J.A., Damaj, M.I., 2021. Deficit in voluntary wheel running in chronic inflammatory and neuropathic pain models in mice: Impact of sex and genotype. Behav. Brain Res. 399, 113009.

Cowie, A.M., Stucky, C.L., 2019. A mouse model of postoperative pain. Bio-Protocol 9 (2), e3140. https://doi.org/10.21769/BioProtoc.3140.

De Rantere, D., Schuster, C.J., Reimer, J.N., Pang, D.S.J., 2016. The relationship between the Rat Grimace Scale and mechanical hypersensitivity testing in three experimental pain models. Eur. J. Pain 20 (3), 417–426.

Deacon, R.M.J., 2006. Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. Nat. Protoc. 1 (1), 118–121. https://doi.org/10.1038/nprot.2006.19.

Dolan, S., Nolan, A.M., 2007. Blockade of metabotropic glutamate receptor 5 activation inhibits mechanical hypersensitivity following abdominal surgery. Eur. J. Pain (London, England) 11 (6), 644–651. https://doi.org/10.1016/j.ejpain.2006.10.002.

Gan, T.J., 2017. Poorly controlled postoperative pain: prevalence, consequences, and prevention. J. Pain Res. 10, 2287–2298. https://doi.org/10.2147/JPR.S144066.

Jirkof, P., Cesarovic, N., Rettich, A., Nicholls, F., Seifert, B., Arras, M., 2010. Burrowing behavior as an indicator of post-laparotomy pain in mice. Front. Behav. Neurosci. 4, 165. https://doi.org/10.3389/fnbeh.2010.00165.

Jirkof, P., Cesarovic, N., Rettich, A., Fleischmann, T., Arras, M., 2012. Individual housing of female mice: influence on postsurgical behaviour and recovery. Lab. Anim. 46 (4), 325–334. https://doi.org/10.1258/la.2012.012027.

Kendall, L.V., Wegenast, D.J., Smith, B.J., Dorsey, K.M., Kang, S., Lee, N.Y., Hess, A.M., 2016. Efficacy of sustained-release buprenorphine in an experimental laparotomy model in female mice. J. Am. Assoc. Lab. Anim. Sci. : JAALAS 55 (1), 66–73.

Ladha, K.S., Neuman, M.D., Broms, G., Bethell, J., Bateman, B.T., Wijeysundera, D.N., Bell, M., Hallqvist, L., Svensson, T., Newcomb, C.W., Brensinger, C.M., Gaskins, L.J., Wunsch, H., 2019. Opioid prescribing after surgery in the United States, Canada, and Sweden. JAMA Network Open 2 (9), e1910734.

Martin, T.J., Buechler, N.L., Kahn, W., Crews, J.C., Eisenach, J.C., 2004. Effects of laparotomy on spontaneous exploratory activity and conditioned operant responding in the rat: a model for postoperative pain. Anesthesiology 101 (1), 191–203. https://doi.org/10.1097/00000542-200407000-00030.

Mogil, J.S., Pang, D.S.J., Silva Dutra, G.G., Chambers, C.T., 2020. The development and use of facial grimace scales for pain measurement in animals. Neurosci. Biobehav. Rev. 116, 480–493. https://doi.org/10.1016/j.neubiorev.2020.07.013.

Neddenriep, B., Bagdas, D., Contreras, K.M., Ditre, J.W., Wolstenholme, J.T., Miles, M.F., Damaj, M.I., 2019. Pharmacological mechanisms of alcohol analgesic-like properties in mouse models of acute and chronic pain. Neuropharmacology 160, 107793. https://doi.org/10.1016/j.neuropharm.2019.107793.

Negus, S.S., Neddenriep, B., Altarifi, A.A., Carroll, F.I., Leitl, M.D., Miller, L.L., 2015. Effects of ketoprofen, morphine, and kappa opioids on pain-related depression of nesting in mice. Pain 156 (6), 1153–1160. https://doi.org/10.1097/j.pain.0000000000000171.

Oliver, V.L., Thurston, S.E., Lofgren, J.L., 2018. Using cageside measures to evaluate analgesic efficacy in mice (Mus musculus) after surgery. J. Am. Assoc. Lab. Anim. Sci.: JAALAS 57 (2), 186–201.

Pogatzki, E.M., Raja, S.N., 2003. A mouse model of incisional pain. Anesthesiology 99 (4), 1023–1027. https://doi.org/10.1097/00000542-200310000-00041.

Roughan, J.V., Bertrand, H.G.M.J., Isles, H.M., 2016. Meloxicam prevents COX-2-mediated post-surgical inflammation but not pain following laparotomy in mice. Eur. J. Pain (London, England) 20 (2), 231–240. https://doi.org/10.1002/ejp.712.

Schiene, K., Schröder, W., Linz, K., Frosch, S., Tzschentke, T.M., Christoph, T., Xie, J.Y., Porreca, F., 2019. Inhibition of experimental visceral pain in rodents by cebranopadol. Behav. Pharmacol. 30 (4), 320–326.

Sufka, K.J., 1994. Conditioned place preference paradigm: a novel approach for analgesic drug assessment against chronic pain. Pain 58 (3), 355–366. https://doi.org/10.1016/0304-3959(94)90130-9.

Tappe-Theodor, A., Kuner, R., 2014. Studying ongoing and spontaneous pain in rodents—Challenges and opportunities. Eur. J. Neurosci. 39 (11), 1881–1890. https://doi.org/10.1111/ejn.12643.

Toma, W., Kyte, S.L., Bagdas, D., Alkhlaif, Y., Alsharari, S.D., Lichtman, A.H., Chen, Z.J., Del Fabbro, E., Bigbee, J.W., Gewirtz, D.A., Damaj, M.I., 2017. Effects of paclitaxel on the development of neuropathy and affective behaviors in the mouse. Neuropharmacology. 117, 305–315.

Weiser, T.G., Haynes, A.B., Molina, G., Lipsitz, S.R., Esquivel, M.M., Uribe-Leitz, T., Fu, R., Azad, T., Chao, T.E., Berry, W.R., Gawande, A.A., 2016. Size and distribution of the global volume of surgery in 2012. Bull. World Health Organ. 94 (3), 201–209F. https://doi.org/10.2471/BLT.15.159293.

Zhang, H., Lecker, I., Collymore, C., Dokova, A., Pham, M.C., Rosen, S.F., Crawhall-Duk, H., Zain, M., Valencia, M., Filippini, H.F., Li, J., D'Souza, A.J., Cho, C., Michailidis, V., Whissell, P.D., Patel, I., Steenland, H.W., Virginia Lee, W.-J., Moayedi, M., Sterley, T.-L., Bains, J.S., Stratton, J.A., Matyas, J.R., Biernaskie, J., Dubins, D., Vukobradovic, I., Bezginov, A., Flenniken, A.M., Martin, L.J., Mogil, J.S., Bonin, R.P., 2021. Cage-lid hanging behavior as a translationally relevant measure of pain in mice. Pain 162 (5), 1416–1425.