Title	Characterization of the affective component of acute postoperative pain associated with a novel rat model of inguinal hernia repair pain
Author(s)	Bree, Dara; Moriarty, Orla; Broom, Daniel C.; Kelly, John P.; Roche, Michelle; Finn, David P.
Publication Date	2015-12-10
Publication Information	Bree, Dara, Moriarty, Orla, Broom, Daniel C., Kelly, John P., Roche, Michelle, & Finn, David P. (2016). Characterization of the Affective Component of Acute Postoperative Pain Associated with a Novel Rat Model of Inguinal Hernia Repair Pain. CNS Neuroscience & Therapeutics, 22(2), 146-153. doi: 10.1111/cns.12483
Publisher	Wiley
Link to publisher's version	https://doi.org/10.1111/cns.12483
Item record	http://hdl.handle.net/10379/15082
DOI	http://dx.doi.org/10.1111/cns.12483

Characterisation of the affective component of acute postoperative pain associated with a novel rat model of inguinal hernia repair pain

Running title: Affective component of postoperative in rats

Dara Bree[1,2,3], [+]Orla Moriarty[1,2,3 4], Daniel C. Broom[4], John P. Kelly[1,3], Michelle Roche[2,3], David P. Finn[1,3].

[1]Pharmacology and Therapeutics, [2]Physiology, School of Medicine [3]Galway Neuroscience Centre and Centre for Pain Research, NCBES, National University of Ireland, Galway, Ireland, [4]Research and Development, Covidien, USA

The first two authors contributed equally to this work

Corresponding authors:

Professor David Finn (david.finn@nuigalway.ie), Pharmacology and Therapeutics, and Galway Neuroscience Centre and Centre for Pain Research, NCBES, National University of Ireland Galway, University Road, Galway, Ireland. Tel: +353 (0)91 495280

Dr. Michelle Roche (michelle.roche@nuigalway.ie), Physiology, and Galway Neuroscience Centre and Centre for Pain Research, NCBES, National University of Ireland Galway, University Road, Galway, Ireland. Tel: +353 (0)91 495427

Abstract

Aims: Acute postoperative pain remains a significant healthcare issue. Historically the assessment of postoperative pain in rodents has relied on evoked withdrawal or reflexive measures. Using a recently developed, anatomically relevant rat model of acute postoperative pain [1], the present experiments sought to investigate the affective component of acute postoperative pain associated with inguinal hernia repair.

Methods: Male Lister-Hooded rats underwent surgery to model Lichtenstein inguinal hernia repair (without hernia induction), or a sham procedure. Postsurgical characterisation involved a modified place escape avoidance paradigm (mPEAP), as well as home cage and open field locomotor activity monitoring. In pharmacological validation studies, rats received either morphine, or carprofen prior to mPEAP testing.

Results: Surgery was associated with a significantly increased proportion of the trial duration in the light compartment of the mPEAP arena, in avoidance of the noxious stimulus, compared with sham animals. When re-tested in the mPEAP at Day 7 post-surgery, there was no difference between sham and surgery animals for time spent in either compartment, but surgery animals displayed a persistent increase in the percentage response to noxious stimulation. Morphine and carprofen treatment in surgery animals reduced escape/avoidance behaviour at discrete time points over the trial. Surgery-induced reductions in home cage and open field locomotor activity were also observed.

Conclusion: The present studies report for the first time the characterisation of the affective component of acute postoperative pain using the mPEAP in a rodent model, which may facilitate development of improved understanding and treatment of postoperative pain.

Key Words: Affect; Hernia repair; Postsurgical pain; Place preference; Morphine; Carprofen; Locomotor activity

Introduction

Up to 41% of patients who undergo surgery experience moderate to severe acute postoperative pain despite the use of acute pain management protocols [2]. Inguinal hernia repair is one of the most common surgical procedures [3] with approximately 40% of patients experiencing moderate to severe acute pain in the early hours and days following surgery that can negatively impact on their physical and mental well-being [4-6]. We have recently developed and reported on a novel, anatomically relevant, rat model of acute postoperative pain associated with inguinal hernia repair [1]. Cardinal features of the model include surgery-induced deficits in locomotor activity and activation of c-Fos expression in the dorsal horn of the spinal cord that are reversible upon analgesic administration.

In the present study, we sought to further characterise this novel model of postoperative pain with an emphasis on the affective component of pain. The affective dimension of pain includes pain-related unpleasantness, fear and arousal, with a strong urge to escape or avoid this unpleasantness [7]. The severity of acute postoperative pain can have a significant negative impact on a patients overall well-being, causing increased psychological distress [6,8,9]. In patients who underwent ventral hernia repair 38.7% reported strong affective components associated with their postoperative pain 24 months following surgery [10]. The place escape/avoidance paradigm (PEAP) is a well-validated model of pain affect and aversion in rodents [11,12]. The PEAP is used to dissociate the multidimensional pain experience in rodents by measuring the affective/motivational component of pain processing independent of the sensory component [13,14]. In this paradigm, animals demonstrate avoidance of a preferred test location (dark arena) which is associated with the application of a noxious mechanical stimulus to the injured area. The PEAP has been validated in numerous pain

models including those for inflammatory [15,16] and neuropathic [14,17,18] pain. However, to our knowledge, it has yet to be validated in a rodent model of postoperative pain.

Thus, the initial aim of the present study was to characterise the affective component of acute postoperative pain in the novel rat model of acute postoperative pain associated with inguinal hernia repair using a modified PEAP (mPEAP), further validating it as a model of pain experience. A second aim was to investigate the ability of opioid and non-opioid analgesics to modulate the affective dimension of postoperative pain in this model. Escape/avoidance behaviour in the mPEAP model is amenable to treatment with morphine when administered systemically [14,17] and so was used herein. The non-steroidal anti-inflammatory drug (NSAID) carprofen has not previously been characterised in the PEAP but was efficacious in this model of inguinal hernia repair pain [1] and so was also used herein.

Materials and Methods

Animals

Male Lister-Hooded rats (200-250g on arrival; Charles River, Margate, UK) were used in all studies and were individually housed in clear polycarbonate cages (L x W x H = 480 x 265 x 210 mm; floor area 940 cm^2) containing wood shavings as bedding in a home cage monitoring system (Opto-M3 Dual Axis System, Columbus Instruments, USA). Food and water were available *ad libitum*. Rats were maintained under standard laboratory conditions of temperature (21 ± 3 °C), humidity (45-65%), and lighting (12:12 hour light/dark cycle lights on at 08:00 h). In all experiments, the sequencing of surgery and testing was pseudo-randomised to control for any possible confounding effects of order of testing. The experimental protocols were carried out in accordance with the guidelines and approval of the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under license from the Irish Department of Health and in compliance with the European Communities Council directive 86/609 as well as the ARRIVE guidelines from the National Centre for the Replacement Refinement and Reduction of Animals in Research [19].

Surgery

The surgical procedure employed was based on the most commonly used clinical open-surgery Lichtenstein indirect inguinal hernia repair technique [20-22] and has been described in detail previously [1]. Briefly, rats were anaesthetized using isoflurane and an incision of 3cm in length was made in the right inguinal area (located in the lower abdomen adjacent to the pubic area) through the skin, fascia and muscle. The spermatic cord and surrounding structures were identified, excess fat removed and the spermatic cord and surrounding area was elevated from the posterior wall of the canal with the aid of polyethylene PTFE tubing (OD 0.97mm, ID 0.58mm). A piece of sterile polyester textile mesh (Covidien, USA) was cut to the required

size (2.5cm length and 2cm width) and shape and was implanted on the floor of the canal, sutured to the pubic tubercle with polyprolene 5-0 suture (5-0 Surgipro II, Covidien USA). The tails of the mesh were then crossed and sutured. The ends of the tails were then trimmed and placed into the inguinal canal. The fascia was closed by continuous suture polyprolene 5-0 (5-0 Surgipro II, Covidien USA) and the skin by continuous synthetic absorbable suture (Polysorb 5-0 Covidien USA). Rats in the sham group were anaesthetized, and then left under anesthesia (without any incision) for an identical duration (1 h) to the surgery groups.

Home cage monitoring system

The Opto-M3 Dual Axis system (Columbus Instruments, USA) was used to monitor horizontal and vertical locomotor activity continuously in the home cage environment (25 lux) as described previously [1]. Any differences in home cage activity in the present study were expected to be less than previously reported [1], as the early postoperative period occurred in the light phase as opposed to the dark phase to allow for mPEAP testing.

Open field system

The open field system (Opto-M3 Triple Axis System, Columbus Instruments, USA) was identical to that described previously [1]. The arenas (7 lux) were cleaned with mild detergent and dried to remove odour cues between successive rats. Open field testing was conducted at 1 h prior to mPEAP testing (2 h post-surgery) and again at 7 days post-surgery for the initial characterisation study (Experiment 1) and at 1 h prior to mPEAP testing (2 h post-surgery) for the analgesic study (Experiment 2). Strong surgery-induced deficits in locomotor activity were apparent at 2h post-surgery in previous studies [1] and thus this time point was chosen herein.

Place escape/avoidance paradigm testing

The place escape avoidance paradigm (PEAP) measures and dissociates the multidimensional pain experience in rodents [12] and we used a modified version of this paradigm (mPEAP). Our arena consisted of a 64 x 31 x 32cm wooden arena positioned on top of a wire mesh. The arena is comprised of a light side (lux 100) and a dark side with a central wooden partition with a small opening allowing unrestricted movement between the two sides during the 30 minute behavioural test. To begin, the animal is placed into the light arena, facing away from the dark chamber. When in the dark chamber, the animal receives a noxious stimulation (6g von Frey filament applied for 5s, or until a response is observed), to the site of incision in the inguinal area at an interval of every 15s. No stimulation was applied when the animal was in the light chamber. This is a slight modification on the traditional PEAP protocol which is used in conjunction with paw-directed pain models and where the contralateral paw is traditionally stimulated in the light arena. The decision not to stimulate the animals when in the light arena was taken for a number of reasons. First, previous von Frey filament studies in this model suggested that stimulation of the contralateral side of the inguinal area may have been noxious/aversive in itself and might have confounded the mPEAP results. Secondly, we reasoned that sham animals would serve as the more important control in this experiment rather than contralateral stimulation in surgery animals, as sham animals are devoid of any incision or injury and thus should display no injury-related mechanical hypersensitivity. The 6g von Frey filament was chosen as a noxious stimulus, based on pilot work which demonstrated that it consistently elicited a response in both sham and surgery animals. A response was defined as a jump, scratch, or biting/licking of stimulated area. The behaviour of the animal was recorded using a camera placed above the light-side chamber and time that the animal spent in the light chamber was subsequently measured. Whether or not the animal responded to the

noxious stimulus was also recorded during the trial and a percentage response for each animal was subsequently calculated. 24 h prior to surgery, the animals were placed into the arena and allowed to freely explore it for 30 min without receiving any stimulation. Place escape/avoidance testing was performed at 3 h and 7 days after inguinal hernia repair surgery for the initial characterisation study (Experiment 1) and at 3 h post-surgery for the analgesic study (Experiment 2).

Drugs

Carprofen (Pfizer, Ireland) at a dose of 5 mg/kg or saline vehicle, were administered subcutaneously (s.c.) in a volume of 1ml/kg 1 hour prior to surgery (4 h prior to mPEAP testing). Morphine sulphate (Antigen Pharmaceuticals, Ireland) or saline vehicle were administered s.c at a dose of 3 mg/kg 2 h and 30 min post-surgery (30 minutes prior to mPEAP testing) in a volume of 1 ml/kg. Drug doses and times of administration were based on the pharmacokinetics of the drugs, previous work with the hernia pain model [1] as well published studies demonstrating their efficacy in animal studies modelling the affective component of pain processing [14,17]. Three rats that received morphine had to be excluded from the final analysis as they displayed stereotyped behaviours such as biting and licking of the wooden partition and wire floor. This was to such an extent that it is very likely to have distracted the rats' attention away from the noxious von Frey stimulus, thereby resulting in a lack of response to the noxious stimulus and hence these rats were excluded from the analysis.

Experimental protocol

Two experiments were carried out, namely, an initial characterisation study (Experiment 1) and an analgesic study (Experiment 2). For the initial characterisation study there were two groups: sham (n=10) and surgery (n=8). For the analgesic study there were 6 treatment groups in total; sham saline (n=8), sham morphine 3mg/kg (n=7), sham carprofen 5mg/kg (n=7), surgery saline (n=7), surgery morphine 3mg/kg (n=9) and surgery carprofen 5mg/kg (n=7). For both experiments, rats were randomly assigned to each group. For the initial characterisation experiment, animals were monitored in the home cage throughout the entire duration of the study. Open field testing was conducted at 2 h and 7 days post-surgery. mPEAP testing was performed at 3h and 7 days post-surgery. For the analgesic study, animals were again monitored in their home cage for the entire duration of the study and were subject to open field testing at 2 h and testing in the mPEAP at 3 h post-surgery. Animals were killed by either decapitation or transcardial perfusion (heparinised saline followed by 4% paraformaldehyde in a phosphate buffered saline solution) at 8 days post-surgery for the initial characterisation study and at 3 h post-surgery for the analgesic study. A male experimenter performed all surgical procedures and open field testing while a female experimenter conducted all mPEAP testing. The experimental protocol for both studies is depicted in Figure 1.

Data analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS Inc., USA). Data were analysed using parametric statistics if they were found to follow a normal or approximately normal distribution within group (Shapiro-Wilks $p>0.05$) and if the between-group variances were homogeneous or approximately homogeneous (Levene's Test $p>0.05$). If data were found to follow a normal distribution, repeated measures analysis of variance (ANOVA) was performed to determine main effects of time, surgery or drug treatment, or their interactions. Fisher's least significant difference (LSD) *post-hoc* tests or unpaired two-tailed t-tests with or

without Bonferroni's correction were used as appropriate to determine where the differences lay between the groups and across time points. Non-parametric data were analysed by Friedman's ANOVA by ranks or Kruskal-Wallis tests, followed by Mann-Whitney *U* test or Wilcoxon signed-ranks test. Data are presented as mean + SEM. $p<0.05$ was considered statistically significant. Results were depicted graphically with the aid of GraphPad Prism 5.0 (GraphPad Software, USA).

Results

Experiment 1: Characterisation of the effects of inguinal hernia repair surgery in the mPEAP

Percentage time spent in the light compartment

The entire 30 min trial duration was split into 5 min bins and the percentage time spent in the light compartment was calculated for each interval and subsequently plotted.

At 3 h post-surgery, there was a significant main effect of time ($F_{5,80}=11.97, p<0.001$), surgery ($F_{1,60}=47.74, p<0.001$) and a significant time x surgery interaction ($F_{1,16}=20.53, p<0.001$) on the percentage time spent in the light compartment (Figure 2A). The surgery group spent a significantly higher percentage of time in the light compartment compared to the sham group at the 20 min, 25 min and 30 min time points ($p<0.05$), confirming a higher degree of aversion to noxious stimulation of the inguinal area in the surgery group compared with the sham group. On day 7 post-surgery, no differences were observed between the groups at any of the time points (Figure 2B).

Percentage response to noxious stimulation

Student's unpaired two-tailed t-test was used to compare the percentage response to the noxious stimulus applied in the dark between the two groups over the entire 30 min trial. At 3 h post-surgery, the surgery group had a significantly increased percentage response when compared to the sham control group ($t_{(16)}=3.9$, $p<0.01$) (Figure 3A). On day 7 post-surgery the surgery group also had a significantly increased percentage response compared to the sham control group ($t_{(16)}=4.96$, $p<0.01$) (Figure 3B).

Assessment of locomotor activity following surgery

Home cage activity following surgery

For the initial mPEAP characterisation study, home cage activity was analysed during the period 1-3 h post-surgery, as previous work has demonstrated that this time frame is where the peak effects of surgery on locomotor activity is most apparent [1]. Analysis focused on vertical activity as it proved to be the most robust behavioural phenotype associated with the model [1].

Student's unpaired two-tailed t-test was used to compare home cage vertical activity during the period 1-3h post-surgery before mPEAP testing. The surgery group displayed significantly decreased levels of vertical activity compared to the sham group ($t_{(16)}=3.3$, $p<0.01$) (Figure 4).

Open field activity following surgery

Animals underwent open field testing before each of the mPEAP trials in order to confirm that the previously characterised behavioural phenotype associated with this model, surgery-induced deficits in open field locomotor activity, were apparent in this study. Similar to home cage activity, analysis focused on vertical activity.

Repeated measures ANOVA revealed a significant effect of time ($F_{(1, 16)}=56.91$, $p<0.001$), surgery ($F_{(1, 16)}=4.41$, $p<0.05$) and a time x treatment interaction ($F_{(1, 16)}=11.71$, $p<0.05$) on open field vertical activity following surgery. *Post-hoc* analysis revealed that the behavioural phenotype associated with the model was again apparent at 2h post-surgery, with the surgery group displaying significantly decreased levels of activity compared to the sham group ($p<0.01$). There was no difference between the groups at 7 days post-surgery (Figure 5).

Experiment 2: Effect of morphine and carprofen on mPEAP testing following surgery

Percentage time spent in the light compartment

The entire 30 min trial was split into 5 minute bins and the percentage time spent in the light compartment was calculated for each time bin and subsequently plotted.

Two-way repeated measures ANOVA with surgery and drug as factors, revealed a significant effect of time ($F_{(5, 195)}=10.55$, $p<0.001$) and a time x drug treatment interaction ($F_{(10, 195)}=2.41$, $p<0.05$) but no main effects of surgery ($F_{(1, 39)}=1.93$, $p>0.05$) or drug treatment ($F_{(2, 39)}=1.55$, $p>0.05$) on the percentage time spent in the light compartment (Figure 6). *Post-hoc* analysis

revealed that the surgery saline group spent a significantly greater percentage of time in the light compartment compared to the sham saline group at the 4th and 6th time bins (p<0.05). Percentage time in the light compartment was significantly lower in the surgery morphine group compared to the surgery saline group at the 4th time bin (p<0.01). Like morphine, carprofen also attenuated surgery-induced increases in percentage time spent in the light compartment at the 4th (p<0.01) and 6th (p<0.05) time bins where the surgery carprofen group exhibited significantly lower percentage time in the light compartment compared to the surgery saline group. There was no significant effect of drug treatment on time in the light compartment for sham animals (Figure 6).

Percentage response to noxious stimulation

Two-way ANOVA revealed a significant effect of surgery ($F_{(1, 41)}$=29.93, p<0.05) and drug treatment ($F_{(2, 41)}$=5.49, p<0.05) on the percentage response to noxious stimulation during the mPEAP trial. *Post-hoc* analysis revealed that the surgery saline group displayed an increased percentage response compared to the sham saline group (p<0.01). Morphine reduced the percentage response to noxious stimulation irrespective of surgery, with both the sham morphine group and the surgery morphine group displaying a decreased percentage response, compared to respective saline-treated controls (p<0.01) (Figure 7).

Assessment of home cage and open field locomotor activity following surgery

For the mPEAP analgesic study (Experiment 2), home cage activity was analysed for the period 1-3h post-surgery only, owing to the short duration of the post-surgical period in this

experiment, as well as being the period where surgery-induced deficits in locomotor activity are most apparent. Analysis focused on vertical activity.

A Kruskal-Wallis test revealed an overall significant effect between all groups ($K=22.33$, $p<0.001$) on home cage vertical activity during the period 1-3h post-surgery. Mann-Whitney U tests revealed that the surgery saline group displayed significantly reduced activity compared to the sham saline group ($p<0.01$), while the surgery 5mg/kg carprofen group displayed significantly increased activity compared to the surgery saline group ($p<0.01$), indicating an attenuation of the surgery-induced locomotor deficits (Figure 8). Morphine had no significant effects in sham or surgery animals which was not unexpected given that it was administered 2.5 h post-surgery.

Surgery-induced reductions in OF activity were observed as for Exp 1 but carprofen had no effect and morphine was administered after OF testing (data not shown).

Discussion

This manuscript reports the behavioural and pharmacological characterisation of the affective dimension of postoperative pain following inguinal hernia repair using the mPEAP. In the mPEAP, the aversive/unpleasant nature of pain is measured by the avoidance of a preferred location i.e. the dark chamber, and an increase in the amount of time spent in the light chamber reflects the degree of aversion to noxious stimulation [12]. At 3 h post-surgery, animals displayed a significantly increased percentage time in the light compartment, choosing to reside in the aversive light chamber within 20 minutes of test onset and spending nearly 100% of their

time in that compartment by the end of the trial. These results correlate strongly with the results obtained with rodent models of pain in the PEAP [12,14]. Systemic administration of either morphine or carprofen decreased the time spent in the light arena at 3 h post-surgery at discrete time points during the 30 min trial. The percentage response to noxious stimulation was significantly increased in surgery animals compared to sham controls at 3 h post-surgery and also at 7 days post-surgery. However, there was no difference in time spent in the light chamber between the two groups at day 7, suggesting that the sensory-discriminative response to noxious stimulation persisted up to 7 days post-surgery, while the affective-motivational dimension did not.

Three major nerves innervate the inguinal area; ilioinguinal, iliohypogastric and genitofemoral, therefore it is possible that the inguinal area is more sensitive to mechanical stimulation than the plantar surface of the hindpaw in the absence of any intervention, making sham animals more likely to respond to any stimulation, noxious or non-noxious. This may explain the high response in sham animals at both 3 h and 7 days post-surgery, approximately 70% in both cases. Previous PEAP studies have reported a lower response to stimulation of uninjured hindpaw with the use of a noxious von Frey stimulus [15,16,23], but to our knowledge this is the first study to investigate stimulation of the inguinal area. Indeed the decision not to stimulate the contralateral side in the mPEAP was taken with the above reason in mind. The lack of avoidance of the stimulus observed in surgery animals at 7 days post-surgery could be because the stimulus may not have been of sufficient intensity to drive the animals into the aversive light context after such a long post-surgery recovery period, i.e. at 7 days post-surgery, test animals found the 6g von Frey stimulus less unpleasant, or they were better able to tolerate it than at 3 h post-surgery, where active avoidance was observed. In addition, it could be explained by the observation that animals that undergo surgery display acute pain-related

anxiety in the early postoperative period [24,25]. In the case of the mPEAP, such pain-related fear-avoidance may exacerbate a painful response to a noxious stimulus in the early hours following surgery and hence the active avoidance of the stimulus. However by day 7, the pain-related fear avoidance may have dissipated and active avoidance is not observed suggesting no anxiety–induced exacerbation of pain. Exacerbation of pain by anxiety has been well documented in both the clinical [26,27] and pre-clinical [28,29] literature.

Our results demonstrate that both morphine and carprofen were efficacious during the mPEAP trial, significantly decreasing the time spent in the light arena at discrete time points during the 3h post-surgery test period. These data indicate the ability of these analgesics to attenuate the affective component of acute postoperative pain in this model. These results are in agreement with previous reports which have demonstrated attenuated escape/avoidance behaviour with morphine [14,30]. Morphine has traditionally been the gold standard for postoperative analgesia and is often used as a comparator with which putative analgesics are assessed [31,32]. No overt effects of drug treatment were observed on mPEAP performance in sham animals. Thus, although time spent in the light compartment was not completely attenuated by either drug, both do modulate affective behavioural responding in this model at discrete time points and further characterise it as a novel model of acute postoperative pain with both somatic and affective pain dimensions. Morphine also reduced the percentage response to noxious stimulation in both sham and surgery animals compared to corresponding controls, suggesting that the 3mg/kg dose was effective in relieving both the sensory and affective dimensions of pain. Carprofen failed to attenuate mechanical hyperalgesia but did reduce time spent in the light area in the mPEAP. This dissociation of the analgesic effects of carprofen on somatic and affective dimensions of pain have been observed previously in the mPEAP with another NSAID, aspirin [15]. Indeed an attenuation of the affective/motivational component of pain

has consistently been observed at lower doses of analgesics than that required for mechanical hypersensitivity in the mPEAP [13,14,18,30], suggesting that the paradigm is primarily sensitive to the affective dimension of pain.

The inguinal hernia repair model of acute postoperative pain produced a robust and reproducible behavioural response in the mPEAP, as evidenced by an increased time spent in the light compartment in surgery animals in both experiment 1 and 2. When the magnitude and duration of effect in the mPEAP for this model of acute pain is compared to more chronic neuropathic and inflammatory models, a number of differences are apparent. In a model of L5 spinal nerve ligation (SNL), differences in duration spent in the light arena between sham and SNL groups emerged much sooner, after only 5 minutes into the test and persisted for the entire 30 minute trial duration [12]. This is in contrast to the model reported here, where differences emerged between the sham and surgery groups 20 minutes into the trial. Similar effects to that seen with SNL have also been reported in other neuropathic [14,18] and chronic inflammatory pain models [12,15]. All of these models report an earlier emergence and greater magnitude of difference (particularly in the early part of the trial) between sham and injured groups compared to the model of inguinal hernia repair pain reported herein. However it should be noted that by the end of the mPEAP trial, inguinal hernia repair animals are spending 100% of their time in the light compartment, similar to those following neuropathic and inflammatory procedures.

Locomotor activity following surgery was also assessed to confirm the presence of the surgery-induced deficits in activity which is a hallmark of this model [1]. For the present study, animals were placed on a traditional light-dark cycle (lights on at 0800 h) in their home cage as this was more compatible with mPEAP testing which was performed at 100 lux and during the

animals' light phase. This contrasts with our previous report on the model [1] where animals were kept on a reverse light-dark cycle to provoke a high baseline of locomotor activity in the early postoperative period, thereby giving a bigger window over which to see surgery-induced locomotor deficits. Open field testing was carried out as described previously and performed under dim light (7 lux). The surgery-induced deficits in both home cage and open field vertical locomotor activity observed previously [1] were also evident in the present study, confirming that this phenotype is retained on a normal light:dark cycle. The surgery-induced reduction in home cage activity was attenuated by carprofen, corroborating our previous results [1] and confirming that the surgery-induced locomotor impairment is pain-related.

The PEAP was developed to help dissociate the complex and multidimensional nature of pain and to address limitations associated with the use of traditional tests for sensory hypersensitivity. The major benefit of the paradigm is that it requires no pre-training and measures drug effects on the acquisition of behaviour [13]. However, the paradigm is not without its caveats, an important one being that a noxious stimulus still has to be applied to the injured area to initiate the affective conditioning of the pain response [14]. As a result, it is not possible to conclusively rule out that analgesic-induced manipulation of mPEAP behaviour is not solely due to effects on the sensory dimension of the pain experience – unless the analgesic affects time spent in the light compartment without affecting the sensory hypersensitivity response, as was the case for carprofen herein. In conclusion, the novel findings described here expand upon the characterisation of this model of acute postoperative pain to include an affective dimension. An affective dimension of postoperative pain was previously demonstrated using a Conditioned Place Preference paradigm in the hindpaw incision model [33]. However the present study demonstrates for the first time an affective dimension of acute postoperative pain in rodents using the mPEAP. With a significant proportion of patients

experiencing moderate to severe postoperative pain up to 7 days post-surgery that negatively affects their physical and mental well-being [6], this study may help to better explain the mechanisms underlying the multidimensional nature of acute postoperative pain.

Acknowledgements

This project was funded by a grant from the Irish Industrial Development Agency in partnership with Covidien.

Disclosure: D.C.B. and O.M. were employees of Covidien, a commercial sponsor of the study. The other authors have no conflicts of interest to declare.

References

1. Bree D, Moriarty O, O'Mahony CM, Morris B, Bannerton K, Broom DC, et al. Development and characterization of a novel, anatomically relevant rat model of acute postoperative pain. J Pain. 2015;**16**:421-35 e6.

2. Dolin SJ, Cashman JN, Bland JM. Effectiveness of acute postoperative pain management: I. Evidence from published data. Br J Anaesth. 2002;**89**:409-23.

3. Jenkins JT, O'Dwyer PJ. Inguinal hernias. BMJ. 2008;**336**:269-72.

4. Massaron S, Bona S, Fumagalli U, Battafarano F, Elmore U, Rosati R. Analysis of post-surgical pain after inguinal hernia repair: a prospective study of 1,440 operations. Hernia. 2007;**11**:517-25.

5. McGrath B, Elgendy H, Chung F, Kamming D, Curti B, King S. Thirty percent of patients have moderate to severe pain 24 hr after ambulatory surgery: a survey of 5,703 patients. Can J Anaesth. 2004;**51**:886-91.

6. Taylor RS, Ullrich K, Regan S, Broussard C, Schwenkglenks M, Taylor RJ, et al. The impact of early postoperative pain on health-related quality of life. Pain practice : the official journal of World Institute of Pain. 2013;**13**:515-23.

7. Melzack RaCKL. Sensory, motivational, and central control determinants of pain: A new conceptual model. In: Thomas CC, editor. The Skin Senses. Springfield, IL: In Kenshalo, D.; 1968. p. 423-43.

8. Ali M, Winter DC, Hanly AM, O'Hagan C, Keaveny J, Broe P. Prospective, randomized, controlled trial of thoracic epidural or patient-controlled opiate analgesia on perioperative quality of life. Br J Anaesth. 2010;**104**:292-7.

9. Levy BF, Scott MJ, Fawcett W, Fry C, Rockall TA. Randomized clinical trial of epidural, spinal or patient-controlled analgesia for patients undergoing laparoscopic colorectal surgery. Br J Surg. 2011;**98**:1068-78.

10. Gronnier C, Wattier JM, Favre H, Piessen G, Mariette C. Risk factors for chronic pain after open ventral hernia repair by underlay mesh placement. World J Surg. 2012;**36**:1548-54.

11. Sufka KJ. Conditioned place preference paradigm: a novel approach for analgesic drug assessment against chronic pain. Pain. 1994;**58**:355-66.

12. LaBuda CJ, Fuchs PN. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. Experimental neurology. 2000;**163**:490-4.

13. Boyce-Rustay JM, Zhong C, Kohnken R, Baker SJ, Simler GH, Wensink EJ, et al. Comparison of mechanical allodynia and the affective component of inflammatory pain in rats. Neuropharmacology. 2010;**58**:537-43.

14. Pedersen LH, Blackburn-Munro G. Pharmacological characterisation of place escape/avoidance behaviour in the rat chronic constriction injury model of neuropathic pain. Psychopharmacology. 2006;**185**:208-17.

15. LaBuda CJ, Fuchs PN. Low dose aspirin attenuates escape/avoidance behavior, but does not reduce mechanical hyperalgesia in a rodent model of inflammatory pain. Neurosci Lett. 2001;**304**:137-40.

16. Uhelski ML, Fuchs PN. Naltrexone fails to increase pain affect in response to inflammatory pain in a novel escape/avoidance paradigm. Physiology & behavior. 2009;**98**:263-7.

17. LaBuda CJ, Fuchs PN. Morphine and gabapentin decrease mechanical hyperalgesia and escape/avoidance behavior in a rat model of neuropathic pain. Neurosci Lett. 2000;**290**:137-40.

18. Baastrup C, Jensen TS, Finnerup NB. Pregabalin attenuates place escape/avoidance behavior in a rat model of spinal cord injury. Brain Res. 2011;**1370**:129-35.

19. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS biology. 2010;**8**:e1000412.

20. Amid PK, Shulman AG, Lichtenstein IL. The Lichtenstein open "tension-free" mesh repair of inguinal hernias. Surg Today. 1995;**25**:619-25.

21. Amid PK. The Lichtenstein repair in 2002: an overview of causes of recurrence after Lichtenstein tension-free hernioplasty. Hernia. 2003;**7**:13-6.

22. Lichtenstein IL, Shulman AG, Amid PK, Willis PA. Hernia repair with polypropylene mesh. An improved method. AORN J. 1990;**52**:559-65.

23. LaBuda CJ, Fuchs PN. Attenuation of negative pain affect produced by unilateral spinal nerve injury in the rat following anterior cingulate cortex activation. Neuroscience. 2005;**136**:311-22.

24. Li CQ, Zhang JW, Dai RP, Wang J, Luo XG, Zhou XF. Surgical incision induces anxiety-like behavior and amygdala sensitization: effects of morphine and gabapentin. Pain research and treatment. 2010;**2010**:705874.

25. Roeska K, Doods H, Arndt K, Treede RD, Ceci A. Anxiety-like behaviour in rats with mononeuropathy is reduced by the analgesic drugs morphine and gabapentin. Pain. 2008;**139**:349-57.

26. Ploghaus A, Narain C, Beckmann CF, Clare S, Bantick S, Wise R, et al. Exacerbation of pain by anxiety is associated with activity in a hippocampal network. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2001;**21**:9896-903.

27. Asmundson GJ, Taylor S. Role of anxiety sensitivity in pain-related fear and avoidance. Journal of behavioral medicine. 1996;**19**:577-86.

28. Greenwood-Van Meerveld B, Gibson M, Gunter W, Shepard J, Foreman R, Myers D. Stereotaxic delivery of corticosterone to the amygdala modulates colonic sensitivity in rats. Brain Res. 2001;**893**:135-42.

29. Roeska K, Ceci A, Treede RD, Doods H. Effect of high trait anxiety on mechanical hypersensitivity in male rats. Neurosci Lett. 2009;**464**:160-4.

30. LaGraize SC, Borzan J, Peng YB, Fuchs PN. Selective regulation of pain affect following activation of the opioid anterior cingulate cortex system. Experimental neurology. 2006;**197**:22-30.

31. McEvoy A, Livingstone JI, Cahill CJ. Comparison of diclofenac sodium and morphine sulphate for postoperative analgesia after day case inguinal hernia surgery. Annals of the Royal College of Surgeons of England. 1996;**78**:363-6.

32. Pettersson N, Berggren P, Larsson M, Westman B, Hahn RG. Pain relief by wound infiltration with bupivacaine or high-dose ropivacaine after inguinal hernia repair. Reg Anesth Pain Med. 1999;**24**:569-75.

33. Navratilova E, Xie JY, Okun A, Qu C, Eyde N, Ci S, et al. Pain relief produces negative reinforcement through activation of mesolimbic reward-valuation circuitry. Proc Natl Acad Sci U S A. 2012;**109**:20709-13.

Figure legends

Figure 1 Experimental protocol for PEAP characterisation experiments.

Figure 2 Percentage time spent in the light compartment (A) 3 h post-surgery and (B) 7 days post-surgery during the mPEAP trial. Data are mean + SEM (n=8-10). ** p<0.01 vs. sham.

Figure 3 Percentage response to noxious von Frey hair stimulation during the 30 min mPEAP trial at (A) 3 h post-surgery and (B) 7 days post-surgery. Data are mean + SEM (n=8-10). ***p*<0.01 vs. sham.

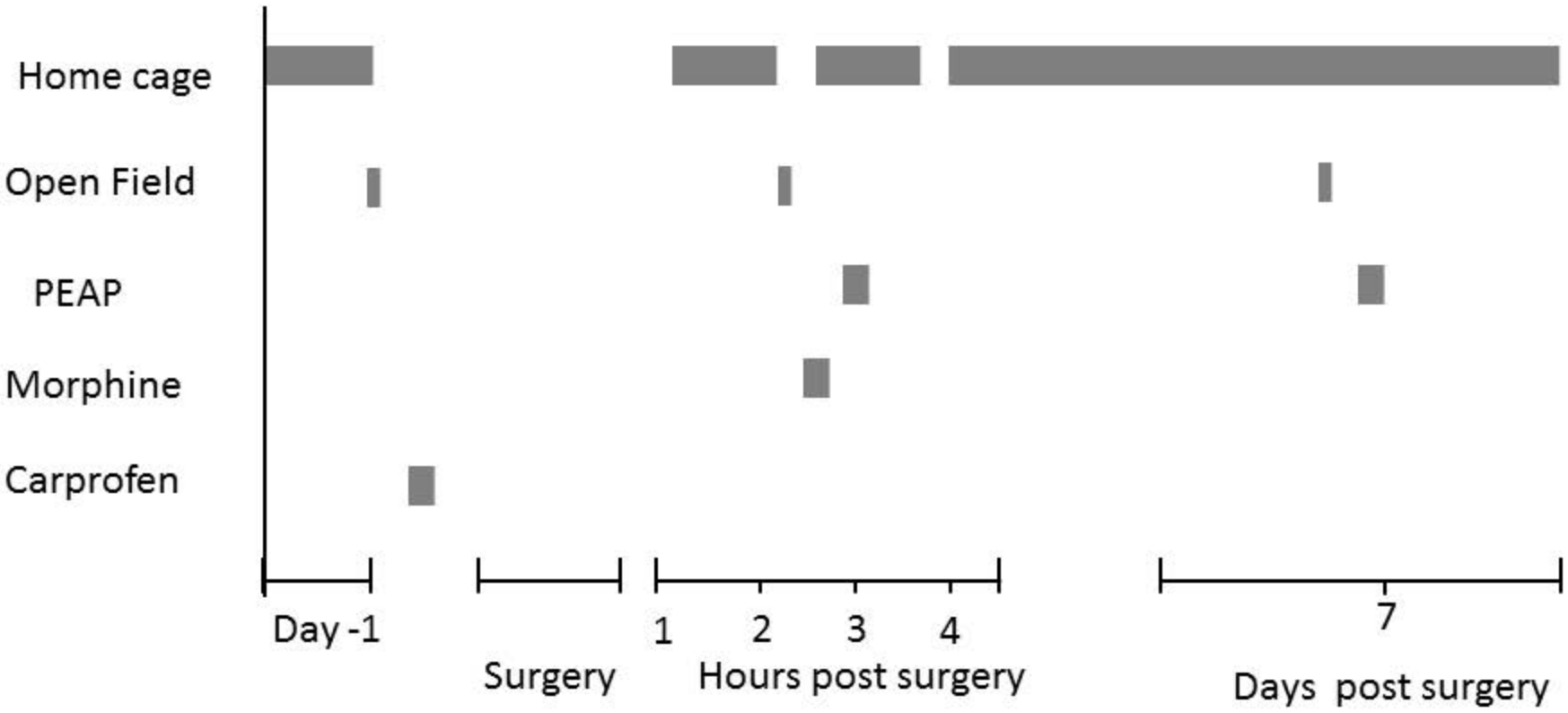
Figure 4 Effect of inguinal hernia repair surgery on home cage vertical activity during the period 1-3 h post-surgery. Data are mean + SEM (n=8-10). ***p*<0.01 sham vs. surgery.

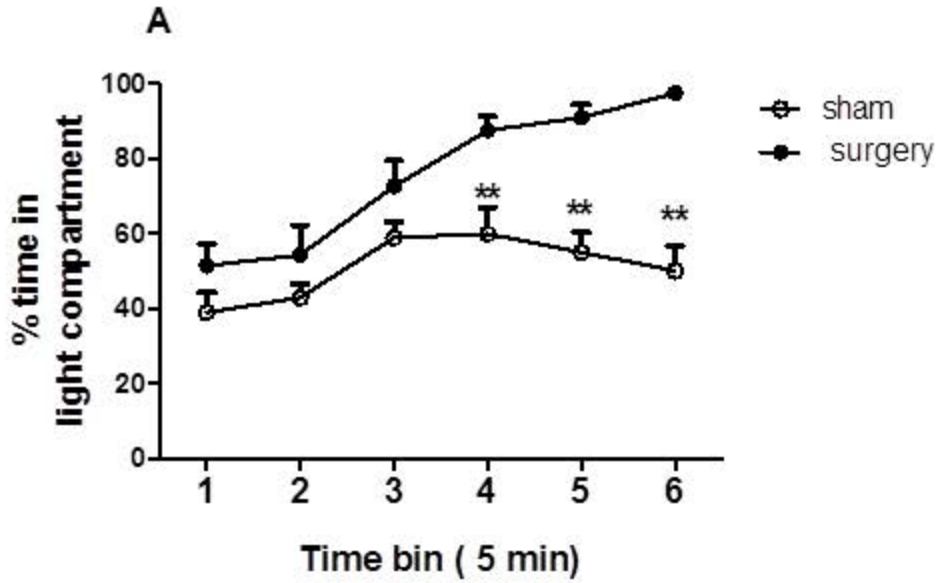
Figure 5 Effect of inguinal hernia repair surgery on vertical activity in the open field at 2 h or 7 days following surgery. Data are mean + SEM (n=7-9). **p*<0.05, ***p*<0.01 sham vs. surgery.

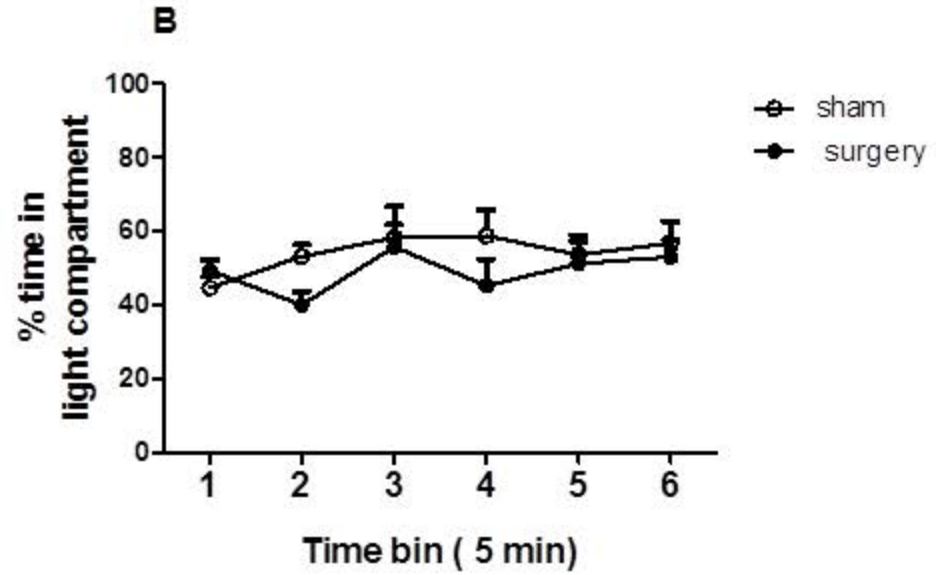
Figure 6 Effect of morphine (Morph) and carprofen (Carp) on the percentage time spent in the light compartment during the mPEAP trial in sham and surgery rats. Data are mean + SEM (n=7-9). * p<0.05 sham saline vs. surgery saline; ##p<0.01 surgery morph vs. surgery saline; +p<0.05, ++p<0.01 surgery carprofen vs. surgery saline.

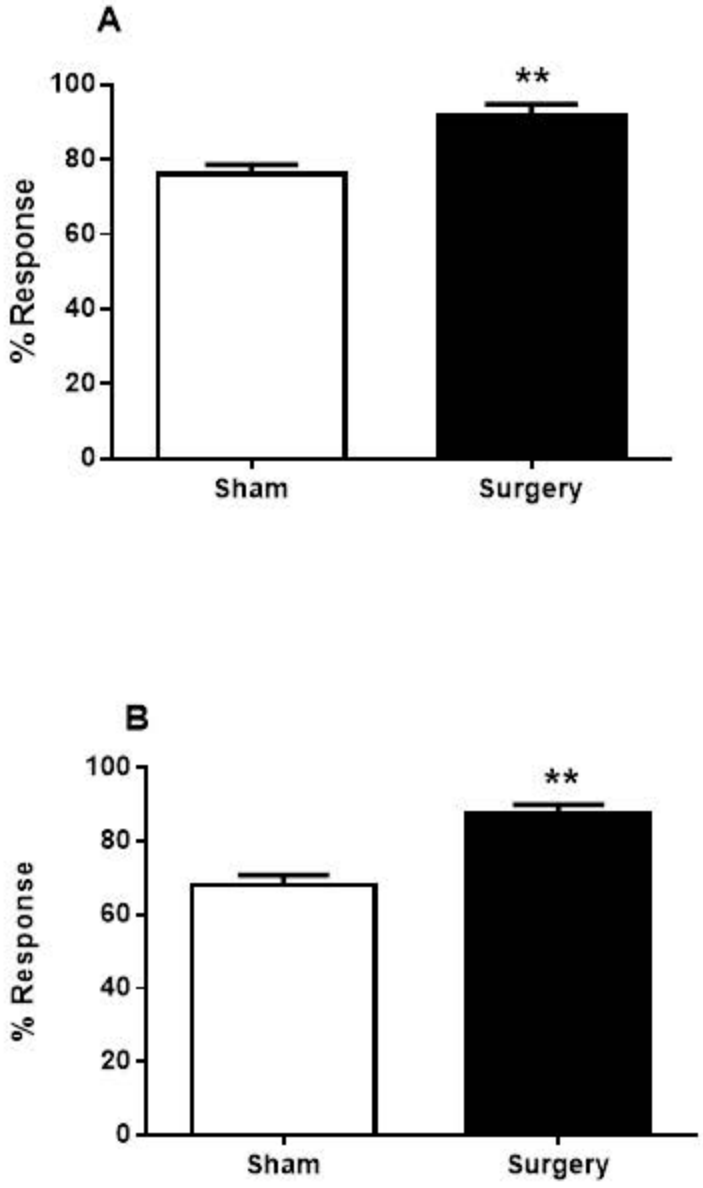
Figure 7 Effect of morphine and carprofen on the percentage response to noxious von Frey hair stimulation following surgery during mPEAP trial. Data are mean + SEM (n=7-9). ***p*<0.01, *p*<0.01 vs sham saline #*p*<0.05 vs. surgery saline.

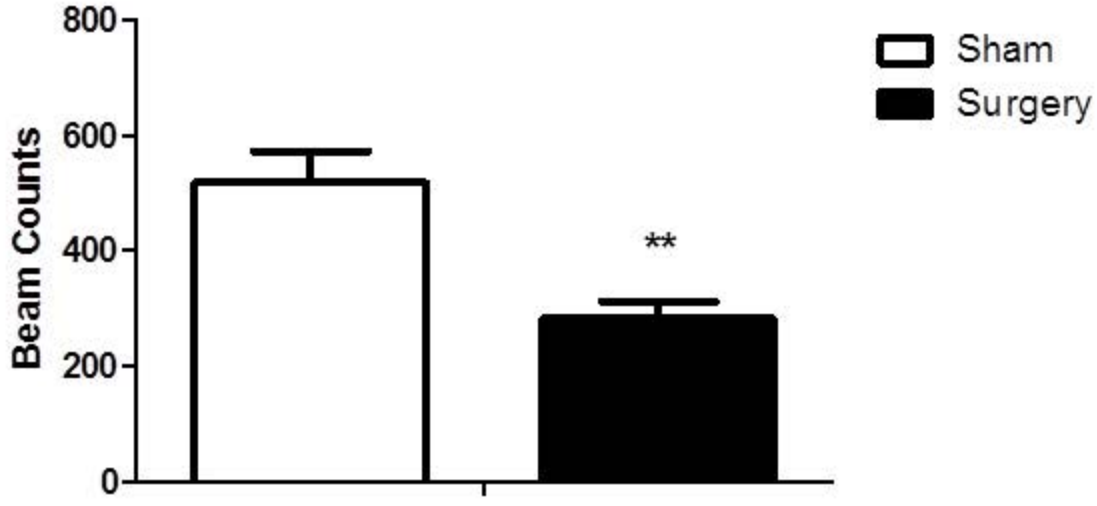
Figure 8 Effect of morphine and carprofen in sham and surgery animals on home cage vertical activity during the period 1-3h post-surgery. Data are mean + SEM (n=7-9) ***p*<0.01 vs. sham saline ++*p*<0.01 vs. surgery saline.

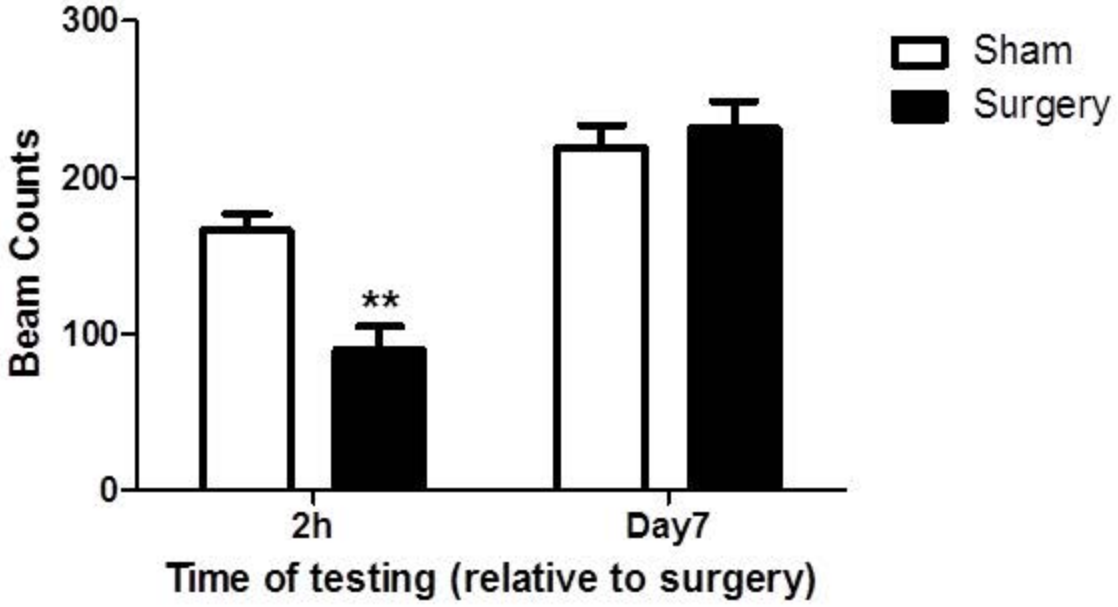

Home cage
Open Field
PEAP
Morphine
Carprofen
Day -1
Surgery
1
2
3
4
Hours post surgery
7
Days post surgery

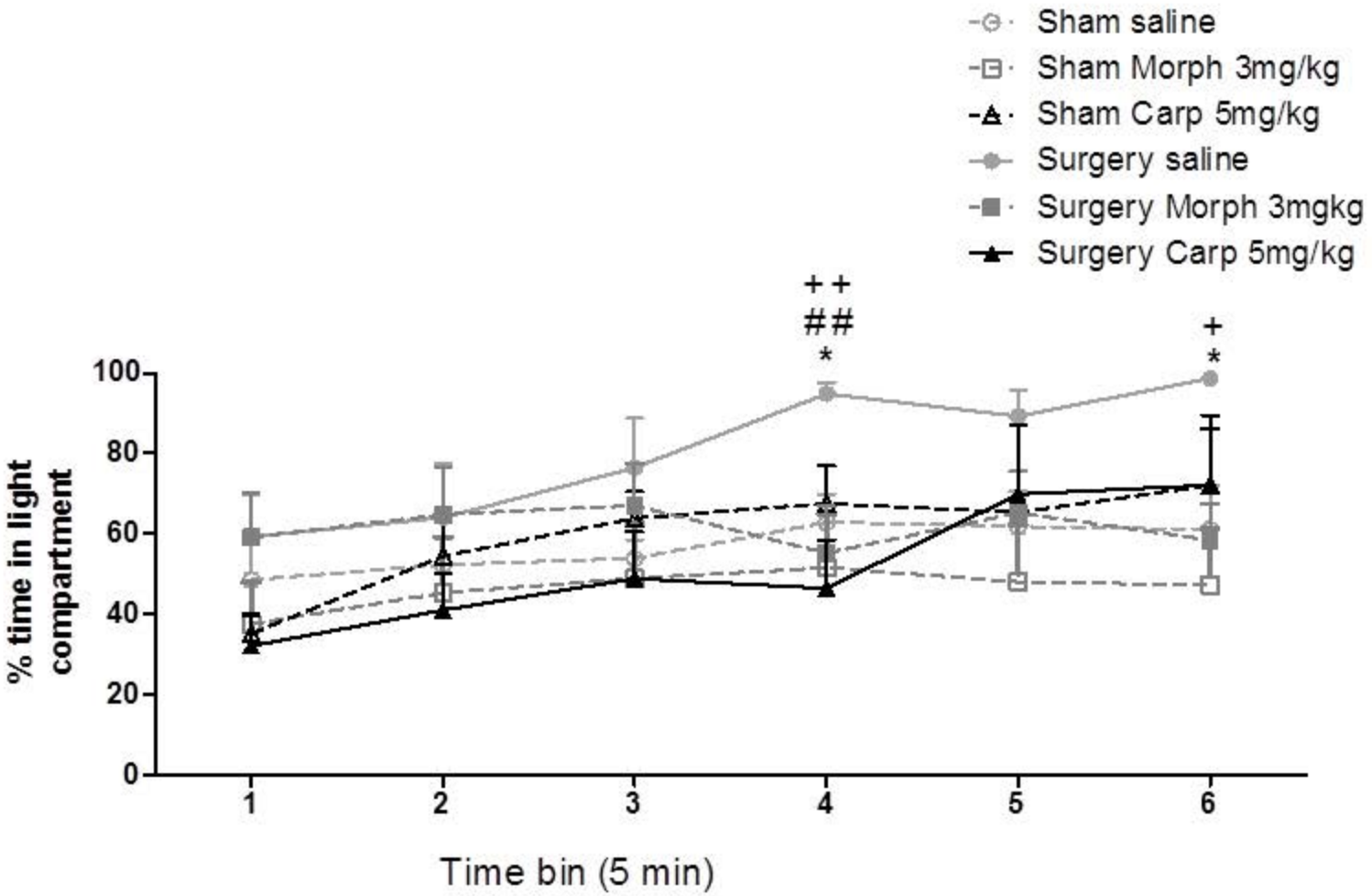

A
% time in light compartment
100
80
60
40
20
0
1
2
3
4
5
6
Time bin (5 min)
**
**
**
sham
surgery

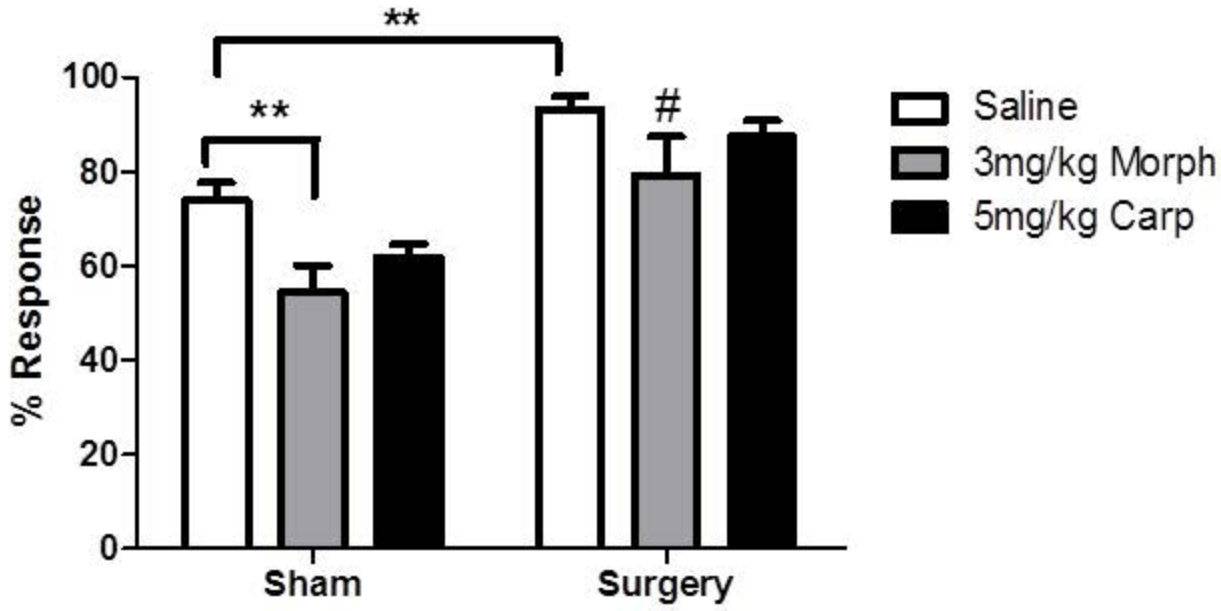

B
% time in light compartment
100
80
60
40
20
0
1
2
3
4
5
6
Time bin (5 min)
sham
surgery

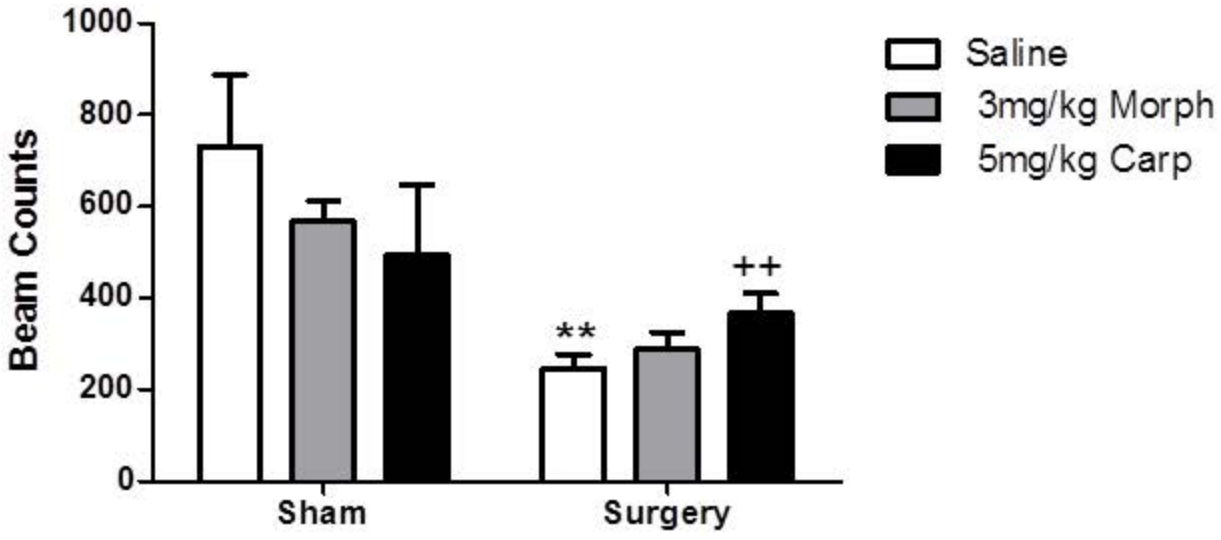

A
100
80
60
40
20
0
% Response
**
Sham
Surgery
B
100
80
60
40
20
0
% Response
**
Sham
Surgery


800
600
400
200
0
Beam Counts
**
Sham
Surgery


300
200
100
0
Beam Counts
**
2h
Day7
Time of testing (relative to surgery)
Sham
Surgery


Sham saline
Sham Morph 3mg/kg
Sham Carp 5mg/kg
Surgery saline
Surgery Morph 3mgkg
Surgery Carp 5mg/kg
++
##
*
+
*
% time in light compartment
100
80
60
40
20
0
1
2
3
4
5
6
Time bin (5 min)


**
**
#
100
80
60
40
20
0
% Response
Sham
Surgery
Saline
3mg/kg Morph
5mg/kg Carp


1000
800
600
400
200
0
Beam Counts
Sham
Surgery
**
++
Saline
3mg/kg Morph
5mg/kg Carp