<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Development and characterization of a novel, anatomically relevant rat model of acute postoperative pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>Bree, Dara; Moriarty, Orla; O’Mahony, Cliona M.; Morris, Bradley; Bannerton, Karen; Broom, Daniel C.; Kelly, John P.; Roche, Michelle; Finn, David P.</td>
</tr>
<tr>
<td><strong>Publication Date</strong></td>
<td>2015-01-30</td>
</tr>
<tr>
<td><strong>Publication Information</strong></td>
<td>Bree, D; Moriarty, O; Mahony, CM; Morris, B; Bannerton, K; Broom, DC; Kelly, JP; Roche, M; Finn, DP (2015) ‘Development and Characterization of a Novel, Anatomically Relevant Rat Model of Acute Postoperative Pain’. Journal Of Pain, 16 :421-435.</td>
</tr>
<tr>
<td><strong>Publisher</strong></td>
<td>Elsevier</td>
</tr>
<tr>
<td><strong>Link to publisher’s version</strong></td>
<td><a href="https://doi.org/10.1016/j.jpain.2015.01.010">https://doi.org/10.1016/j.jpain.2015.01.010</a></td>
</tr>
<tr>
<td><strong>Item record</strong></td>
<td><a href="http://hdl.handle.net/10379/15365">http://hdl.handle.net/10379/15365</a></td>
</tr>
<tr>
<td><strong>DOI</strong></td>
<td><a href="http://dx.doi.org/10.1016/j.jpain.2015.01.010">http://dx.doi.org/10.1016/j.jpain.2015.01.010</a></td>
</tr>
</tbody>
</table>
Development and characterisation of a novel, anatomically relevant rat model of acute postoperative pain

Dara Bree1,2,3, Orla Moriarty1,2,3,4, Cliona M. O’Mahony1,2,3,4, Bradley Morris1,2,3, Karen Bannerton1,3, Daniel C. Broom4, John P. Kelly1,3, Michelle Roche2,3, *David P. Finn1,3.

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

Running title: A new rat model of acute postoperative pain

*Corresponding author

Prof. David P. Finn, Pharmacology and Therapeutics, School of Medicine, University Road, National University of Ireland, Galway, Ireland. Tel. +353 91 495280; Fax +353 91 495586 Email: david.finn@nuigalway.ie
URL: http://www.nuigalway.ie/pharmacology/Dr_David_Finn.html

Disclosure: The authors have no conflicts of interest to declare. This project was funded by a grant from the Irish Industrial Development Agency in partnership with Covidien. The Irish Industrial Development Agency played no part in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. Co-authors OM, COM and DCB at Covidien did contribute to these elements.
Abstract

Acute postoperative pain remains a significant healthcare issue. Development of anatomically relevant animal models of postoperative pain, with improved predictive validity, would advance understanding of postoperative pain mechanisms and improve treatment outcomes. This study aimed to develop, characterise and validate a rat model of acute postoperative pain associated with inguinal hernia repair based on the Lichtenstein inguinal hernia repair procedure (without hernia induction). We hypothesised that the surgery would result in reduced spontaneous locomotor activity which would represent a pain-related phenotype. Post-surgical characterisation involved extensive monitoring of home cage and open field locomotor activity, as well as mechanical hypersensitivity and assessment of c-Fos expression in the dorsal horn of the spinal cord. In pharmacological validation studies, rats received morphine or carprofen 1h before, and/or immediately after, surgery. Rats that underwent hernia repair surgery exhibited significantly lower horizontal and vertical activity in the home cage and open field in the early post-surgical period, compared with sham rats or rats that underwent skin incision only. Morphine, carprofen and paracetamol attenuated the surgery-induced reductions in locomotor activity, to varying degrees. Surgery was associated with significantly increased c-Fos expression in the ipsilateral dorsal horn of the spinal cord, an effect attenuated by carprofen treatment. These results support the development and characterisation of a novel, anatomically relevant model of acute postoperative pain which may facilitate development of improved treatment regimes.
Perspective

Acute pain following inguinal hernia repair can be difficult to treat. Here we report, for the first time, the development of a novel, anatomically relevant rat model to facilitate improved understanding and treatment of acute postoperative pain following inguinal hernia repair.

Keywords: postsurgical pain; hernia repair; morphine; carprofen; paracetamol; locomotor activity; behaviour; c-Fos

Introduction

Acute postoperative pain remains a significant healthcare problem. Inguinal hernia repair is one of the most common surgical procedures, and increasingly it is performed on an ambulatory (day-patient) basis. However, ambulatory surgery can be associated with clinically significant pain, with approximately 40% of patients experiencing moderate to severe acute pain in the early hours and days following inguinal hernia repair surgery, even after administration of opioid and non-opioid analgesics. There is a need for improved understanding of the mechanisms mediating acute postoperative pain and for development of more effective pain management protocols. Current well-characterised models of postoperative pain are based upon paw, thigh, abdominal and back incision injuries. These models have contributed significantly to our understanding of postoperative pain mechanisms and assessment of pain evoked by mechanical or thermal stimuli. However, development of a clinically and anatomically relevant animal model of postoperative pain, with improved face, construct and predictive validity for a surgical procedure performed in humans, would further facilitate research in this area.
The purpose of this study was to develop and characterise an anatomically relevant rat model of the acute postoperative pain associated with inguinal hernia repair. We hypothesised that surgery to mimic inguinal hernia repair would result in reduced spontaneous locomotor activity, particularly vertical activity (rearing), in rats, with such a reduction representing a pain-related phenotype. Reduced spontaneous locomotor activity in rodents has previously been proposed as a measure of non-evoked pain-related behaviour following laparotomy and knee surgery. Following ambulatory surgery in humans, locomotor activity is correlated with spontaneous and movement-evoked pain, as well as time to ambulation. Thus, the initial aim was to develop a surgical procedure in rats that closely mirrored the Lichtenstein inguinal hernia repair procedure most commonly performed in humans, and to complete an extensive characterisation of post-surgical locomotor activity (horizontal and vertical) in the home cage and open field environments, as well as postoperative mechanical hypersensitivity using von Frey filaments. A second aim was to investigate the ability of opioid and non-opioid analgesics to attenuate the surgery-induced phenotype, which would further validate its relevance as a pain model. Current clinical recommendations for management of acute postoperative pain include systemic administration of opioids and/or non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol. Systemic administration of morphine, carprofen and paracetamol are efficacious in animal models of postoperative pain, therefore these drugs were used to validate our model. A third aim was to investigate whether surgery was associated with altered expression of the immediate early gene and marker of pain-related neuronal activity, c-Fos, in the dorsal horn of the spinal cord.
Materials and Methods

Animals

Male Lister-Hooded rats (200-250g on arrival; Charles River, UK) were used in all studies and were individually housed in clear polycarbonate cages (LxWxH = 48x26.5x21cm) 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. On arrival, animals were immediately placed on a 12h:12h reverse light:dark cycle (lights on at 20:00). The temperature of the room was maintained at 21 ± 3 °C and relative humidity at 35-65%. Animals were habituated to the animal unit and entrained to the reverse light:dark cycle for a minimum of 10 days prior to commencement of experiments and surgery. This acclimatisation period was selected as pilot studies in our laboratory demonstrated that Lister-Hooded rats entrain fully to a reverse light:dark cycle within 10 days. 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 and in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, under license from the Irish Department of Health and Children and in compliance with the European Communities Council directive 86/609.

Modelling inguinal hernia repair surgery in the rat

A video of the surgical technique is presented in Supplemental Digital Content 1. The surgical procedure employed was based on the most commonly used clinical open-surgery
Lichtenstein indirect inguinal hernia repair technique. The key principles of this technique include using a sheet of mesh, and crossing the tails of the mesh behind the spermatic cord to avoid re-occurrence of the hernia after repair. The mesh is then secured in place to the floor of the inguinal canal with sutures to avoid movement within the inguinal space.

There are a number of differences between the anatomy of the human and rat inguinal areas which had to be considered. Firstly, the location of the testicle between human and rat differs, the human being retroperitoneal and the rat being intraperitoneal which allows access of the testicle into the peritoneal space. Therefore, before and during surgery, it was important to ensure that the testicle was confined to the scrotum and not allowed to access the peritoneal space. Secondly, in clinical surgery the implanted mesh may be sutured to both the pubic tubercle and to the inguinal ligament. However, because of the small size of the inguinal ligament in the rat and the difficulty in locating it, it was only possible to suture the mesh to the pubic tubercle in our model. Clinically, the size of the mesh used is dependent on the size of the hernia in patients and is therefore variable. However, the present model does not involve the induction of a hernia since we wished to model pain associated with repair surgery only, devoid of any confounding effects of pain associated with a hernia itself. Thus, the size and shape of the mesh was kept consistent between rats.

Rats (280-350g at the time of surgery) were anaesthetized using isoflurane: 5% induction, 2-2.5% maintenance in 0.5 L/min O₂, and they remained anaesthetized for precisely 1 hour. The groin region was shaved and disinfected with chlorhexidine and alcohol. 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. Surgery was performed on the right side because this is the side on which the majority of clinical inguinal hernias occur in humans. The incision was enlarged with a blunt scissors to expose the contents of the inguinal canal. 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 implanted on the floor of the canal, and sutured to the pubic tubercle with polypropylene 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 polypropylene 5-0 (5-0 Surgipro II, Covidien USA) and the skin by continuous synthetic absorbable suture (Polysorb 5-0 Covidien USA). All rats received a 2ml injection of sterile saline (s.c.) following completion of surgery. Rats in the sham group were anaesthetized, the groin region was shaved and disinfected with chlorhexidine (BCM, UK) and alcohol swabs (Fleming Medical, Ireland), and rats were then left under anesthesia (without any incision) for an identical duration to the surgery groups (1 hour). A separate cohort of rats was used to compare the effects of skin incision only with those of the full surgical procedure. In all studies, surgery/sham/incision only procedures were carried out during the early dark phase from 0900h – 14.00h. This period was chosen because locomotor activity in the critical first few hours post-surgery could then be monitored while the animals were still in the dark phase, when their activity levels would be naturally higher, and thus the scope to detect surgery-induced reductions in locomotor activity would be greater. Each hour, one rat from a surgery group and one rat from a sham group were subjected to either procedure simultaneously and then tested as a pair in subsequent behavioural assays to control for time of day and order of testing. All rats
were returned to their home cage where standard bedding was replaced with an absorbent padding Vlesi bedsheets (Fleming Medical, Ireland) for the first 24h of the post-surgery recovery period, after which it was replaced with regular bedding. Body weight and food and water intake were monitored daily following surgery. Across all of the experiments, a small number of animals were excluded due to death during surgery from isoflurane overdose (n=3) or excessive bleeding and wound dehiscence after surgery (n=1).

*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. The system evaluated the movement of animals in 2 horizontal planes, 5 cm and 11.75 cm from the floor of the cage. Each plane was monitored by 16 beams spaced 2.54 cm apart and there was one infrared emitter and detector per beam located on either side of each cage to monitor movement across the width of the cage. The total number of beams broken per interval was calculated and data sent to a central computer which displayed outputs as X-total/horizontal activity (total number of beams broken in the lower plane) and Z-total/vertical activity (total number of beams broken in the upper plane) over 5 minute time bins, which were then totalled and analysed in 1h time bins. Activity in the lower (X) plane represents horizontal locomotor activity and activity in the upper (Z) plane represents vertical or rearing activity. Average light intensity in the home cages during the light phase was 25 lux.
Open field monitoring system

The Opto-M3 Triple Axis System (Columbus Instruments, USA) was used to monitor horizontal and vertical locomotor activity in an open field environment. The system consisted of a 4 arena set-up which employed similar detector-emitter pairs as the home cage (X and Z axes), with an additional detector-emitter pair monitoring horizontal activity across the width of the arena (Y axis). Plane heights were 5 cm and 11.75 cm monitoring horizontal activity and vertical activity in a clear 43 x 43 x 43 cm arena. Data were sent from the 3 sets of detector-emitters to a central computer displaying outputs in XY-total and Z-total activity counts over 5 second intervals and were analysed as a total over 5 minutes. Each of the 4 open field arenas was lit with an individual incandescent light bulb on a dimmer switch to maintain homogenous lighting across the arenas (7 lux). All open field testing was carried out during the dark phase. The arenas were cleaned with mild detergent and dried to remove odour cues between successive rats. Pilot studies confirmed that no habituation to the open field arena occurred after multiple 5 min tests, enabling us to perform repeated testing following surgery.

von Frey testing

The method used was adapted from several test protocols used in our laboratory and others\(^9,\)\(^{17,25}\) which are commonly used to assess the sensitivity of the plantar surface of the hind paw following intervention but filaments were instead applied perpendicular to the left or right of the surgical wound site. The arena used for von Frey testing consisted of a six-chambered arena made of clear Perspex and white, melamine-coated chipboard and the dimensions of the chambers were such that rats could move freely (14 x 20 x 25cm). A Perspex lid with air-holes was placed on top of the arena during the habituation and testing periods. The arena
was placed on a raised wire-mesh flooring so that the experimenter could access the inguinal area from below. Rats were habituated to the arena for 20 min prior to testing. A range of von Frey filaments (Touch-Test ®Sensory Evaluators, North Coast Medical, Inc., USA) of different weights (0.07g – 15g) were used, starting with filament number 10 (2g). The filament was applied perpendicular to the left or right of the surgical wound site with sufficient force to cause slight buckling of the filament, for approximately 5 seconds or until a positive result was observed. A positive result was recorded if flinching, licking or withdrawal of the area occurred on application of the filament or immediately after removal of the filament. Filaments were applied to either side of the wound site five times (alternating between sides). If a positive response was observed to any of the five applications using the 2g filament, filaments of lower weights were applied in descending order until no positive responses were observed. Filaments were then applied in ascending order of weight until positive responses to all five applications were observed on two consecutive filaments. The arena was cleaned with ethanol (20% v/v) between each session. The filament weight eliciting a 50% response was calculated by plotting a non-linear regression curve of the % response versus filament weight for each rat (using GraphPad Prism® software).

Drugs
Carprofen (Pfizer, Ireland) at doses of 5 or 10mg/kg, or saline vehicle, were administered subcutaneously (s.c.) in a volume of 1ml/kg 1h prior to surgery. Morphine sulphate (Antigen Pharmaceuticals, Ireland) or saline vehicle were administered s.c. at doses of 7 or 10mg/kg 1h prior to surgery and again immediately after surgery in a volume of 1ml/kg. Paracetamol (acetaminophen; Pfizer, Ireland) at doses of 75 or 150mg/kg, or 50:50 PEG 400 and sterile water vehicle, were administered s.c. immediately after surgery in a volume of 2ml/kg. Drug doses and times of administration were based on the pharmacokinetics of the drugs as well as
in-house pilot work and published studies demonstrating their efficacy in animal models of acute pain. ², ²², ²⁴

Experimental Protocols

Experiment 1: Characterisation of postoperative behaviour in hernia repair model

Experiment 1A: Comparison of surgery versus sham

The experimental protocol is summarised in Figure 1A. Twenty-four rats were allocated into two experimental groups, sham and surgery (n=12/group), on the basis of their home cage and open field activity at pre-surgery baseline (24h prior to surgery) such that the group mean baseline locomotor activities were equivalent. Surgery and sham procedures were carried out as described above.

Home cage activity was monitored throughout the post-surgical period. Animals were placed in the open field for 5 minutes, at 24h pre-surgery (baseline), 1, 2 and 4h post-surgery on day 0 (surgery day) and on days 1, 2, 3 and 6 post-surgery and locomotor activity was monitored as described above. von Frey testing was performed immediately following open field exposure 24h pre-surgery (baseline) and at 4h, and days 2 and 6 post-surgery. Animals were killed by decapitation under isoflurane anaesthesia at the day 6 time point as previous pilot work showed no behavioural differences between sham and surgery groups at later time points (up to day 28).
**Experiment 1B: Comparison of surgery versus incision only**

The contribution of the initial skin incision to the surgery-induced behavioural phenotype was investigated with an experimental protocol identical to that for Experiment 1A above and summarised in Figure 1A. Twenty-four rats were allocated into two experimental groups, incision only (n=12) and surgery (n=12), on the basis of their home cage and open field activity at pre-surgery baseline (24h prior to surgery) such that the group mean baseline locomotor activities were equivalent. Surgery and incision only procedures were carried out as described above. Animals were killed by decapitation under isoflurane anaesthesia at the day 6 time point.

**Experiment 2: Pharmacological characterization of hernia repair model with analgesics**

The experimental protocol is summarised in Figure 1B. Morphine, carprofen or paracetamol (acetaminophen) were used in the pharmacological characterisation of the hernia repair model. Animals underwent either the surgery procedure as described above or the sham procedure. Animals were assigned to one of the treatment groups (n = 9-10 rats per group) in a pseudo-random manner and there were no baseline differences between groups for home cage or open field activity. Morphine, carprofen or paracetamol were administered at the doses and time points described above and home cage and open field monitoring and von Frey testing were carried out at the time points illustrated in Figure 1B. Animals were killed by decapitation under isoflurane anaesthesia at the 24h post-surgery time point. Both male and female experimenters performed the surgical procedures and behavioural testing.
Experiment 3: Investigation of the effect of surgery on spinal cord c-Fos expression and ATF-3 expression in the dorsal root ganglia

Animals underwent either surgery or sham procedure (n = 6/group) and were returned to their home cage for 2h immediately following surgery, at which point they were killed by terminal anaesthesia (100mg/0.5ml pentobarbital i.p) and transcardial perfusion with 100ml of heparinised saline followed by 500ml of 4% (w/v) paraformaldehyde fixative in 0.1M phosphate buffer (PB) at pH 7.4 and 4 °C. Spinal cords were rapidly removed and stored in the same fixative for 48h followed by immersion in 30% (w/v) sucrose solution in 0.1M PB containing 1% sodium azide until sectioning. A separate cohort of rats was used to investigate the effect of a single acute administration of the NSAID carprofen on spinal cord c-Fos expression following surgery. Rats were habituated to handling and s.c. injections by receiving a single saline injection per day from 4 days prior to surgery or sham procedure. On the day of surgery, carprofen (5 mg/kg) or saline vehicle were administered s.c. 1h prior to surgery or sham procedure and animals were sacrificed 2h and 15 mins post-surgery by transcardial perfusion (n=5/6 per group). A separate cohort of animals was used to assess expression of the marker of nerve injury, ATF-3, in the dorsal root ganglia (T13-L2) ipsilateral to the side of surgery at 24h post-surgery.

Immunohistochemical staining for c-Fos in the dorsal horn of the spinal cord

Serial sections of 30µm thickness were taken at the level of the spinal cord at which the three major inguinal area nerves (ilioinguinal, iliohypogastric, genitofemoral) enter the cord, T13-L2 and collected in 0.1M PB containing 0.1% sodium azide. The procedure for c-Fos
immunolabelling was similar to that previously used in our laboratory 27, 28, 35. Briefly, sections were washed in phosphate buffered saline (PBS) and then placed in 0.75% hydrogen peroxide (H₂O₂) for 20 min in order to quench endogenous peroxide in tissue. Sections were then incubated for 24h at room temperature under constant agitation in PBS containing a polyclonal antibody directed against residues 4-17 of human c-Fos (Calbiochem, Merck Biosciences, UK; c-Fos antisera raised in rabbit (1:10000), 0.2% (v/v) Triton X, 1% (w/v) normal goat serum). The incubated sections were washed and incubated for 90 min in biotinylated goat anti-rabbit antisera (1:200; Jackson ImmunoResearch Europe, UK). The secondary antibody had minimal cross-reactivity to non-target species. This was followed by incubation in the avidin–biotin–peroxidase complex (1:200; ABC Elite Kit; Vector Laboratories Ltd, Peterborough, UK) for a further 90 min, after which sections were immersed in 0.02% (w/v) 3,3-diaminobenzidine-4HCl (DAB) containing 0.01% (v/v) H₂O₂ in PBS until a brown colour developed. Sections were mounted onto Superfrost® Plus slides (Thermo Scientific, Ireland), dehydrated in an ascending series of alcohols, cleared in xylene (Lennox, Ireland) and coverslipped using DPX mountant (BDH chemicals, UK).

Quantification of Fos-like immunoreactivity

Relevant levels of the spinal cord were identified in accordance with a rat spinal cord atlas 37 and photomicrographs of the relevant sections taken with a digital camera linked to an Olympus X5 microscope. The number of Fos-like immunoreactive neurons for each rat was calculated as the mean of three sections per level of spinal cord (T13, L1, L2), and an overall group mean was then calculated. Counting of Fos-like immunoreactive neurons was performed with the aid of NIMH Image J software (Bethesda, MD) by an experimenter blind to treatment.
ATF-3 staining in dorsal root ganglia

Serial 16µm dorsal root ganglia sections were cut on a cryostat (Microm International, Germany) and immediately mounted on Superfrost®-charged microscope slides (Thermo Scientific, Ireland). Following 3x10 minute washes, non-specific secondary antibody binding was blocked for 2h in solution containing 3% normal goat serum (Sigma Aldrich, Ireland) and 0.2% triton X (Sigma Aldrich, Ireland) in PBS. Sections were then incubated overnight in blocking solution containing a polyclonal antibody (1:500 dilution) targeted at the C19 residue of human ATF-3 (Santa Cruz Biotechnology, Germany). The incubated sections were then washed and incubated for 3h in goat-anti-rabbit Alexa Fluor 546 fluorescent secondary antibody (Bio-Sciences, Ireland). Sections were left to dry before cover slipping using a fluorescent counting medium Fluoromount ™ (Sigma-Aldrich, Ireland). Sections were visualised using fluorescence microscopy (Olympus Inverted Microscope with structured light illumination) and ATF-3 staining was assessed qualitatively throughout the section by an experimenter blind to the treatments.

Data analysis

Statistical analysis was performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene test, respectively. Two-way repeated measures analysis of variance (ANOVA) was performed to determine main effects of time, surgery, drug treatment, or their interaction, on behaviour in the open field or home cage. Two-way ANOVAs were used toanalyse total home cage locomotor activity collapsed over 1-10h and c-Fos data. Student Newman Keuls post-hoc test (Experiments 2 and 3) or Student’s unpaired two-tailed t-tests (Experiments 1a and 1b) were
used, as appropriate, to assess differences between the groups and across time points. Data are presented as mean ± SEM. p<0.05 was considered statistically significant.

Results

Experiment 1: Characterisation of postoperative behaviour in hernia repair model

Experiment 1A: Comparison of surgery versus sham

Locomotor activity in the home cage for the first 48 hours post-surgery

Locomotor activity in the home cage was examined up to day 5 post-surgery and surgery-induced effects on locomotor activity were evident within the first 2 days post-surgery but not thereafter (Supplementary Digital Content 3). As surgery-induced effects on locomotor activity were evident in the early post-surgery period, the temporal profile of home cage activity over the first 48h post-surgery was analysed further.

Horizontal and vertical activity of the surgery group was significantly lower at the 2, 3, 5, 6, 8, 9 and 10h time bins compared to activity of the sham group (Figure 2A&B). Activity of the surgery group was also significantly lower at 4h for horizontal and at 11h for vertical activity. Horizontal activity of the surgery group was higher at the 15h and 16h time bins compared to activity of the sham group (Figure 2A&C). For 1-10h collapsed data, repeated measures ANOVA revealed that the surgery group displayed significantly decreased horizontal and vertical activity compared to the sham group and significantly decreased levels of vertical activity compared to its baseline value, while the sham group displayed increased levels of activity compared to baseline levels (Figure 2B&D).
Locomotor activity in the open field test

Horizontal and vertical activity did not differ between the groups at Day -1 baseline (Figure 3A&B). Horizontal and vertical activity of the sham group was significantly lower at the 1h time point. Vertical activity was also higher in the sham group at Day 3 and Day 6 post-surgery compared to baseline pre-surgery values. Horizontal and vertical activity in the surgery group was significantly lower at 1h, 2h, and 4h time points and higher at the Day 6 post-surgery for vertical activity, compared to baseline pre-surgery activity. Vertical activity was also significantly higher in the surgery group at Day 3 post-surgery compared to baseline pre-surgery activity. Horizontal and vertical activity in the surgery group were significantly lower than the sham group at the 2h time point and vertical activity in the surgery group was also significantly lower than the sham group at 1h and 4h post-surgery (Figure 3A&B).

Body weight and sensory responding to von Frey stimulation over the course of the experiment were not significantly different between sham and surgery groups and data are presented as Supplementary Digital Content 2 and 4, respectively.

Experiment 1B: Comparison of surgery versus incision only

Locomotor activity in the home cage for the first 48 hours post-surgery

Horizontal and vertical activity of the surgery group was significantly lower at 2, 3, 4, and 5h time bins compared to activity in the incision only group (Figure 4A&C). Vertical activity in the surgery group was also significantly lower at the 6, 8, 9 and 10, 27 and 28h time bins compared to the incision only group. For 1-10h collapsed data, repeated measures ANOVA revealed that the surgery group displayed significantly decreased horizontal and vertical
activity compared to the incision only group and significantly decreased levels of vertical activity compared to its baseline levels, while the incision only group displayed increased levels of activity compared to baseline levels (Figure 4B&D).

**Locomotor activity in the open field test**

Horizontal and vertical activity did not differ between the groups at Day -1 baseline (Figure 5A&B). Horizontal activity of the incision only and surgery groups was significantly lower at 1h post-surgery compared to baseline pre-surgery activity. Horizontal activity in the surgery group was also significantly lower at 2h, 4h time points compared to its baseline pre-surgery values. Vertical activity of the incision only and surgery groups was significantly lower at 1h and 2h, 4h and day 1 compared to its baseline pre-surgery values. Vertical activity in the surgery group was significantly lower compared to the incision only group at 2h and 4h post-surgery (Figure 5A&B).

Sensory responding to von Frey stimulation over the course of the experiment was not significantly different between the incision only and surgery groups and data are presented as Supplementary Digital Content 5.

**Experiment 2: Pharmacological characterization of hernia repair model with analgesics**

Experiment 1(A and B) revealed that the main surgery-induced deficits in locomotor activity were observed over the first 24h post-surgery. Thus, subsequent pharmacological experiments assessed locomotor activity over this period. We sought to determine whether the surgery-induced phenotype was indeed pain-related by investigating whether it could be
attenuated by administration of analgesics. Because surgery-induced deficits in Experiment 1 were most prominent for vertical activity, we present only the data for vertical activity herein for Experiment 2 and we refer readers to Supplemental Digital Content 6 and 7 for the horizontal activity results which were generally similar to those for vertical activity.

Home cage activity was analysed during the 1-10h period post-surgery because initial characterisation work revealed that the surgery-induced behavioural phenotype was most apparent in the home cage during this period.

Effect of morphine, carprofen or paracetamol on home cage vertical activity over first 10h post-surgery

Vertical activity in the surgery saline and surgery morphine groups was significantly lower than activity in the corresponding sham groups. Thus, morphine did not attenuate the surgery-induced reduction in vertical locomotor activity in the home cage and had no effect in sham animals (Figure 6A).

The surgery saline and surgery carprofen groups displayed significantly lower levels of vertical activity compared to the corresponding sham groups. Surgery rats that received 5 or 10mg/kg carprofen displayed significantly increased levels of vertical activity compared to the surgery saline group, indicating that carprofen attenuated the surgery-induced reduction in home cage vertical activity (Figure 6B).
Vertical activity in the surgery saline and surgery paracetamol groups was significantly lower than activity in the corresponding sham groups. Thus, paracetamol did not attenuate the surgery-induced reduction in vertical locomotor activity in the home cage and had no effect in sham animals (Figure 6C).

**Effect of morphine administration on vertical locomotor activity in the open field**

All of the groups displayed significantly lower levels of activity at 1h and 2h post-surgery compared to baseline pre-surgery activity levels (Figure 7A). The surgery saline and 7mg/kg morphine groups also displayed significantly lower levels of activity at 4h and Day 1 post-surgery compared to baseline pre-surgery activity levels in these groups. The surgery saline group displayed significantly lower levels of activity compared to the sham saline group at, 4h post-surgery. However, no surgery-induced decreases in vertical activity were observed in the surgery animals receiving morphine 7 or 10 mg/kg at 4h post-surgery, indicating that morphine was capable of preventing the surgery-induced reduction in locomotor activity at this time point, with no effects in sham animals (Figure 7A).

**Effect of carprofen administration on vertical locomotor activity in the open field**

Activity in all groups was significantly lower at 1h and 2h post-surgery compared to baseline pre-surgery activity in these groups (Figure 7B). Activity in all surgery groups (saline and carprofen treated) was also significantly lower at 4h and on Day 1 post-surgery compared to baseline pre-surgery activity in these groups. The sham saline group displayed decreased levels of activity at 4h post-surgery compared to its baseline pre-surgery activity. Activity of the sham 5mg/kg group was significantly lower on Day 1 post-surgery compared with
baseline pre-surgery activity in this group. The surgery saline group displayed significantly lower levels of activity compared to its sham counterpart at 2h and 4h post-surgery, while the surgery carprofen 10mg/kg group displayed significantly lower levels of activity compared to its sham counterpart at 4h post-surgery. However, no surgery-induced decreases in vertical activity were observed in the surgery 5mg/kg carprofen group at 2h and in particular at 4h post-surgery, indicating that this dose of carprofen was capable of preventing the surgery-induced reduction in locomotor activity at this time point, with no significant effect in sham animals (Figure 7B).

Effect of paracetamol administration on vertical locomotor activity in the open field

Activity in all groups was significantly lower at 1h and 2h post-surgery compared to baseline pre-surgery activity and activity in the surgery saline and 75mg/kg paracetamol groups remained significantly lower at 4h post-surgery. The surgery saline group displayed significantly lower levels of activity compared to its sham counterpart at 4h post-surgery, while the surgery paracetamol 75mg/kg group displayed significantly lower levels of activity compared to its sham counterpart at 2h and 4h post-surgery. However, no surgery-induced decreases in vertical activity were observed in the surgery 150mg/kg paracetamol group at 2h or 4h post-surgery, indicating that this dose of paracetamol was capable of preventing the surgery-induced reduction in locomotor activity at these time points, with no significant effect in sham animals (Figure 7C).

In summary, all three analgesics attenuated surgery-induced reductions in vertical locomotor activity to various degrees. Morphine prevented surgery-induced deficits in locomotor activity, but it also had some discrete effects on locomotor activity in sham animals. The
NSAID carprofen and paracetamol also prevented surgery-induced reductions in vertical locomotor activity at discrete time points and doses that had negligible effects in sham rats.

**Experiment 3: Investigation of the effect of surgery and carprofen treatment on spinal cord c-Fos expression**

Expression of Fos-like immunoreactivity was examined in the superficial dorsal horn of the spinal cord of sham and surgery animals sacrificed 2h post-surgery (Figure 8A&B). Surgery animals exhibited a significant increase in c-Fos expression at all 3 levels (L1, L2, T13) of the dorsal horn ipsilateral to the side of surgery compared with the contralateral side and also compared to the ipsilateral side of sham controls (Figure 8A&B). Carprofen administration significantly reduced c-Fos expression at the level of T13 in surgery rats, compared with vehicle-treated surgery controls (Figure 9). No difference in ATF-3 expression in T13-L2 dorsal root ganglia was observed between sham and surgery animals at 24h post-surgery, suggesting that this model of inguinal hernia repair pain is not associated with any surgery-induced nerve damage (Supplementary Digital Content 8).
Discussion

This manuscript reports, for the first time, the development and characterisation of an anatomically and clinically relevant animal model of acute postoperative pain associated with inguinal hernia repair. A surgical procedure based upon the human Lichtenstein procedure was developed in rats and significant surgery-induced reductions in home cage and open field locomotor activity were observed. These were most notable within the first 24h post-surgery, and attenuated, to varying degrees, by the analgesics morphine, carprofen and paracetamol, suggesting that the surgery-induced reductions in locomotor activity represent a pain-related phenotype. c-Fos expression was increased in levels of the spinal cord receiving innervation from the inguinal area, suggesting that surgery resulted in the activation of this nociceptive pathway. Moreover, surgery-induced c-Fos expression at the level of T13 was reduced by carprofen pre-treatment. This novel, anatomically-relevant rat model of post-operative pain following hernia repair will facilitate future studies aimed at improving our understanding of the mechanisms underlying acute postoperative pain and the development of improved therapeutic approaches.

In the home cage, the most striking surgery-induced reductions in activity were observed during the first 24h post-surgery (particularly during the first 10h). Activity, as expected, decreased sharply with the onset of the light phase (12-13h) in both sham and surgery groups. However, on re-entry into the dark phase on the day after surgery, a trend for the re-emergence of the surgery-induced differences in locomotor activity between the groups was observed, most notably in vertical activity. In the open field environment, we found surgery-induced reductions in both horizontal and vertical activity over the first 4h post-surgery, compared to sham controls. These surgery-induced differences were most pronounced for
vertical activity. The results of this initial characterisation suggest that a surgery-induced behavioural phenotype is apparent with robust and reproducible reductions in locomotor activity (especially vertical activity) in both the home cage and open field environments. Our results are in line with previous work demonstrating surgery-induced reductions in vertical activity following laparotomy and knee surgery in rodents.\textsuperscript{8, 20} In contrast, there were no differences between surgery and sham animals for quantitative sensory testing with von Frey filaments, indicating that the pain-related phenotype in this model manifests as a deficit in non-evoked measures such as locomotor activity rather than differences in evoked sensory responsivity. The model developed has low sensitivity to von Frey testing relative to the robust and reliable deficits observed in locomotor activity, particularly vertical activity. The examination of locomotor activity in animals following surgery is of clinical and translational relevance as time to ambulation and resumption of normal activities are primary clinical outcomes following inguinal hernia repair surgery.\textsuperscript{1, 11} An important consideration for any animal putative model of postoperative pain is which component(s) of the surgical procedure produce the observed pain-related phenotype. To this end, we compared rats undergoing our full surgical procedure with those undergoing only the initial skin incision component of the procedure. Deficits in home cage and open field locomotor activity of a significantly greater magnitude were observed in rats undergoing the full surgical procedure, compared to those undergoing skin incision only, suggesting that the full procedure, including deep tissue manipulation, is necessary to produce the postoperative behavioural phenotype. These findings are in agreement with those for other models of postoperative pain which have shown that both incision and manipulation of deep tissues is required to produce the complete pain-related syndrome.\textsuperscript{10, 12}
Pharmacological studies were employed to determine whether the surgery-induced behavioural phenotype could be prevented or attenuated with analgesic treatment, a result that would strongly suggest that the phenotype was pain-related. Systemic administration of morphine (10mg/kg) prevented the surgery-induced reduction in horizontal activity in the home cage over the first 10 hours post-surgery without affecting activity in sham animals. Moreover, morphine (7 or 10mg/kg) also prevented the surgery-induced reductions in horizontal and vertical locomotor activity in the open field at 2h and/or 4h post-surgery. However, interpretation of these open field data is complicated by the fact that morphine treatment in sham animals increased locomotor activity compared to saline-treated controls. Morphine can elicit a bi-phasic locomotor response consisting of marked akinesia lasting 1.5-2h followed by a hyperactive phase lasting approximately 2h, corresponding to the 2h and 4h open field time points assessed herein, and this may explain the increased activity observed in sham animals. However, our data also provide evidence that the effects of morphine on locomotor activity in the open field in sham versus surgery rats are dissociable. For example, at 2h post-surgery, morphine reduced vertical activity of sham rats but had the opposite effect in surgery rats, increasing vertical activity. And the 10 mg/kg dose of morphine increased horizontal activity of surgery rats in the open field at 2h post-surgery, while having no significant effect in sham rats. Moreover, morphine also prevented the reduction in home cage horizontal locomotor activity in surgery rats, without having any effect on home cage activity in sham rats. Thus, home cage activity and early open field testing may be better indices of morphine-induced analgesia in this model, devoid of the complications associated with morphine-induced hyperactivity apparent at later open field testing points.
The NSAID carprofen (5 or 10mg/kg) attenuated the surgery-induced decrease in home cage activity (horizontal and vertical activity) over the first 10h post-surgery, without altering sham activity. While the effect of carprofen on home cage activity appeared dose-dependent, this was not the case for open field activity where 5mg/kg, but not 10mg/kg, carprofen prevented the surgery-induced reductions in activity at 4h post-surgery. Analgesic effects of carprofen in a dose range of 5-10 mg/kg have been reported in other postoperative pain models. Paracetamol, a clinically relevant analgesic for postoperative pain, resulted in a partial attenuation of the surgery-induced deficits in home cage vertical activity during the first 1-10h post-surgery, however, this effect did not reach statistical significance. In the open field environment, however, paracetamol (150mg/kg) prevented the surgery-induced reduction in vertical activity at 4h post-surgery, with a trend for the same effect apparent at 2h post-surgery. In the plantar incision model of postoperative pain, paracetamol has been shown to be efficacious at a similar dose range to that used in the current study. Our data suggest that the surgery-induced reductions in locomotor activity associated with the present model are pain-related, as they can be reversed following the administration of both an NSAID and a clinically relevant analgesic that are devoid of overt effects on locomotor activity per se. Clinically, NSAIDs/paracetamol are preferred for the treatment of less invasive and ambulatory surgeries as they can act as opiate-sparing agents. While it was the case that, in general, carprofen was more active in the home cage environment and morphine and paracetamol were more active in the open field test, it was also the case that all 3 analgesics tested exhibited some efficacy in both the home cage and open field environments. Differences in the magnitude or duration of efficacy of the 3 analgesics in the home cage vs. open field may reflect their differing mechanisms of action, with the drugs that possess a degree of anxiolytic activity (i.e. morphine and paracetamol) being more active than carprofen in the open field. Differences may also be explained by the fact that carprofen...
has a longer half-life (~8h) than either morphine (~2h) or paracetamol (~1.5-2h). Thus, carprofen may be more likely to produce a more sustained antinociceptive effect that would be discernible over the 10h home cage postoperative period.

Fos-like immunoreactivity was found to be increased in the dorsal horn of the spinal cord at the level of T13-L2 in the side ipsilateral to injury at 2h post-surgery. T13-L2 corresponds to the levels at which the nerves innervating the inguinal canal terminate in the spinal cord (illiohypogastric nerve: T13-L1, ilioinguinal: L1, genitofemoral: L1-L2). These data suggest that the surgery was associated with activation of primary afferent fibres leading to increased neuronal activity in the dorsal horn of the spinal cord and likely activation of ascending pain pathways. Following treatment with carprofen, surgery-associated c-Fos expression was reduced at the level of T13. Thus, it is possible that the majority of primary afferents that innervate the area of the inguinal canal affected by surgery in our model terminate at the level of T13 in the spinal cord. The surgery-induced c-Fos expression observed herein likely represents an additional marker of the pain-related phenotype in our model. While nociceptive primary afferent neurons innervating the inguinal canal appear to be activated by surgery, they do not appear to be damaged in this model, as evidenced by similar ATF-3 staining in the dorsal root ganglia of sham versus surgery rats.

In conclusion, we have established a novel rat model of the acute postoperative pain associated with inguinal hernia repair surgery, characterised by reductions in horizontal and vertical locomotor activity in the early hours post-surgery, and a corresponding increase in spinal c-Fos expression. An opioid, an NSAID and paracetamol all attenuated/prevented the surgery-induced behavioural alterations at discrete time points post-surgery. The surgery procedure can be performed quickly and with relative ease and other laboratories should be
able to replicate the model without difficulty. These results demonstrate that the model described herein is an appropriate and anatomically relevant novel animal model which may be used to study mechanisms underlying acute postoperative pain associated with inguinal hernia repair and novel therapeutic interventions.

Acknowledgements

The advice and assistance of veterinary surgeon Dr. Yolanda Garcia (Anatomy, School of Medicine, NUI Galway) and general surgeon Mr. Karl Sweeney (Galway University Hospitals) are gratefully acknowledged, as is technical assistance from Mr. Ambrose O’Halloran (Pharmacology and Therapeutics, NUI Galway).

References


14. Gilron I, Orr E, Tu D, O'Neill JP, Zamora JE, Bell AC. A placebo-controlled randomized clinical trial of perioperative administration of gabapentin, rofecoxib and their


**Figure 1.** (A) Outline of protocol for Experiment 1: Characterisation of postoperative behaviour in hernia repair model (B) Outline of protocol for Experiment 2: Pharmacological characterisation of model.

**Figure 2.** The effect of inguinal hernia repair surgery on home cage locomotor activity in rats. (A) horizontal activity and (B) vertical activity over the first 48 hours post-surgery; (C) horizontal activity and (D) vertical activity presented as collapsed data for the period 1-10h post-surgery, and showing the corresponding baseline period. Data are mean + SEM (n = 12 per group). For horizontal activity, there were significant main effects of time ($F_{47,1034}=9.46$, $p<0.001$), time x surgery interaction ($F_{47,1034}=2.32$, $p<0.001$) but not surgery. For vertical activity there were significant main effects of time ($F_{50,1050}=11.12$, $p<0.001$), surgery ($F_{1,21}=5.06$, $p<0.001$) and time x surgery interaction ($F_{50,1050}=2.3$, $p<0.001$). * $p<0.05$, **$p<0.01$ vs. respective baseline, +$p<0.05$, ++$p<0.01$ sham vs. surgery. Arrows indicate when open field testing occurred. Shaded background represents dark phase data.

**Figure 3.** The effect of inguinal hernia repair surgery on (A) horizontal and (B) vertical activity in the open field over 6 days. Data are mean + SEM (n = 12 per group). Repeated measures ANOVA revealed a significant main effect of time ($F_{7,154}=76.46$, $p<0.001$), time x surgery interaction ($F_{7,154}=6.78$, $p<0.001$) but no effect of surgery on horizontal activity and a significant main effect of time ($F_{7,154}=83.31$, $p<0.001$), surgery ($F_{1,22}=8.38$, $p<0.05$) and time x surgery interaction ($F_{7,154}=6.260$, $p<0.001$) on vertical activity in the open field. *$p<0.05$, **$p<0.01$ vs. respective baseline, +$p<0.05$, ++$p<0.01$ sham vs. surgery at specific time points.
Figure 4. The effect of inguinal hernia repair surgery or an incision only procedure on home cage locomotor activity in rats. (A) horizontal activity and (B) vertical activity over the first 48 hours post-surgery; (C) horizontal activity and (D) vertical activity presented as collapsed data for the period 1-10h post-surgery, and showing the corresponding baseline period. Data are mean ± SEM (n = 12 per group). For horizontal activity, there were significant main effects of time (F_{46,1012}=11.18, p<0.001, a time x surgery interaction (F_{46,1012}=2.23, p<0.001) but not surgery, for vertical activity there were significant main effects of time (F_{46,1012}=7.46, p<0.001), surgery (F_{1,22}=27.07, p<0.001) and a time x surgery interaction (F_{46,1012}=3.65, p<0.001). * p<0.05, **p<0.01 vs. respective baseline, +p<0.05, ++p<0.01 incision only vs. surgery. Arrows indicate when open field testing occurred. Shaded background represents dark phase data.

Figure 5. The effect of inguinal hernia repair surgery or an incision only procedure on (A) horizontal activity or (B) vertical activity in the open field over 6 days. Data are mean ± SEM (n=12 per group). Repeated measures ANOVA revealed a significant main effect of time (F_{7,126}=22.42, p<0.001), but not of surgery or time x surgery interaction for horizontal activity and a significant effect of time (F_{7,126}=64.28, p<0.001) and surgery (F_{1,18}=6.05, p<0.05) but no time x surgery interaction for vertical activity in the open field. *p<0.05, **p<0.01 vs. respective baseline; + p<0.05; ++p<0.01 incision only vs. surgery at specific time point.

Figure 6. The effect of (A) morphine (7 or 10mg/kg, s.c. administered 1h before and immediately after surgery) (B) carprofen (5 or 10mg/kg s.c. administered 1h before surgery) and (C) paracetamol (75 or 150mg/kg s.c. administered immediately after surgery) on home
cage vertical activity over the first 10h following surgery. Data are mean ± SEM (n=8-10 per group). A: Two-way ANOVA revealed a significant main effect of surgery (F_{1,48}=53.29, p<0.001) but not morphine on home cage vertical activity during the period 1-10h post-surgery. B: Two-way ANOVA revealed a significant main effect of surgery (F_{1,51}=67.47, p<0.001) and carprofen treatment (F_{2,51}=8.057, p<0.01) on home cage vertical activity 1-10h post-surgery. C: Two-way ANOVA revealed a significant main effect of surgery (F_{1,42}=112.23, p<0.001) but not paracetamol treatment on home cage vertical activity during the period 1-10h post-surgery. *p<0.05, **p<0.01 vs. sham counterpart. #p<0.05 vs. surgery saline.

Figure 7. The effect of (A) morphine (7 or 10mg/kg, s.c. administered 1h before and immediately after surgery) (B) carprofen (5 or 10mg/kg s.c. administered 1h before surgery) and (C) paracetamol (75 or 150mg/kg s.c. administered immediately after surgery) on open field vertical activity following surgery. Data are mean + SEM (n=8-10 per group). A: Repeated measures ANOVA revealed a significant main effect of time (F_{4,192}=180.78, p<0.001), surgery (F_{1,48}=19.79, p<0.001), time x surgery interaction (F_{4,192}=6.39, p<0.001) and time x morphine interaction (F_{8,192}=3.39, p<0.05) on vertical activity in the open field. B: Repeated measures ANOVA revealed a significant main effect of time (F_{4,204}=204.14, p<0.001), surgery (F_{1,51}=34.18, p<0.001), time x surgery interaction (F_{4,204}=8.38, p<0.001) but not carprofen treatment on vertical activity in the open field. C: Repeated measures ANOVA revealed a significant main effect of time (F_{4,168}=188.36, p<0.001), surgery (F_{1,42}=13.22, p<0.001) and time x surgery interaction (F_{4,168}=6.4, p<0.001) but no significant main effect of paracetamol in the open field. *p<0.05, **p<0.01 vs. respective baseline. +p<0.05, vs. sham saline at specific time points. @p<0.05 vs. sham 10mg/kg carprofen. $p<0.05 vs. sham 75mg/kg paracetamol at specific time point.
**Figure 8.** (A) Effects of surgery on Fos-like immunoreactivity in the superficial dorsal horn in rats 2h following surgery. Data are mean ± SEM (n=6). Data were analysed by two-way ANOVA (with side and group as factors) at each spinal level (T13-L2). For each level, a significant main effect of surgery (T13: $F_{1,20}=21.72, p<0.001$; L1: $F_{1,20}=31.12, p<0.001$; L2: $F_{1,20}=15.00, p<0.001$), side (T13: $F_{1,20}=18.89, p<0.01$; L1: $F_{1,20}=33.14, p<0.01$; L2: $F_{1,20}=16.34, p<0.01$) and a surgery x side interaction (T13: $F_{1,20}=15.97, p<0.05$; L1: $F_{1,20}=23.23, p<0.05$; L2: $F_{1,20}=11.90, p<0.05$) was observed. *$p<0.05$, **$p<0.01$ surgery ipsilateral vs. sham ipsilateral; #$p<0.05$, ##$p<0.01$ surgery ipsilateral vs. surgery contralateral

(B) Photomicrographs showing representative Fos-like immunoreactivity in the superficial dorsal horn of the spinal cord of surgery rats. Scale bar 200µm.

**Figure 9.** Effect of carprofen (5mg/kg s.c. administered 1h before surgery) on c-Fos expression in rats 2h following surgery. Data are mean ± SEM (n=5-6). Data were analysed by Student’s unpaired two-tailed t-tests at each level of the spinal cord. #$p<0.05$, surgery saline vs. surgery carprofen.
Highlights

- A novel rat model of acute postoperative pain has been developed
- Represents the first animal model of acute pain following inguinal hernia repair
- Model is characterized by deficits in locomotor activity, reversible by analgesics
- Pain-related phenotype is driven primarily by activation of deep somatic or visceral nociceptors
- Model is associated with increased c-Fos expression in the dorsal horn
A

Home cage
Open field
Von Frey
Tissue Harvesting

(Baseline)

(Hours post-surgery)

(Days post-surgery)

Animals: reverse dark/light phase, light on at 8pm

B

Home cage
Open field
Von Frey
Morphine
Carprofen
Paracetamol
Tissue Harvesting

(Baseline)

(surgery)

(Hours post-surgery)

Time

24 hrs
A  Horizontal Activity

B  Vertical Activity

** Beam Counts/5 min

Day -1  1h  2h  4h  Day 1  Day 2  Day 3  Day 6

Time of testing (relative to surgery)

Sham  Surgery

*  **  +  ++  *  *  *

*  **  +  ++  *  *  *
**Horizontal Activity**

- Incision Only
- Surgery

**Vertical Activity**

- Incision Only
- Surgery

**Horizontal Activity**

<table>
<thead>
<tr>
<th>Hours Post Surgery</th>
<th>Beam Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>0-1000</td>
</tr>
<tr>
<td>3-4</td>
<td>1000-2000</td>
</tr>
<tr>
<td>5-6</td>
<td>2000-3000</td>
</tr>
<tr>
<td>7-8</td>
<td>3000-4000</td>
</tr>
<tr>
<td>9-10</td>
<td>4000-5000</td>
</tr>
</tbody>
</table>

**Vertical Activity**

<table>
<thead>
<tr>
<th>Hours Post Surgery</th>
<th>Beam Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>0-2000</td>
</tr>
<tr>
<td>3-4</td>
<td>2000-4000</td>
</tr>
<tr>
<td>5-6</td>
<td>4000-6000</td>
</tr>
<tr>
<td>7-8</td>
<td>6000-8000</td>
</tr>
<tr>
<td>9-10</td>
<td>8000-10000</td>
</tr>
</tbody>
</table>

**Horizontal Activity**

- Baseline: 0-5000
- Surgery Day: 10000-20000

**Vertical Activity**

- Baseline: 0-1000
- Surgery Day: 15000-25000

**Horizontal Activity**

- Baseline: 1000-2000
- Surgery Day: 3000-4000

**Vertical Activity**

- Baseline: 5000-7000
- Surgery Day: 15000-20000

**Horizontal Activity**

- Baseline: 5000-7000
- Surgery Day: 10000-15000

**Vertical Activity**

- Baseline: 0-1000
- Surgery Day: 15000-20000

* Significance at p < 0.05
** Significance at p < 0.01
A

Vertical Activity

Beam Counts

Time of Testing (Relative to Surgery)

B

Vertical Activity

Beam Counts

Time of Testing (Relative to Surgery)

C

Vertical Activity

Beam Counts

Time of Testing (Relative to Surgery)
**Number of FLI cells**

<table>
<thead>
<tr>
<th></th>
<th>Ipsi</th>
<th>Contra</th>
<th>Ipsi</th>
<th>Contra</th>
<th>Ipsi</th>
<th>Contra</th>
</tr>
</thead>
<tbody>
<tr>
<td>T13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B**

Contralateral | Ipsilateral

T13

L1

L2
**Bodyweight**

Repeated measures ANOVA revealed a significant main effect of time ($F_{7,154}=39.54$, $p<0.001$) and time x surgery interaction ($F_{7,154}=4.831$, $p<0.001$) but not surgery alone on body weight over 6 days post-surgery.

Both the sham and the surgery group maintained and increased their body weight post-surgery, compared with pre-surgery baseline bodyweight. There was a trend for slightly lower post-surgery body weight in the surgery group compared with the sham group but this difference did not reach statistical significance.

Body weight in sham and surgery animals over a 6 day period. Surgery occurred on Day 0. Data are Mean + SEM (n=12).
Locomotor activity in the home cage over 5 days post-surgery

Home cage activity data, including X axis (horizontal activity) and Z axis (vertical activity) beam counts, were collected post interventions (i.e. following baseline open field and von Frey testing on day -1, post-surgery/sham on day 0 and post-open field exposure on subsequent days 1-5) during both the light and the dark phase and the average beam count per hour was calculated for each phase. As expected, activity was lower during the light phase compared with the dark phase, irrespective of treatment. The results of ANOVAs are detailed in the figure legends while the results of post-hoc pairwise comparisons are described in the text below.

Sham vs. Surgery Comparison

Dark phase home cage activity: Horizontal and vertical activity did not differ between the groups at Day -1 baseline. Levels of horizontal and vertical activity were significantly higher in the sham group and significantly lower in the surgery group on the day of surgery (Day 0) compared with their respective Day -1 baseline values. Horizontal activity in the surgery group was significantly lower compared with the sham group on the day of surgery. Vertical activity in the surgery group was significantly lower compared with the sham group on the day of surgery and day 1 post-surgery.

Light phase home cage activity: Horizontal and vertical activity did not differ between the groups at Day -1 baseline. Both the sham and surgery groups displayed significantly higher levels of vertical activity on days 3, 4 and 5 post-surgery compared to baseline activity levels.
Vertical activity in the surgery group was significantly lower compared with the sham group on the day of surgery.

The effect of inguinal hernia repair surgery on home cage locomotor activity in rats. (A) horizontal activity (B) vertical activity. Data are mean ±SEM (n=12 per group) beam counts per hour. Surgery or Sham procedures were carried out on Day 0 and only post-procedure data are presented for this day. For horizontal activity in the dark phase there was a significant main effect of time ($F_{6,132}=2.32$, $p<0.001$) and a time x surgery interaction ($F_{6,132}=8.65$, $p<0.001$) but no effect of surgery and for vertical activity in the dark phase there was a significant effect of time ($F_{6,132}=2.32$, $p<0.001$), surgery ($F_{1,22}=6.76$, $p<0.001$) and a time x surgery interaction ($F_{6,132}=9.18$, $p<0.001$). For horizontal activity in the light phase there was a significant main effect of time ($F_{6,132}=8.07$, $p<0.001$) but no significant effect of surgery or time x surgery interaction.
and for vertical activity in the light phase there was a significant main effect of time \((F_{6,132}=29.74, \ p<0.001)\) and a time x surgery interaction \((F_{6,132}=5.13, \ p<0.001)\). 

\( *p<0.05, **p<0.01, \) vs. respective baseline (Day -1); \( +p<0.05 \) ++\( p<0.01 \) sham vs. surgery.

**Incision only vs. Surgery Comparison**

**Dark phase home cage activity:** Horizontal and vertical activity did not differ between the groups at Day -1 baseline. Levels of horizontal and vertical activity were significantly higher on days 4 and 5 post-surgery in both the incision only and surgery groups compared with their respective Day -1 baselines. Horizontal activity in the surgery group was also significantly higher on day 3 post-surgery and vertical activity in the surgery group was significantly lower on the day of surgery compared with their respective Day -1 baselines. Vertical activity in the surgery group was significantly decreased compared to the incision only group on the day of surgery.

**Light phase home cage activity:** Horizontal and vertical activity did not differ between the groups at Day -1 baseline. Vertical activity in the surgery group was significantly decreased compared to the incision only group on the day of surgery.
The effect of inguinal hernia repair surgery or an incision only procedure on home cage locomotor activity (A) horizontal vertical and (B) vertical activity. Data are mean + SEM (n=12 per group). For horizontal activity in the dark phase there was a significant effect of time (F<sub>6,132</sub>=29.88, p<0.001), but not surgery or a time x surgery interaction and for vertical activity there was a significant effect of time (F<sub>6,132</sub>=19.95, p<0.001) and surgery (F<sub>1,22</sub>=5.68, p<0.05) but no time x surgery interaction during the dark phase.

For horizontal activity in the light phase there was a significant effect of time (F<sub>6,132</sub>=5.42, p<0.001) but not surgery or a time x surgery interaction. For vertical activity during the light phase there was a significant effect of time (F<sub>6,132</sub>=19.95, p<0.001) and surgery (F<sub>1,22</sub>=5.68, p<0.05) but no a time x surgery interaction  *p<0.05,
**p<0.01 vs. respective baseline; +p<0.05, ++p<0.01 incision only vs. surgery at specific time point.
**Von Frey testing**

Repeated measures ANOVA revealed a significant main effect of time ($F_{3,66}=25.727$, $p<0.001$) but no significant effect of surgery or time x surgery interaction on the 50% response threshold in the von Frey test.

*Post-hoc* analysis revealed that withdrawal thresholds did not differ between the groups at baseline. Withdrawal threshold in the surgery group was significantly lower at the 4h ($p<0.05$) and day 2 ($p<0.05$) time points compared to baseline pre-surgery thresholds in this group. A similar trend for reduced thresholds was observed in sham rats compared with pre-surgery thresholds, however this failed to reach statistical significance. There were no significant differences in withdrawal threshold between sham and surgery rats in this experiment or between vehicle-treated sham and surgery rats in subsequent pharmacological studies (data not shown) and thus von Frey thresholds do not appear to be a useful index of post-surgical pain in this particular model.

![Graph](image)

**The 50% withdrawal threshold to Von Frey filaments. Results are mean ± SEM (n=12 per group). *p<0.05 vs respective baseline.**
von Frey testing

Repeated measures ANOVA revealed a significant effect of time (F\(_{3,66}=53.18, p<0.001\)) but not surgery (F\(_{1,22}=3.79, p>0.05\)) or a time x surgery interaction (F\(_{3,66}=2.08, p>0.05\)). Post-hoc analysis revealed that both groups displayed a significantly decreased response threshold at 4h, day 2 and day 6 post-surgery (p<0.01).

The effect of inguinal hernia repair surgery or an incision only procedure on the 50% withdrawal threshold to von Frey filaments. Data are mean + SEM (n=12 per group). *p<0.05, **p<0.01 vs respective baseline.
Effect of morphine, carprofen or paracetamol on home cage horizontal activity over first 10h post surgery

Morphine

Two-way ANOVA, with surgery and morphine treatment as factors, revealed a significant main effect of surgery ($F_{1,48}=21.85$, $p<0.001$), but not drug treatment or surgery x drug treatment interaction, on home cage horizontal activity during the 1-10h period post surgery. Post-hoc analysis revealed that the surgery saline and surgery morphine 7mg/kg groups displayed significantly lower levels of activity compared to their respective sham groups ($p<0.05$). However, this was not the case for the surgery 10mg/kg morphine group, indicating that this dose of morphine prevented the surgery-induced reduction in horizontal locomotor activity, with no significant effect in sham animals.

Carprofen

Two-Way ANOVA revealed a significant main effect of surgery ($F_{1,51}=25.103$, $p<0.001$) and a surgery x drug treatment interaction ($F_{2,51}=3.208$, $p<0.05$) on home cage horizontal activity over the first 10h post surgery. Post-hoc analysis revealed that the surgery saline group displayed significantly lower levels of horizontal activity compared to sham saline group ($p<0.05$). Carprofen dose-dependently attenuated this surgery-induced reduction in home cage horizontal activity, with the surgery 10mg/kg group displaying significantly higher levels of horizontal activity compared to the surgery saline group ($p<0.05$). Carprofen had no significant effects at either dose on horizontal activity in sham animals.
Two-Way ANOVA revealed a significant main effect of surgery ($F_{1,42}=49.05$, $p<0.001$) but not drug treatment or a surgery x drug interaction on home cage horizontal activity during the period 1-10h post-surgery. *Post-hoc* analysis revealed that the surgery vehicle group displayed significantly lower levels of activity compared to the sham vehicle group ($p<0.05$). Paracetamol had no significant effects on this surgery-induced reduction in horizontal locomotor activity in the home cage. Paracetamol had no significant effects at either dose on horizontal activity in sham animals.
The effect of (A) morphine (7 or 10mg/kg, s.c.) (B) carprofen (5 or 10mg/kg) and (C) paracetamol (75 or 150mg/kg s.c.) on home cage horizontal activity over the first 10h following surgery. Data are mean ± SEM (n=9-10 per group). *p<0.05 vs. sham counterpart. #p<0.05 vs. surgery saline
Effect of morphine, carprofen or paracetamol administration on horizontal locomotor activity in the open field

Morphine

Repeated measures ANOVA revealed a significant main effect of time ($F_{4,192}=76.85, p<0.001$), surgery ($F_{1,48}=5.92, p<0.05$), drug treatment ($F_{2,48}=5.69, p<0.05$), time x surgery interaction ($F_{4,192}=5.18, p<0.05$) and time x drug treatment interaction ($F_{8,192}=6.95, p<0.001$) on horizontal activity in the open field. Post-hoc analysis revealed that all groups (both sham and surgery, saline and morphine-treated groups) displayed significantly lower levels of activity at 1h post-surgery compared to baseline pre-surgery activity in these groups ($p<0.05$). The surgery saline group displayed lower levels of activity at 1h, 2h, 4h and Day 1 post-surgery compared to baseline pre-surgery activity in this group ($p<0.05$). The surgery 7mg/kg morphine group displayed significantly lower levels of activity at 1h, 2h, 4h and Day 1 post-surgery compared to baseline pre-surgery activity in this group ($p<0.05$). The surgery rats treated with 10mg/kg morphine displayed significantly lower levels of activity at 1h and day 1 post-surgery compared to baseline pre-surgery activity in this group ($p<0.05$). The sham saline group displayed significantly lower levels of activity at 1h and 2h post-surgery compared to baseline pre-surgery activity in this group ($p<0.05$). The sham rats treated with 10mg/kg morphine displayed significantly higher levels of activity at 4h post surgery compared to baseline pre-surgery levels ($p<0.05$). The surgery saline group displayed significantly lower levels of activity compared to the sham saline group at 2h post-surgery ($p<0.05$). At 2h post-surgery the 7 and 10mg/kg morphine surgery animals displayed significantly higher levels of activity ($p<0.05$) while the sham 7mg/kg morphine group displayed increased levels of activity compared to the saline-treated animals.
**Carprofen**

Repeated measures ANOVA revealed a significant main effect of time (\(F_{4,204}=181.42, p<0.001\)), surgery (\(F_{1,51}=4.98, p<0.001\)), and time x surgery interaction (\(F_{4,204}=7.63, p<0.001\)) on horizontal activity in the open field. *Post-hoc* analysis revealed that activity in all of the groups was significantly lower at the 1h and 2h time points compared to baseline pre-surgery activity in these groups (\(p<0.05\)). Activity of the surgery groups (saline and carprofen treated) was also significantly lower at 4h and Day 1 post surgery compared to baseline pre-surgery activity in these groups (\(p<0.05\)). Sham rats that received 5mg/kg carprofen displayed significantly lower activity at 4h and Day 1 when compared to baseline pre-surgery activity (\(p<0.05\)). Activity of surgery saline treated rats was significantly lower than that of sham saline treated animals at the 2 and 4h time points (\(p<0.05\)). This surgery-induced reduction in horizontal activity at the 2h time point was unaffected by carprofen treatment. At the 4h time point, the surgery carprofen 10mg/kg group (\(p<0.05\)) displayed significantly lower levels of activity compared to its respective sham group. However this was not the case for the surgery 5mg/kg group, indicating that this dose of carprofen prevented the surgery-induced reduction in horizontal locomotor activity, with no significant effect in sham animals.

**Paracetamol**

Repeated measures ANOVA revealed a significant main effect of time (\(F_{4,168}=122.14, p<0.001\)), surgery (\(F_{1,42}=8.41, p<0.01\)) and time x surgery interaction (\(F_{4,168}=5.09, p<0.001\)) but no significant main effect of drug treatment (\(F_{2,42}=0.36, p>0.05\)) on horizontal activity in the open field. *Post-hoc* analysis revealed that horizontal activity in all groups was significantly lower at the 1h and 2h time points compared to baseline pre-surgery activity in these groups (\(p<0.05\)). Activity in the surgery groups (vehicle- and paracetamol-treated) was
also lower at the 4h time point compared to baseline pre-surgery activity in these groups ($p<0.05$). Horizontal activity in the surgery vehicle group was significantly lower than activity in the sham vehicle group at the 2h and 4h time points ($p<0.05$). Paracetamol had no significant effects on these surgery-induced reductions in horizontal locomotor activity in the open field. Activity in the sham 75 and sham 150mg/kg groups was significantly higher than activity in the sham vehicle group on day 1 post-surgery ($p<0.05$).
The effect of (A) morphine (7 or 10mg/kg, s.c.) (B) carprofen (5 or 10mg/kg) and (C) paracetamol (75 or 150mg/kg s.c.) on open field horizontal activity following surgery.
Data are mean + SEM (n=9-10 per group). *p<0.05 vs. respective baseline. #p<0.05 vs. surgery saline at specific time point. +p<0.05 vs. sham saline at specific time points. @p<0.05 vs. sham 10mg/kg carprofen at specific time point.
**ATF-3 Staining 24h post-surgery**

Qualitative analysis revealed that there was no difference in ATF-3 expression between surgery and sham rats in the ipsilateral DRGs at the levels corresponding to the entry of the 3 inguinal nerves (T13-L2).

Representative images of ATF-3 stained T13 DRG in sham and surgery rats. Scale bar = 120 µm.