Housing environment influences the need for pain relief during post-operative recovery in mice

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A R T I C L E   I N F O

Article history:
Received 22 December 2009
Received in revised form 28 January 2010
Accepted 29 January 2010

Keywords:
Self-administration
Pain relief
Environmental enrichment
Social housing
Mice
Post-operative recovery

A B S T R A C T

The impact of invasive experimental procedures on perceived stress and pain may be dependent on both physical and social environmental conditions. The aim of this study was to evaluate the effects of a physically and a socially enriched environment on the need for pain relief following painful experimental procedures. A non-invasive method to administer analgesics post-operatively is by means of self-administration which is a feasible objective method to measure perceived pain during the post-operative recovery period. In the present study eight groups of mice housed in different conditions underwent the surgical procedure of caecal manipulation or only exposure to anaesthesia. After surgery the mice were given the choice to self-administer an analgesic available in one of their water bottles during two post-operative weeks. It was shown that socially enriched mice drank i.e. self-administered, less from the analgesic containing water than the non-enriched and socially deprived groups. Mice that underwent operation self-administered more analgesic than mice that received only anaesthesia without operation. The findings indicate that the recovery environment can contribute positively to attenuate the need for pain relief in animals submitted to invasive procedures.

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1. Introduction

Laboratory mice and rats are frequently subjected to a variety of surgical and other painful procedures in order to serve as models for the understanding of diseases, development and testing of treatments and medicines and improvement of surgical methods. As most of these invasive procedures involve stress and pain, animals are generally anaesthetised during these procedures. However, post-operatively animals may perceive pain, but assessing the grade of pain in these small animals is difficult, because there is no universal objective indicator which can be applied. Moreover the impact of invasive experimental procedures on perceived stress and pain may be dependent on both physical and social environmental conditions. Environmental conditions have a significant impact on the well-being of laboratory animals including neurochemical, physiological and behavioural functions [1,2]. It is known that both cage enrichment and social housing can influence stress response, and increase the ability to adapt to novel situations in rodents. In general, cage enrichment leads to a reduction of fear and an increased ability to adapt to new situations in mice [3–6]. Enriched mice appear less stressed and less frightened; they also have a broader behaviour repertoire and are easier to handle [5]. Furthermore, differential housing such as cage enrichment for rats has been shown to play an important role in functional recovery after experimentally induced brain injuries [7–10].

Group housing of social living animals like mice, rats and guinea pigs reduces the stress response, when compared to single housing [11–14]. In an earlier experiment we found that both ovariectomised and control mice socially housed in groups showed significantly more locomotion and rearing in an open field test compared to singly housed mice during post-operative recovery phase [13]. In another study where several physiological and behavioural parameters were assessed after surgery for telemetry transmitter implantation, it was shown that mice benefit most from social housing in terms of post-operative recovery and distress [15]. This experiment indicated that social housing indeed may enhance post-operative recovery.

Although measuring persistent pain in laboratory rodents is extremely difficult, Roughan and Flecknell [16,17] have demonstrated that laparotomised rats and mice perform behaviours that can be associated with pain and that providing analgesia reduces these behaviours. Similarly, a study assessing post-operative pain in mice [18] showed that the mice receiving no analgesic treatment after laparotomy demonstrated significant changes in telemetry electrocardiogram recordings. Furthermore, Colpaert et al. [19] demonstrated that rats with adjuvant arthritis would self-administer opiates.
Although analgesics might interfere with experimental results, unnecessarily prolonging pain might do so as well. A quick recovery after surgical interventions with the least possible use of analgesics will thus benefit both animal and experiment. Analgesics, administered post-operatively after intestinal manipulation, reduced pain and discomfort, resulting in a quicker recovery in rats in terms of an increased food intake [20,21]. Prolonged recovery after surgery may lead to prolonged lack of appetite, hypothermia, reduction in food intake and weight loss [16,17,22], which affect the well-being of the animal and consequently the experimental outcome. Laboratory rats can learn to self-administer analgesics via the drinking water, some studies reported that rats suffering from painful adjuvant-induced arthritis consumed more of an analgesic solution than control non-arthritic animals [19,23]. Chicken (meat poultry) which experienced lameness due to problems associated with breeding, growth and husbandry, selected more food drugged with analgesics than non-lame birds, and when lameness increased, these lame birds consumed a greater proportion of the drugged food [24]. These studies support the idea that the presence of pain would modulate the behaviour of drug self-administration which in turn can be objectively and quantitatively measured as indicator of pain. Also clinical studies in humans have demonstrated the importance and reliability of patient controlled analgesic drug intake as an important objective to measure pain in humans [25,26]. Primarily since the early 1980s, patient-controlled analgesia (PCA) has been extensively used for treatment of post-operative pain with opioids. This self-administer paradigm permits individuals to regulate their dosing intervals through operant behaviour [27]. In the same way we hypothesized that mice are able to balance their intake of analgesia in order to match their level of pain. Since both the physical and social environment play a crucial role in the well-being of an animal [28], we hypothesized that a physically or socially enriched environment might influence the animals’ well-being in a positive way, and when given the choice to self-administer pain medication after a surgical procedure, they might require less analgesia in terms of amount and duration than the animals from a non-enriched environment. To investigate this, mice housed under differential physical and social conditions were subjected to laparotomy and caecal manipulation. During the post-operative recovery, self-administrations of analgesics as well as parameters indicative of recovery were measured. In this study Ibuprofen (Iprem®, a nonsteroidal anti-inflammatory drug (NSAID)) was used as post-operative analgesic. Iprem is also available in oral suspension formula, which can easily be dissolved in the drinking water. The analgesic effect, however, might not only be dependent on the choice of the analgesic, amount and time-interval, but also on the state of well-being of the animal. According to the 3R principle the number of animals used for experimentation should be reduced to a minimum. Hence we used animals which were part of another experiment where caecal manipulation was required for a post-operative shock model.

2. Materials and methods

Seventy-two female C57BL/6Sca inbred mice, about 21–25 g at arrival were used. The mice were purchased from a commercial breeder (B&K Universal AB, Sollentuna, Sweden). They were housed in Makrolon arrival were used. The mice were purchased from a commercial breeder

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2.2. Self-administration

At the start of the study, all animal cages were equipped with 2 × 250 ml bottles of drinking water (SCANBUR A/S, Denmark). During 1 week (baseline) prior to surgery, both bottles contained only tap water. On the day of surgery and for a period of 2 weeks post-operatively, following caecal manipulation or anaesthesia, all mice received in one of the bottles the analgesic that they could self-administer. The analgesic used was Ibuiprofen (Iprem®, 20 mg/ml, McNeil, Sweden) a non-steroidal anti-inflammatory drug (NSAID) in oral suspension form, mixed with tap water for the maximum recommended daily dose of 40 mg/kg body weight based on the literature [29]. For each cage Ibuiprofen solution was available in one of the bottles, the other bottle contained tap water only. Half of the groups received Iprem in the bottle at the right side of the cage, the other half at the left side. New Iprem solution and fresh tap water were provided during scheduled water changing routine twice per week.

2.3. Surgical trauma model

All surgical procedures were performed by the same skilled lab technician (BH). The mice were brought to the surgical room at 08:00 h, the same time for all operating days. For each operation, two mice were anaesthetised simultaneously: one mouse underwent laparotomy and caecal manipulation, and the control mouse received only anaesthetic during the time the surgical procedure was performed on the caecal manipulated mouse. Two animals from each of the 8 experimental groups were treated per day. The procedures took place between 8–12 am. For induction, mice were put in airtight anaesthetising chambers containing 4% isofluorane. For maintenance the animals were removed from the chamber and allowed to breathe isoflurane (1.5–2.0%) through a nose cone connected to the vaporizer (Univentor 400, Aghtho’s, Lidhö, Sweden). The depth of anaesthesia was controlled during all procedures by means of the toe pinch reflex.

During anaesthesia and surgery, mice were placed on heating pads to maintain body temperature. A 2-cm mid-abdominal incision was made; the caecum was then exteriorized and gently held in moist

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Socially housed</th>
<th>Enrichment</th>
<th>Only anaesthesia</th>
<th>Surgery</th>
<th>N</th>
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<td>1-EC-Op</td>
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<tr>
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<td>No</td>
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<tr>
<td>5-EL-Op</td>
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<td>Yes</td>
<td>Yes</td>
<td>n = 6</td>
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<tr>
<td>6-EL-An</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>n = 6</td>
</tr>
<tr>
<td>7-NEI-OP</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>n = 6</td>
</tr>
<tr>
<td>8-NEI-An</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>n = 6</td>
</tr>
</tbody>
</table>
gauze for 3 min. The caecum was then returned to the abdominal cavity, the peritoneal cavity filled with 0.5 ml saline and the incision closed using two layers of absorbable suture (Vicryl®, 4-0, Ethicon Inc., Johnson & Johnson, Langhorne PA, USA). The duration of the surgical procedure was about 15 min. When the surgical procedure was completed, isoflurane anaesthesia was switched off for both the operated mouse and the control mouse. After surgery, animals were returned to their cages and had free access to tap water and ipren water. All experimental procedures in this study are in compliance with National Institutes of Health Guide for Care and Use of Laboratory Animals, the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by The Southern Stockholm Animal Ethics Committee.

2.4. Parameters

The individual body weight of the mice was measured daily. The amount of fluid intake from each cage was monitored by weighing the drinking bottles everyday over the entire period of the study. From post-operative (POD) day 1, checking for wound healing was carried out before body weight was taken and scored as intact or damage/broken wound sutures; presence or absence of infection/sore; and sign of wound biting. Additionally, before weighing of the animals and drinking bottles, daily observations of the shape and condition of the nest were carried out as indicators for pain. In the non-enriched cages any nest made in the bedding was noted.

2.5. Statistics

Statistical analysis was performed using STATISTICA v.8 software (StatSoft Inc., Tulsa, Oklahoma, USA). Body weight data was analyzed for both the baseline and post-operative period by means of repeated measures analysis of variance (ANOVA) with day as the within factor and housing, social condition and surgery as between factors. When appropriate, ANOVA was followed by Tukey HSD post-hoc test. The preference for the left or the right bottle provided during the baseline was assessed by running an independent paired t-test on the total amount of water consumed from the left and respectively the right side during the baseline condition. Descriptive statistics were used for wound healing, suture nibbling, and nest site and nest complexity.

For the analysis of all independent factors on repeatedly measured variables (post-op. body weight and water consumption) the GLM module was used with Housing condition (E/NE), Social condition (G/I) and Caecal Manipulation (Op/An) as independent variables.

Differences were considered significant when $p < 0.05$.

3. Results

3.1. Body weight

All mice gradually increased their body weight during the baseline period. To rule out any systematic bias in bodyweight between animals that were assigned to the enriched and non-enriched groups the data for the 8 days prior to operation (D8) was analyzed by repeated measures ANOVA with enrichment (E/NE) as the independent variable. Although a trend was found in body weight between the enriched and non-enriched groups with a lower bodyweight for the non-enriched groups, no significant difference was found ($p = 0.0708$). As can be expected in young adult mice, a significant day effect was apparent ($F(7, 441) = 10.191, p < 0.001$) due to a slight increase in body weight during the first week of the experiment.

The body weight was significantly affected by the surgical procedure. A significant interaction was seen between Day and Manipulation ($F(14, 882) = 7.76, p < 0.001$) and between Day and Social condition ($F(14, 882) = 1.79, p < 0.05^*$). Two main effects of Day ($F(14, 882) = 12.57, p < 0.001$) and Caecal Manipulation ($F(1, 63) = 6.15, p = 0.05$) were revealed by the same analysis. Tukey post hoc test confirmed a significant difference between the body weight of operated and anaesthesia only mice ($p = 0.05^*$) with the operated mice weighing less than the anaesthetised mice (Fig. 1a, b). Further post hoc analysis showed that the body weight of operated mice significantly decreased on POD1 (Tukey $p < 0.001^{***}$) and slowly recovered to baseline over the next 9–12 days. In the anaesthesia-only groups we did not detect any drop in body weight over the 2 week post-experimental period (Table 2).

3.2. Baseline water consumption from the two-bottle system

Prior to the operation day no significant difference in water consumption was detected between the 8 study groups ($p > 0.05$). The mean ($±$ S.E.M.) daily water consumption from the bottles was between 9.48–11.05 ml/cage/day ($± 1.09–1.55$). During this period in each cage there was a clear preference for one of the 2 bottles, with an overall preference for the bottles placed to the right side (paired $t$-test $p < 0.001$, Fig. 2).
3.3. Ipren self-administration

Daily consumption of Ibuprofen solution was analyzed in a General Linear Model with the following factors as independent variables: Housing condition (E/NE), Caecal Manipulation (Op/An), Social condition (G/I) and the Position of the bottle containing the analgesic (Left/Right). This analysis showed a significant 3 way interaction between day, manipulation and housing condition (F(13, 299) = 2.00, p < 0.05), as well as 2 way interactions between (1) Day and Manipulation (F(13, 299) = 3.04, p = 0.001) and (2) Day and Social condition (F(13, 299) = 1.79, p = 0.05) (Fig. 3). Operated mice drank more Ipren water than tap water. Non-enriched and Enriched, individually housed mice (operated and anaesthesia only) drank more Ipren water than group housed animals. As expected from the baseline results, during the post-operative days there was a significant preference for the bottles positioned to the right (F(1, 23) = 71.83, p = 0.001), which did not interact with the other factors. Another significant main effect was due to the Social condition (F(1, 23) = 25.72, p = 0.001) (Fig. 3) with the individually housed mice consuming more ibuprofen daily (Tukey p < 0.001). Further post hoc analysis showed that in the NE anaesthesia only group there was no change in the daily consumption of ibuprofen whereas in the NE operated group there was a slow increase. For the enriched groups that received anaesthesia only i.e. (EG-An; EI-An), these mice consumed slightly less ibuprofen over days whereas for the operated group there were no time dependent changes. Individually housed mice (operated and anaesthesia only) drink more Ipren water than group housed counterparts.

Nest condition/building did not show differences between the groups (data not shown). All operated animals healed properly. Wound healing scores in terms of sore, broken sutures or open wounds showed no differences between groups.

4. Discussion

In this study we investigated the impact of environmental conditions such as cage enrichment and social housing on post-operative recovery and the need for pain relief in mice. Self-administration of an analgesic during post-operative recovery was used as indication of the need for pain relief. The results indicate that the housing environment significantly influenced post-operative recovery and self-administration of analgesics. Mice that underwent abdominal surgery (caecal manipulation) self-administered more analgesic in their drinking water than control mice that received only anaesthesia.

One of the major consequences of the surgical procedure was the drop in body weight in mice that underwent caecal manipulation. The weight loss was consistent in all groups that were operated. However, about one and a half weeks post-operatively, the enriched mice were able to regain body weight and showed similar growth as the control enriched mice that received only anaesthesia. Regain of body weight during the post-operative period was less favourable in the non-enriched groups. In particular, the individually non-enriched housed mice that underwent surgery did not manage to reach their baseline body weight at 2 weeks post-operation.

It could be questioned whether intake of ibuprofen influenced reduced weight gain in the operated mice. Ibuprofen belongs to the non-selective group of NSAIDs known for their ability to block prostaglandin biosynthesis and are used to control post-operative pain mainly due to their analgesic activities [30]. The anti-inflammatory and analgesic effect of NSAIDs interfere with the initiation of pain in the periphery and by reducing the noxious input into the CNS and thereby attenuating the systemic stress response observed after surgical trauma [31]. As a consequence in the operated mice, a reduced overall stress response due to ibuprofen intake would improve post-operative well-being and increase food intake to re-gain lost weight. Therefore, it is less likely that ibuprofen contributed to reduced weight gain in the operated mice.

As expected from previous pilot studies, the mice showed a significant preference for the bottles positioned to the right. The reason for this might be due to the common practice at the breeder and also at our facility to place the drinking bottle on the right side of the food hopper. As in this study we provided half of the groups with the bottles on the right and the other half on the left, this finding did not compromise our results.

Another significant main effect was due to the housing condition. The individually housed mice consumed more ibuprofen, than the operated ones as well as the anaesthesia only animals. Further post hoc analysis showed that in the NE anaesthesia only group there was no change in the daily consumption of ibuprofen whereas in the NE operated group there was a slow increase. For the enriched groups

Table 2
Body weight in anaesthetised groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean BW (g) D8 (baseline)</th>
<th>S.E.M.</th>
<th>Mean BW (g) POD 14</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG-An</td>
<td>25.23</td>
<td>0.47</td>
<td>26.25</td>
<td>0.42</td>
</tr>
<tr>
<td>NEG-An</td>
<td>24.93</td>
<td>0.52</td>
<td>25.34</td>
<td>0.52</td>
</tr>
<tr>
<td>EI-An</td>
<td>25.83</td>
<td>0.99</td>
<td>26.58</td>
<td>0.89</td>
</tr>
<tr>
<td>NEI-An</td>
<td>25.12</td>
<td>0.85</td>
<td>25.52</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Body weight (BW) means and S.E.M. for the anaesthetised groups, measured prior to the operation day and 2 weeks after on post-operative day (POD) 14.
that received anaesthesia only (EG-An; EI-An), these mice consumed slightly less ibuprofen over days whereas for the operated group there were no time dependent changes. The results obtained from this study showed that over the 2 week post-operative recovery time the non-enriched mice that underwent abdominal surgery increased their self-administration of Ipren significantly more than operated mice housed in enriched condition (Fig. 3d, b). This interesting interaction effect of housing and manipulation over 2 weeks of recovery was not seen in the mice that received anesthesia only. The enriched operated mice showed similar levels of Ipren self-administration as both the non-enriched and the enriched groups that were not operated, while in the non-enriched operated mice, the levels of self-administered Ipren water increased over post-operative recovery time. These results support our hypothesis that housing with species specific enrichment such as a soft nest would lessen the need for pain relief during post-operative recovery period. The impact of environmental enrichment found in this study is in agreement with previous enrichment studies in rodents[5,32,33] although the effect of social housing appeared to be more pronounced than the impact of physical enrichment such as nesting material and gnawing sticks which is in agreement with previous results in the rat[33].

Nest building behaviour in mice following surgery as an indication of post-operative pain was described by Arras et al.[18]. Mice that underwent operation destroyed their nests and took a longer time to rebuild their nest than non-operated mice. We chose to provide nesting material from the start of the study, a week before the mice were subjected to caecal manipulation. It was observed and recorded that all enriched mice used the tissues to build and maintain the nest for resting and sleeping, no destruction of any nest was noted on the day following operation (data not shown). The C57BL/6 mice used in this study demonstrated a stable self-administration from the two drinking bottles, and even though there was a preference for the bottle at the right position the amount of total fluid intake did not change from baseline with two tap water bottles to the post-operative period when one of the water bottles contained Ipren solution. Concerning pain, it is known from studies in arthritic rats that pain has a direct effect on the animals’ self-administration behaviour[34,35]. Also the relevance of emotional stress for the animal’s self-administration behaviour has been reported. Several studies have shown that stress altered self-administration of drug behaviour e.g. social isolation enhances oral ingestion of ethanol and opiates[36–38], immobilization stress facilitates the preference for morphine and fentanyl solutions[39], and emotional stress induced by witnessing another rat being subjected to foot shock but not physical stress induced by experiencing foot shock or hot plate, increases intravenous cocaine self-administration[40]. The emotional stress influence on self-administration of analgesics therefore was in line with our findings that the environment has significant impact on the post-operative recovery period. We showed that the difference in the levels of Ipren self-administration between operated and non-operated mice was dependent on the housing conditions and time, such that the non-enriched operated group gradually increased their Ipren intake over time. In a study on housing conditions affecting self-administration of an anxiolytic solution, Sherwin and Olsson[41] reported that mice housed in barren standard cages drank more Midazolam solution than mice housed in enriched cages. Likewise, in this study we found that the social environment of being housed individually or in a group had significant impact on self-administration of Ipren water independent of whether the mice underwent surgery or received anaesthesia alone (Fig. 3b, d). The increased Ipren self-administration in individually and non-enriched mice supports the idea that lack of social support from cage mates and essential cage enrichment that ensures species specific needs such as nest building in mice would lead to greater emotional stress. This might indicate that emotional stress more than physical discomfort made the mice more sensitive to the need for pain relief. These results also substantiate our previous findings where socially housed mice recovered quicker and

![Fig. 3. Daily tap water intake (panels a, c) and Ipren water intake (panels b, d) during the 2 week post-operative period in the operated and control anaesthesia only groups. Operated mice drank significantly more Ipren than tap water; singly housed mice drank more Ipren water than group housed mice (p < 0.001).](image-url)
showed less stress following surgery for implanting a telemetry device compared to single housed mice and mice separated from each other by a grid (Living Apart Together) [15]. The findings also indicate that using the enrichment which has been previously shown to be preferred by mice [42], in this case nesting material and social partners, may modify the need for post-operative pain relief. Assessing post-operative pain in nonverbal experimental rodents is difficult and often subjective since pain behaviour might be masked by the intrinsic natural behaviour of these prey animals not to show clear signs of pain as any signs of weakness or pain can be detrimental for the likelihood of survival of prey animals. The ability to assess behavioural changes in laboratory animals that underwent experimental procedures is necessary, both in order to measure the severity of pain and also to determine the efficacy of the analgesic provided. In this study, we found that self-administration is a useful and easy means to provide post-operative pain relief to laboratory mice and that the potential for using self-administration as an objective method to assess pain depends on the home cage housing conditions.

Acknowledgements

This study was supported by the grants from the Swedish Animals Protection Authority (Djurskyddsmyndigheten) and the Swedish Scientific Research Council (Vetenskapsrådet).

References