Housing of female mice in a new environment and its influence on post-surgical behaviour and recovery

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ABSTRACT

The transportation of mice into a new clean cage after surgery is a standard procedure but might have detrimental effects during the critical post-surgical recovery phase. To analyse the effect of post-surgical housing, female C57BL/6J mice housed in their familiar home cage or in a new environment after minor surgery ± analgesia, anaesthesia only or no treatment were monitored using non-invasive methods during the immediate postsurgical period to assess pain and general impairment. Behavioural investigations and burrowing test revealed no significant differences between housing conditions in untreated mice. While no appearance or posture abnormalities were observed post-experiment, home cage behaviours were affected distinctly. Behavioural rhythmicity was disrupted, and behaviours related to well-being, such as burrowing performance, were less compared to untreated mice. Burrowing latency ranged from an intermediate level following anaesthesia only and surgery with analgesia, to pronounced prolongation after surgery without analgesia in animals housed in their home cage, while after all experimental treatments burrowing latency in animals in new cages was prolonged dramatically. General activity and climbing behaviour in treatment groups housed in new cages tend to be higher compared to animals in familiar cages, leading to significant interactions between housing and treatment conditions ($p = 0.006$; $p = 0.014$). These behavioural differences in animals housed in a new environment compared to animals housed in their familiar environment might be interpreted as signs of reduced well-being, agitation and restlessness in the new cages and may hint that animals cope better with surgical stress when housed in their familiar environment. The post-surgical transport to a new and clean cage might therefore be an additional stressor after an exhausting event and may affect recovery.

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1. Introduction

Laboratory mice are housed under standardized husbandry conditions. In this environment, olfaction probably remains the most significant sense for the animal. Scent marks, originating from urine smears or other glandular sources of secretion such as salivary, plantar or preputial glands and deposited on the substrate, represent a major source of information (Fitchett et al., 2006; Van Loo et al., 2000). Many aspects of mouse behaviour rely on their ability to use odour cues, for example to distinguish among individuals, which is essential for maintenance of stable groups, recognition of offspring or mates, advertisement of dominance over a territory as well as for reproduction (Brennan, 2001; Gray and Hurst, 1995; Hurst et al., 2001). Olfactory cues are also used for orientation and to enhance the detection of novel objects (Hurst, 1987).

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Two common and rather drastic disturbances of these cues that nearly all mice in the laboratory undergo are cage cleaning and in-house transportation. Cage cleaning normally includes the change of the cage, the removal of all its contents and the transport of the mice into a new cage with fresh bedding and other fresh or autoclaved material. While this procedure is essential for hygiene, it disrupts the olfactory cues of mice and has often been described as a repetitive and frequent stressful event in the lives of laboratory rodents (Burn et al., 2006; Gray and Hurst, 1995; Van Loo et al., 2000). It is known that long-term frequent cleaning of cages causes chronic stress and depresses body weight gain in mice (Beynen and van Tintelen, 1990). In-house transport to an experimental laboratory or another animal room results in significant increase in plasma corticosterone concentration in mice and a decrease in thymus gland weight, leucocyte and lymphocyte count, and was therefore considered to be a stressful stimulus in mice (Drozdowicz et al., 1990).

The transportation of an animal after surgery into a new clean cage is a standard procedure in many facilities for several reasons, e.g. the potential health risk of soiled bedding. This procedure combines both stresses of in-house transport and cage cleaning and probably has a comparable or even higher impact on the animal. This procedure may therefore have detrimental effects on the animal during the critical post-surgical recovery phase.

Although the proximate effects of housing conditions on the animal's internal state may not always be obvious, they might affect the way animals respond to additional stressors. For example, Tuli and co-workers have shown that animals in new cages were more sensitive to transportation stress, with mice housed in their home cage recovering faster from this stressor (Tuli et al., 1995). These results led to the suggestion that housing in a new cage may hamper the animal's ability to cope with, and increase the vulnerability to, additional stressful episodes. Surgery and the post-surgical recovery phase represent stressful episodes for mice. Hence, housing conditions may influence an animal's vulnerability to surgical stress and may interfere with post-surgical recovery.

Here, we aimed to analyse the potentially beneficial effect on recovery of post-surgical housing in the home cage by comparing female C57BL/6J mice housed in their familiar home cage or in a new environment after minor surgery. To assess the impact of surgery and different housing conditions on well-being, we used a range of non-invasive behavioural measurements that can be applied in the animals' cage without provoking additional stress. Burrowing performance, changes in home cage behaviours and classical indices like clinical symptoms, overall appearance and body weight should allow recognition not only of post-surgical pain but also impairment of general condition, thus providing a broad picture of the animal's recovery.

We hypothesize that signs of pain and impaired well-being should be reduced in mice housed in their home cage if housing conditions with a stable physical and olfactory environment are beneficial to post-surgical recovery.

2. Animals

2.1. Ethics statement

The animal housing and experimental protocols were approved by the Cantonal Veterinary Department, Zurich, Switzerland, under license no. ZH 120/2008, and were in accordance with Swiss Animal Protection Law. Housing and experimental procedures also conform to European Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes and to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 2011).

2.2. Animals and housing

The animals were 64 female C57BL/6J obtained from our in-house breeding facility at the age of 6–8 weeks.

Animals' health status was monitored by a health surveillance programme according to FELASA guidelines throughout the experiments. The mice were free of all viral, bacterial, and parasitic pathogens listed in FELASA recommendations, except for Helicobacter species (Nicklas et al., 2002).

All animals were housed in groups of three to six animals for at least 3 weeks prior to testing in our animal room. Animals were kept in Eurotype III clear-transparent plastic cages ($425\,mm \times 266\,mm \times 155\,mm$) with autoclaved dust-free sawdust bedding and one nestlet[TM] ($5\,cm \times 5\,cm$), consisting of cotton fibres (Indulab AG, Gams, Switzerland) as nest building material. They were fed a pelleted and extruded mouse diet (Kliba No. 3436, Provimi Kliba, Kaiseraugst, Switzerland) ad libitum and had unrestricted access to sterilized drinking water. The light/dark cycle in the room consisted of 12/12 h with artificial light (approximately 40 lx in the cage). The temperature was $21 \pm 1\,°C$, with a relative humidity of $55 \pm 10\%$, and with 15 complete changes of filtered air per hour (HEPA H 14 filter). The animal room was insulated to prevent electronic and other noise. Disturbances, e.g. visitors or unrelated experimental procedures in the animal room, were not allowed.

3. Materials and methods

3.1. Experiments

3.1.1. Experimental housing and setup

During the whole experimental period animals were housed under standardized conditions as described above with the burrowing test setup in addition. As burrowing apparatus, a plastic bottle (standard opaque water bottle, 250 mL, 150 mm length, 55 mm diameter) filled with 138–142 g of food pellets identical to those of the animal's normal diet was used. An additional empty bottle of the same dimensions was provided to serve as a shelter (for detailed information, see Jirkof et al., 2010).

For acclimatization, animals were housed individually for 3 days under these conditions before experiments

started. The animals had no prior experience with behavioural testing.

3.1.2. Experimental design

Mice were observed directly after the experimental procedure. 32 mice were housed in their familiar home cage during the observation while the other 32 mice were transported directly after the experimental procedure to a new clean cage containing a similar, but clean, set up as during acclimatization. Eight mice of each housing condition were allocated randomly to one of three experimental groups: (1) surgery + anaesthesia (mice underwent anaesthesia and surgery without analgesic treatment); (2) surgery + anaesthesia + analgesia (mice underwent anaesthesia and surgery with analgesic treatment); (3) anaesthesia only; or received no experimental treatment.

3.1.3. Experiments and data acquisition

The experiment began with a subcutaneous injection of 2 μl/g body weight of phosphate buffered saline (PBS) for the surgery + anaesthesia and anaesthesia only groups. In the surgery + anaesthesia + analgesia group, 5 mg/kg body weight of the analgesic carprofen (Rimadyl^TM, Pfizer Inc., New York, NY, USA) was diluted in PBS and injected as 2 μl/g body weight. The animals were transferred 45 minutes later in transport cages to the nearby operating theatre. Mice were anesthetized with sevoflurane (Sevorane^TM, Abbott, Baar, Switzerland) as mono-anaesthesia. The anaesthetic gas was provided with a rodent inhalation anaesthesia apparatus (Provet, Lyssach, Switzerland); oxygen was used as carrier gas. After induction of anaesthesia in a Perspex induction chamber (8% sevoflurane, 600 mL/min gas flow), animals were transferred to a warming mat (Gaymar, TP500, Orchard Park, NY, USA) set at $39 \pm 1\,°C$ to ensure constant body temperature, and anaesthesia was maintained via a nose mask (4.9% sevoflurane, 600 mL/min gas flow). Eye ointment was applied, the fur was clipped and the operating field disinfected with ethanol (70%) in all animals. Mice in both surgery groups underwent a one-side sham embryo transfer. The incision in the abdominal muscle wall was closed with absorbable sutures (Vicryl^TM, 6/0 polyglactin 910, Ethicon Ltd, Norderstedt, Germany), and the skin was closed using skin staples (Precise^TM, 3 M Health Care, St Paul, MN, USA). Surgery was completed within 6–8 min in both surgery groups. Anaesthesia lasted 14–16 min in all groups. Animals were allowed to recover for 15–20 min on the warming mat before being transferred back to the animal room for subsequent behavioural observation.

Experimental treatments were completed at the start of the light phase by returning each mouse from its transport cage to the observation cage. This was the animal's familiar home cage containing the refilled burrowing test apparatus or a new clean cage containing a new and filled burrowing test apparatus. In the case of non-treated mice in their familiar home cage the test apparatus was just refilled. Observation began by starting the digital video recording.

Table 1
Ethogram of home cage behaviours according to Van Oortmerssen (1970).

	Home cage behaviours
Resting	Sitting or lying flat or curled up, sometimes with the eyes closed or nearly closed (includes sleeping)
Locomotion	Walking, running, jumping
Self grooming	Bouts of wiping, licking and nibbling the own fur with forepaws and tongue
Eating	Series of movements resulting in ingesting food
Drinking	Taking in liquids with series of licking movements of the tongue
Climbing	Climbing with all four feet at the cage grid
Burrowing	All behaviours linked with emptying the burrowing apparatus (digging, carrying etc. of material)
Nest building	All behaviours linked with nest building (arranging, pulling in, fraying etc.)

3.2. Behavioural analysis

3.2.1. Home cage behaviours

The recorded video sequences were analysed continuously using ObserverXT^TM software (Noldus, Wageningen, Netherlands) for the first 6 hours of the light phase. Durations of behaviours (resting, locomotion, self grooming, eating, drinking, climbing, burrowing, nest building; Table 1), and numbers of resting bouts were measured. General activity was calculated by summarizing all active behaviours (i.e. all home cage behaviours except resting). Non-defined behaviours were not recorded.

3.2.2. Burrowing performance

The burrowing test determines burrowing performance and can be used as simple method to assess post-surgical impairment in mice. Good performance in this test is defined as short latency to remove items from a tube-like apparatus (burrowing) (Jirkof et al., 2010). Burrowing was defined as the removal of more than three pellets from the apparatus within 10 s. The latency to burrow of each animal was measured. Measurement of latency was continued for 24 h if the animals did not start to burrow within the six hours of behavioural analysis.
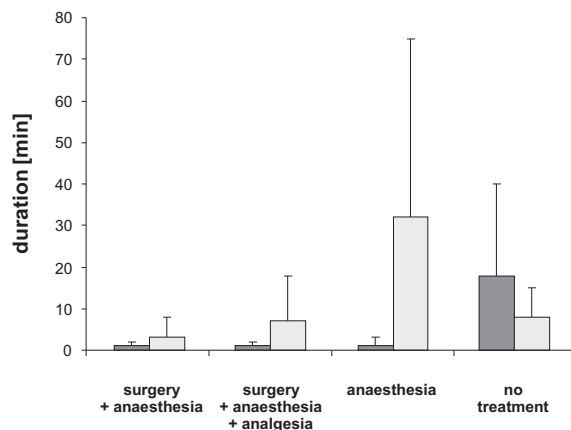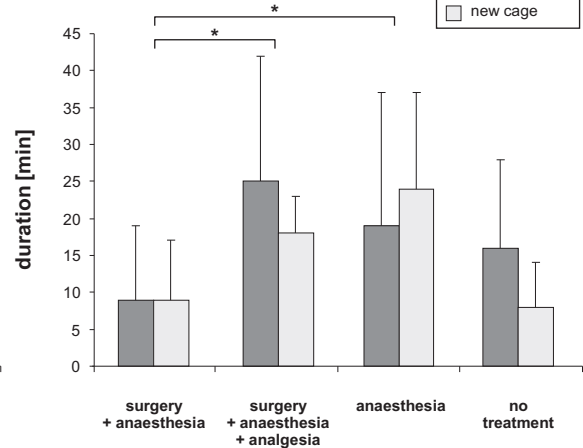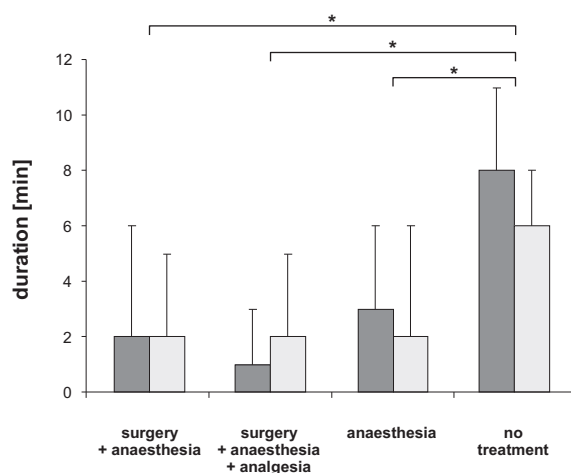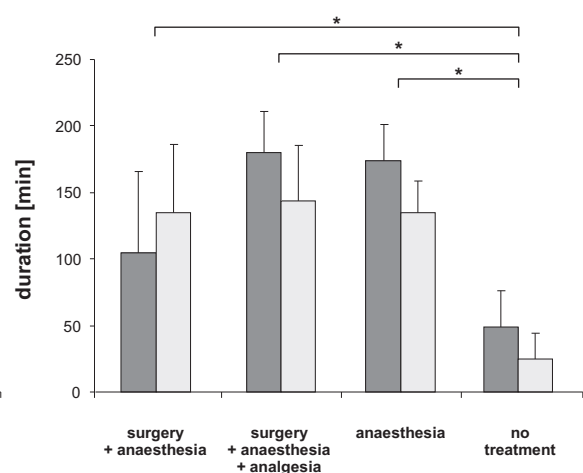
3.2.3. Clinical investigation

Animals were weighed at the beginning of the light phase 24 h before, and 24 and 48 h after experiment and observed for 20–30 s before, during and after weighing. According to a routinely used scoring system documenting the general condition of an animal (Arras et al., 2007), abnormalities of body condition (e.g. sunken flanks), fur condition (e.g. ruffled coat), eyes (e.g. discharge), breathing (e.g. irregular) and posture (e.g. hunched back) were registered, and wound healing, spontaneous behaviour and movement were assessed.

3.3. Statistical analysis

Statistical analyses were performed with SPSS 20.0 software (IBM, Armonk, NY, USA).

Table 2

Mean duration ± SD of home cage behaviours in minutes for animal housed in their familiar home cage or in a new cage after experimental or no treatment.

	Familiar cage				New cage			
	Surgery + anaesthesia	Surgery + anaesthesia + analgesia	Anaesthesia	No treatment	Surgery + anaesthesia	Surgery + anaesthesia + analgesia	Anaesthesia	No treatment
Behaviours (mean ± SD)								
Resting [min]	173 ± 50	114 ± 40	178 ± 79	198 ± 66	155 ± 47	125 ± 31	100 ± 44	247 ± 38
Locomotion [min]	48 ± 27	33 ± 15	56 ± 38	57 ± 23	41 ± 21	46 ± 17	34 ± 30	49 ± 19
Self grooming [min]	105 ± 61	180 ± 31	174 ± 29	49 ± 27	135 ± 51	144 ± 41	135 ± 24	25 ± 19
Eating [min]	9 ± 10	25 ± 17	19 ± 18	16 ± 12	9 ± 8	18 ± 5	24 ± 13	8 ± 6
Drinking [min]	1 ± 1	3 ± 3	2 ± 1	1 ± 1	1 ± 1	3 ± 1	2 ± 1	1 ± 1
Climbing [min]	1 ± 1	1 ± 1	1 ± 2	18 ± 22	3 ± 5	7 ± 11	32 ± 43	8 ± 7
Burrowing [min]	2 ± 4	1 ± 2	3 ± 3	8 ± 3	2 ± 3	2 ± 3	2 ± 4	6 ± 2
Nest building [min]	4 ± 6	1 ± 1	3 ± 5	10 ± 9	8 ± 10	6 ± 10	17 ± 18	11 ± 8

Fig. 1. (A) Climbing: a significant interaction between treatment and housing was found ($p = 0.014$); therefore no post hoc test was conducted for this behaviour. (B) Eating: eating duration showed significant differences between surgery with and without analgesia ($p = 0.028$) and surgery without analgesia and anaesthesia only ($p = 0.022$). (C) Burrowing: burrowing duration was significantly shorter in treatment groups (no treatment vs. surgery $p < 0.001$, no treatment vs. surgery + analgesia $p < 0.001$, and no treatment vs. anaesthesia $p = 0.001$). (D) Self grooming: grooming duration was significantly higher in treatment groups (no treatment vs. surgery $p = 0.009$, no treatment vs. surgery + analgesia $p < 0.001$, no treatment vs. anaesthesia $p < 0.001$).

Table 3

The effects of housing and treatment on analysed behaviours. When interactions were significant, main effects were not reported because they are abundant.

Behaviour (duration in min)	Main effects housing	Main effects treatment	Interaction housing × treatment
Climbing	–	–	$F = 3.859$; $p = 0.014$
Eating	$F = 0.829$; $p = 0.366$	$F = 4.856$; $p = 0.004$	$F = 0.958$; $p = 0.419$
Burrowing	$F = 0.363$; $p = 0.549$	$F = 10.946$; $p < 0.001$	$F = 0.716$; $p = 0.547$
Self grooming	$F = 0.899$; $p = 0.347$	$F = 10.877$; $p < 0.001$	$F = 0.789$; $p = 0.505$
Activity	–	–	$F = 4.521$; $p = 0.006$
Resting bouts	$F = 0.030$; $p = 0.864$	$F = 21.375$; $p < 0.001$	$F = 0.626$; $p = 0.601$

All data was tested for normal distribution and homogeneity of variance (Shapiro-Wilks, Levene's test). If necessary, data was log $(X + 1)$ transformed to meet assumptions of statistical tests.

Mean and standard deviation (SD) of durations of home cage behaviours and numbers of resting bouts were calculated.

Discriminant analysis was used to determine behaviours mainly responsible for group separation. The determined behaviours were further analysed using multivariate general linear model (GLM) with experimental group and housing as fixed factors. Post hoc tests (Bonferroni) were used for comparisons between experimental groups.

Mean duration of resting bouts was calculated by dividing resting duration by number of resting bouts. Activity duration and mean duration of resting bouts were compared between groups using a multivariate general linear model (GLM) with experimental group and housing as fixed factors. Post hoc tests (Bonferroni) were used for comparisons between experimental groups.

Mean and standard deviation (SD) of latency to burrow were calculated. Kaplan–Meier survival analysis was performed to examine the distribution of time to effect (latency to burrow). To test whether latency to burrow differed statistically between experimental groups or housing conditions, a log rank significance test was performed.

Significance for all statistical tests was established at $p \leq 0.05$.

4. Results

4.1. Influences of housing conditions on healthy mice

Behavioural differences between healthy, i.e. non-treated, mice were minor and none of the behaviours analysed showed a significant housing effect (see Figs. 1 and 2, Tables 2 and 3).

In both housing conditions, animals showed a short burrowing latency in no treatment groups (familiar cage 8 ± 6 min; new cage 6 ± 6 min, Fig. 3).
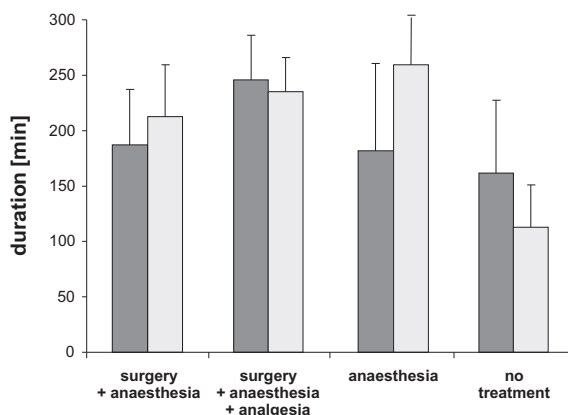
4.2. Influences of experimental treatment on mice

After experimental treatment animals showed no abnormalities in appearance, posture or spontaneous movements. No complications in wound healing after surgery were observed. No significant changes in body weight compared with one day prior to experimental treatments were seen at either one or two days after treatment. Clinical investigation revealed unaltered general condition scores in all groups.
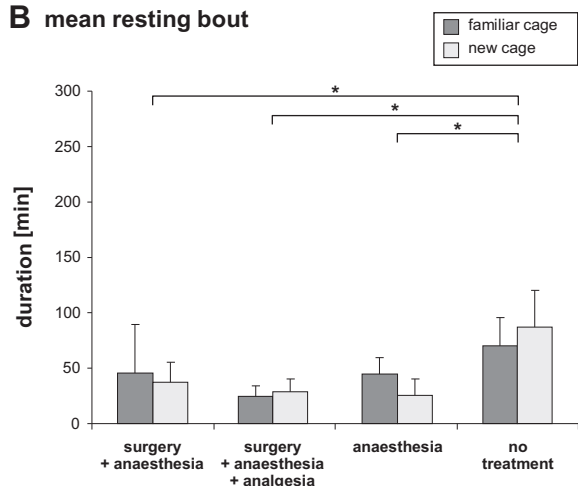
Mean durations of the observed behaviours of treated and non-treated mice in both housing conditions are shown in Table 2.

Discriminant analyses were performed with these behaviours for animals housed in their familiar home cage or a new clean cage revealing that several behaviours contributed to the significant separation of experimental
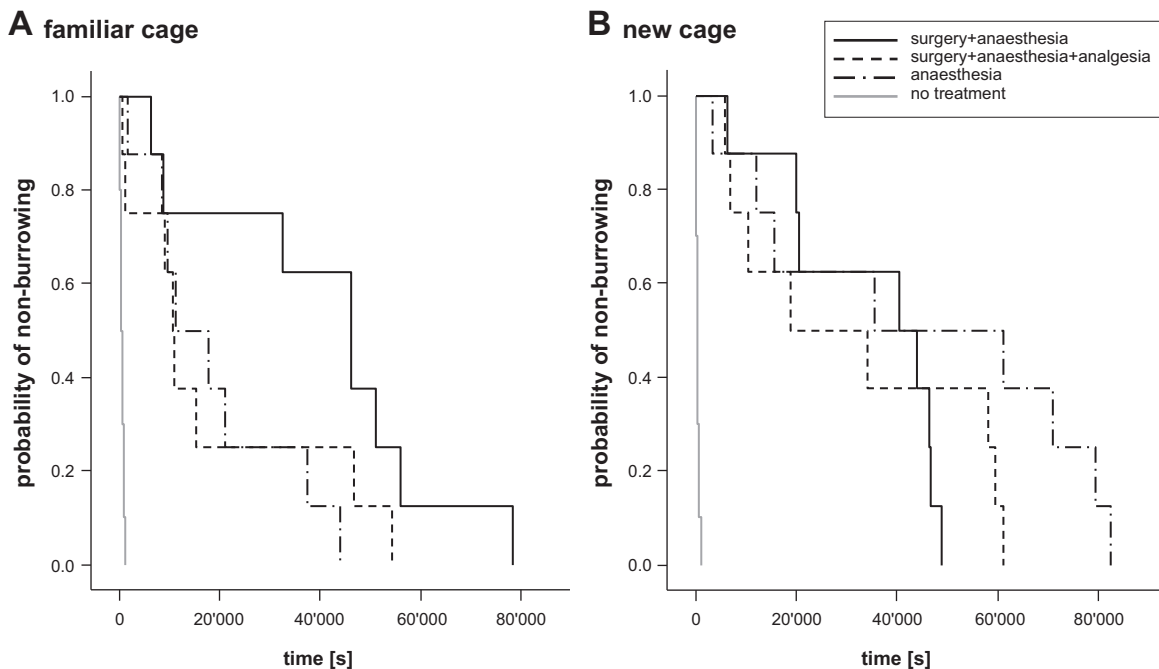

A general activity

B mean resting bout

Fig. 2. (A) General activity: a significant interaction between treatment and housing was found ($p = 0.006$); therefore no post hoc test was conducted for this behaviour. (B) Mean duration of resting bouts: experimental treatment groups had significantly shorter mean resting bout durations (no treatment vs. surgery $p < 0.001$, no treatment vs. surgery + analgesia $p < 0.001$, no treatment vs. anaesthesia $p < 0.001$).

Fig. 3. Kaplan–Meier analysis of latency to burrow. (A) Familiar home cage; (B) new clean cage. Significant differences were found between non-treated animals and the experimentally treated groups under both housing conditions ($p = 0.001$). The difference between surgery without analgesia and anaesthesia only groups was significant in animals housed in their home cage ($p = 0.020$). Comparing both housing conditions, latency after anaesthesia only was shorter for mice housed in their familiar home cage compared with mice housed in an new cage ($p = 0.049$).

groups (familiar cage: Wilks' lambda, function 1, $p = 0.001$; new cage: Wilks' lambda, function 1, $p < 0.001$, function 2 $= 0.017$). GLM was then performed with the main behaviours found to be contributing to experimental group separation in discriminant analyses (duration of climbing, eating, burrowing, self grooming; Fig. 1) and additionally with general activity and mean resting bout duration (Fig. 2) to test for significant differences between treatments and housing conditions.

4.2.1. Main effects and interactions of the factors housing and treatment

No main effect of the factor housing could be shown in any of the analysed behaviours, while the factor treatment had a significant effect on durations of all behaviours with the exception of climbing duration (Table 3).

Significant interactions between the two main factors housing and treatment were found in climbing ($p = 0.014$) and activity ($p = 0.006$) durations (Table 3, Figs. 1A and 2A). Because of the significant interaction post hoc test were not performed for these behaviours but the following tendencies could be observed: While climbing duration was shorter in treated animals housed in familiar cages compared to non-treated mice, the differences were less pronounced in animals in new cages. Climbing durations of animals that underwent anaesthesia only were even higher in this housing condition compared to non-treated animals. General activity was higher following treatments compared to non-treated animals. In the new cages this difference tended to be higher than in the familiar cages.

4.2.2. Effects of specific experimental treatments

Eating durations showed a non-significant tendency towards longer durations in the anaesthesia only and surgery with analgesia groups compared to non-treated animals (n.s., $p = 0.124$; $p = 0.156$), while surgery without pain treatment resulted in durations lower or comparable to non-treated animals (n.s., $p = 1.00$). This resulted in significant differences between surgery with and without analgesia ($p = 0.028$, Fig. 1B) and surgery without analgesia and anaesthesia only ($p = 0.022$, Fig. 1B).

Duration of burrowing was significantly shorter in treatment groups compared to non-treated animals (no treatment vs. surgery $p < 0.001$, no treatment vs. surgery + analgesia $p < 0.001$, and no treatment vs. anaesthesia $p = 0.001$, Fig. 1C).

Grooming behaviour was performed for significantly longer times in treatment groups compared to non-treated animals (no treatment vs. surgery $p = 0.009$, no treatment vs. surgery + analgesia $p < 0.001$, no treatment vs. anaesthesia $p < 0.001$, Fig. 1D).

In experimentally treated animals the mean duration of resting bouts in experimentally treated animals was shorter (no treatment vs. surgery $p < 0.001$; no treatment vs. surgery + analgesia $p < 0.001$; no treatment vs. anaesthesia $p < 0.001$, Fig. 2B).

4.2.3. 4. 2.3 Influences of housing and treatment on burrowing performance

Experimental treatments resulted in prolonged latencies in the burrowing test (Fig. 3 A and B). Log rank test following Kaplan–Meier analyses showed significant

differences between non-treated animals and the experimentally treated groups under both housing conditions ($p = 0.001$). Animals housed in their familiar home cage showed a pronounced gradation of burrowing latency between treatments. The mean latency of animals that underwent surgery without pain relief was distinctly higher (677 ± 402 min) than latencies in animals that received analgesia after surgery (310 ± 340 min) or anaesthesia only (315 ± 246 min). Animals housed in a new cage after treatment showed similar latencies in both surgery groups (surgery 570 ± 267 min; surgery + analgesia 531 ± 411 min) and the highest latency in animals that underwent anaesthesia only (751 ± 538 min). Log rank test showed that the difference between surgery without analgesia and anaesthesia only groups was significant in animals housed in their home cage ($p = 0.020$, Fig. 3A). Comparing both housing conditions, burrowing latency after anaesthesia only was shorter for mice housed in their familiar home cage compared with mice housed in a new cage ($p = 0.049$, Fig. 3).

5. Discussion

This study was set up to determine whether postsurgical housing in the familiar home cage is more beneficial for the recovery and well-being of female mice than housing the animals in a new and clean cage after surgery. For this purpose, animals in both housing conditions were monitored closely during the period immediately after surgery or anaesthesia. Behavioural investigations revealed significant differences in most behaviours in experimentally treated groups (surgery with or without analgesia, or anaesthesia only) compared to non-treated mice, while in contrast behaviours showed no significant differences when comparing housing conditions. Nevertheless, significant interactions between housing and treatment in climbing and activity durations as well as differences in burrowing performance occurred that may hint that animals cope better with surgical stress when housed in their familiar environment.

Clinical investigations, focusing on changes in appearance, posture and body weight, carried out daily are standard monitoring tools after surgery. Since no abnormalities were detected with these investigations, we suggest that our model has only a low impact on condition, health and well-being, particularly in comparison with other models of surgery (e.g. Pham et al., 2010).

Behavioural differences between untreated animals under both housing conditions were minor, and none of the analysed behaviours showed a significant housing effect in the statistical analyses.

In contrast, experimental treatments resulted in significant changes in nearly all analysed behaviours compared to non-treated animals under both housing conditions. These distinct changes were expected as we compared healthy animals that were not treated or manipulated at all with animals that underwent at least transport to the nearby operation theatre and inhalation anaesthesia. We assume that these differences can be explained only partly by restraint procedures and manipulations, as standard restraint and injection procedures have been shown to have only short-term impact on mice (Cinelli et al., 2007; Meijer et al., 2006). Studies from our group instead hint that the behavioural effects are due mainly to the impact of anaesthesia (Cesarovic et al., 2010, 2012; Jirkof et al., 2010, 2012).

While healthy mice mostly rest during the light phase and show a stable circadian rhythm with long resting bouts; disruption of this rhythm might indicate impaired well-being (Kant et al., 1995). In our study, compared to non-treated animals, overall activity was increased, accompanied by significantly more and shorter resting bouts, resulting in a disruption of the activity rhythm in all treated groups, indicating a decrease in animal well-being due to the treatments.

Discriminant analysis showed a significant contribution of the observed home cage behaviours to group separation. The behaviours contributing most to this separation were climbing, eating, self grooming and burrowing. While there was no main effect of treatment on climbing duration, eating duration was affected. Even though eating behaviour is not necessarily identical to food intake, the non-significant tendency to prolonged eating duration compared to non-treated animals in some conditions might indicate that animals increased their food consumption. This may help to reconstitute the animals' health after an exhausting event. Eating increased mainly in animals that were only anaesthetized or received pain treatment after surgery. This resulted in significant differences to animals without pain treatment that did not increase eating duration compared with non-treated mice. This might correlate with a low food intake and is probably a sign of postsurgical pain in these animals. As self-grooming was significantly more prevalent in all treatments compared to untreated animals, it is unlikely to be a specific sign of postsurgical pain. Therefore it could be correlated with the animals' general well-being after anaesthesia as well as increased attention to the shaved operation field (Mogil et al., 2010), the wound or the eye ointment used.

Burrowing behaviour is a highly motivated behaviour that has been shown to decrease after painful surgical interventions (Jirkof et al., 2010, 2012). Burrowing duration compared to non-treated animals was significantly shorter and burrowing latency in the burrowing test was significantly longer in all treatment groups in both housing conditions. In animals housed in their familiar home cage, burrowing performance ranged from short latencies of non-treated animals to an intermediate level following anaesthesia only and surgery with analgesia, to a pronounced prolongation of latency to burrow after surgery without pain relief. In accordance with previous studies (Jirkof et al., 2010, 2012), these findings indicate an anaesthesia effect as well as the occurrence of pain in animals after surgery. While non-treated animals had a similar good burrowing performance with short latencies in both housing conditions, latencies to burrow were dramatically but not in all cases significantly prolonged in animals transferred to a new cage after treatment. In addition to the prolongation of latencies, the transportation of animals to a new cage resulted in latencies to burrow that did not show a clear gradation of the different treatment groups as seen in animals housed in their familiar environment.

Interactions of housing condition and experimental treatment were also seen in other home cage behaviours. The analyses of general activity and climbing behaviour showed significant interactions between housing and treatment. In new cages, activity was higher after treatment compared to non-treated mice, while this difference was distinctly smaller in familiar cages. Climbing durations were shorter in treated animals in familiar cages compared to non-treated mice, whereas higher or comparable in treatment groups in new cages. Because of the significant interactions these treatment differences were not tested for significance. It is known that a disturbed circadian rhythm and decreased burrowing performance might indicate impaired well-being (Deacon, 2012; Jirkof et al., 2010, 2012; Kant et al., 1995). We interpret the relative longer durations of exploratory or flight behaviour (i.e. climbing) and general activity in new cages during the resting phase of the animal as a sign of agitation and restlessness. Increased activity during the natural resting phase might be detrimental for post-surgical recovery. The decreased interest in burrowing activity in animals housed in a new environment might be a consequence of preoccupation with behaviours like climbing. Otherwise, the better performance of animals housed in their home cage may also be a sign that animals in a familiar environment cope better with stressful and exhausting events like surgical procedures, anaesthesia and handling procedures.

These results are in line with other studies that show that even slight changes in a laboratory animal's environment might cause novelty stress and can alter its behaviour during an experiment or produce physiological stress responses (Belz et al., 2003; Dunn et al., 1972; Jain and Baldwin, 2003). Our results suggest that, even though housing female mice in a new and clean cage might be not a distressful event per se, post-surgical transfer to a new environment might act as an additional stressor after an exhausting experimental procedure and might be a detrimental factor for a fast and sound post-surgical recovery.

6. Conclusion

No clear signs of reduced well-being could be observed in healthy female mice placed in new and clean cages. Nevertheless, after experimental treatment, behavioural differences in animals housed in a new environment compared to animals housed in their familiar environment can be interpreted as subtle signs of reduced well-being, agitation and restlessness in the new cages. These results may also hint that animals cope better with surgical stress when housed in their familiar environment. The post-surgical transport to a new and clean cage might therefore be an additional stressor after an exhausting event and detrimental for recovery. We conclude that it might be worthwhile to consider the effects of crucial changes, like cage change, in the animal's physical environment after experimental procedures to minimize distress for the animals as well as to reduce unwanted variation in research findings.

Conflict of interest

The authors have no conflict of interest to disclose.

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