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# Impact of 'living apart together' on postoperative recovery of mice compared with social and individual housing

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## Summary

Social housing is the optimal way of housing female laboratory mice. However, individual housing may be required in experimental designs, for example after surgery. We therefore investigated whether housing two female mice in a cage, separated by a grid partition ('living apart together', LAT), counters the adverse effects of individual housing on postoperative recovery. Ten individually housed (IND) mice, nine socially housed (SOC) mice and nine mice, housed LAT, were surgically implanted with a telemetry transmitter. From one week prior to surgery until three weeks thereafter, several physiological and behavioural parameters were measured in the mice subjected to surgery. The telemetry transmitter measured heart rate (HR), body temperature and activity continuously. Body weight, food and water intake were scored regularly, as were wound healing, ease of handling, nest building and behaviour. Results indicated that SOC mice appear to be less affected by abdominal surgery than IND mice, as indicated by HR and behaviour. LAT, however, did not appear to be beneficiary to the mice. Increased HR levels and differences in behaviour as compared with both SOC and IND animals indicate that LAT may even be the most stressful of the three housing conditions. We therefore conclude that mice benefit most from social housing after surgery. If, however, social housing is not possible, individual housing appears to be a better option than separating mice by a grid partition.

**Keywords** Animal welfare; behaviour; individual housing; mouse; surgery; telemetry; heart rate; social housing

Social interaction can play an important role in the maintenance of normal behaviour in gregarious animals. The effect of individual housing on behavioural and physiological reactivity has been extensively studied in rodents (Brain 1975, Brain & Benton 1979, Giralt & Armario 1989, Ruis *et al.* 1999, Bartolomucci *et al.* 2003). There is general consensus that individual housing of gregarious species has a negative impact on

animal wellbeing. However, periods of individual housing are often required in experimental designs. It can, for example, be necessary to house animals individually after surgery due to damage by cage mates at the animals or the equipment.

After surgery, an animal experiences pain and its ability to perform its normal behavioural repertoire is impaired. Furthermore, recent research has identified several specific pain-related behaviours in rats and mice. Roughan and Flecknell (2003a,b, 2004) have observed these behaviours among

others after surgery, indicating that they may be a useful tool in assessing pain in individual animals. Both the physical and social environment may affect the way in which the animal copes with this stressful situation, which in turn may influence postoperative recovery. Unpublished results suggest that socially housed (SOC) mice need less time to fully recover from telemetry implant surgery (Meijer M, Utrecht University, The Netherlands, personal communication), and that rats subjected to spinal cord injury have a 20% less chance of survival when housed individually after surgery (Olson L, Karolinska Institute, Sweden, personal communication). Group housing is also recommended by the Council of Europe (2006) and the Rodent Refinement Working Party (Jennings *et al.* 1998).

The results and recommendations mentioned above imply that alternatives should be sought for individual housing after surgery. One possibility is housing animals within sight, smell, sound and touch of each other by means of a grid partition in the cage during postoperative recovery, i.e. 'living-apart-together' (LAT). A similar construction is used in the 'partition test', a standard behavioural paradigm used to investigate effects of repeated defeat on stress, anxiety and aggression in unfamiliar male mice (Kudryavtseva 2003). Although studies using the partition test imply that LAT is stressful for unfamiliar male mice, this may not be the case for familiar female mice. Zhu *et al.* (2006) found that female mice are generally more socially interactive than males, indicating that female mice could be more sensitive to social instability, such as individual housing. We therefore hypothesized that SOC female mice and familiar female mice LAT would be less stressed and show a more rapid postoperative recovery than individually housed (IND) female mice.

To induce an animal model for postoperative recovery, and at the same time enabling measurement of heart rate (HR), body temperature (BT) and activity in a conscious, unstressed animal, we implanted radiotelemetry transmitters (Kramer & Kinter 2003) in female mice that were conse-

quently monitored for several behavioural and physiological parameters indicative of pain, stress and general wellbeing until fully recovered while being housed either in pairs, individually or LAT.

## Materials and methods

### *Animals, housing and husbandry*

Forty-six female C57BL/6J<sup>OlaHsd</sup> mice (Charles River, Maastricht, The Netherlands) of approximately nine weeks old were used. C57BL mice were used because of the acquired experience with transmitter implantation in this strain. Prior to surgery, all mice were standard housed in couples, previously unfamiliar to each other, in Makrolon<sup>®</sup> type IIL cages (530 cm<sup>2</sup>; Tecniplast, Milan, Italy) provided with food (CRM-E, SDS, Witham Essex, UK) and water *ad libitum*, aspen chips bedding (ABEDD<sup>®</sup>, Köflach, Austria) and two Kleenex tissues (Kimberly-Clark Corporation<sup>®</sup>, Ede, The Netherlands). Cages were cleaned once a week. All mice had been housed previously in groups of three and used in behavioural research with minor discomfort.

The animal room had a controlled photoperiod. White light (100 lux) was off between 10:00 and 22:00 h, enabling observation of behaviour in the active period of the mouse. Red light (2 lux) was on during the dark period. The temperature (22–24°C), relative humidity (45 ± 10%) and ventilation of the animal room were controlled. A radio (combination of pop songs and spoken word) was playing softly in the background during the dark period. The mice were allowed to acclimatize to the animal room conditions and husbandry procedures for three weeks prior to surgical implantation of the radiotelemetry transmitter, which is described below. Couples were randomly assigned to three experimental groups: IND (5 couples), LAT (9 couples) or SOC (9 couples). Immediately following surgery, the IND mice were housed individually in 10 clean cages. SOC mice and LAT mice were housed in a clean cage with the non-operated cage mate. For the LAT mice, a metal grid partition was inserted in the middle of the



**Figure 1** Living apart together (LAT) mice and its cage mate in a cage with a grid partition

cage (Figure 1). Each mouse was provided with one Kleenex tissue.

#### *Surgery and perioperative care*

At the age of 12 weeks, all 10 IND mice, nine SOC mice and nine LAT mice were surgically implanted with a telemetry transmitter according to the method of Kramer *et al.* (1993), with modifications to optimize peri-surgical care according to Meijer *et al.* (2006). Mice were operated in cohorts (7 animals per day) according to a randomized block design. About 30 min pre-surgery, mice received the analgesic carprofen (5 mg/kg; Rimadyl<sup>®</sup> 20 mg, Pfizer Animal Health BV, Capelle aan de IJssel, The Netherlands) and an antibiotic (enrofloxacin; 30 mg/kg; Baytril<sup>®</sup> 2.5% injection fluid, Bayer BV, Mijdrecht, The Netherlands) subcutaneously. Mice were anaesthetized using isoflurane (ISOFLO<sup>®</sup>, Schering-Plough, Maarsse, The Netherlands), N<sub>2</sub>O and O<sub>2</sub> (induction: isoflurane 5%, N<sub>2</sub>O:O<sub>2</sub> 1:1, 2 L; maintenance isoflurane 1.4–1.6%, N<sub>2</sub>O:O<sub>2</sub> 1:1, 0.5–0.8 L). During surgery, the eyes of the mice were protected with vitamin A eye ointment (Ophtosan<sup>®</sup> Oogzalf, AST Pharma, Oudewater, The Netherlands). After disinfecting the abdomen with alcohol, a 1.5–2 cm long incision was made in the skin along the midline immediately caudal to the abdominal space. Subsequently, the abdominal wall was opened and a radiotelemetry transmitter

(TAT10ETA-F20, Data Science International, St Paul, MN, USA) was implanted in the peritoneal cavity. The electrocardiogram (ECG) electrodes on the transmitter were guided through a small incision in the abdominal wall, and were sutured subcutaneously in the lead II position, i.e. the negative electrode at the right shoulder and the positive electrode at the lower left chest. After both leads were fixed in their places, both transmitter body and leads were sutured to the muscular layer using, respectively, non-absorbable Prolene<sup>®</sup> 4-0 and absorbable Vicryl<sup>®</sup> 4-0 (Johnson & Johnson, Amersfoort, The Netherlands). Before closure, the peritoneal cavity was filled with warm, sterile saline (0.9%, Braun Melsungen AG, Melsungen, Germany). To complete the surgical procedure, the incision in the muscular layer and skin were closed with intradermal sutures (Vicryl<sup>®</sup> 4-0, Johnson & Johnson). In total, surgery took approximately 35 min. Post-surgery, the mice were placed in an incubator of 32°C for one hour and then returned to a clean home cage that was partially placed on a heating pad for at least 24 h. The abdomens of the non-operated cage mates were swabbed with gauze with 70% alcohol, in order to provide a comparable novel odour as the implanted animal.

Post-surgery, mice were treated with carprofen (5 mg/kg subcutaneously, twice daily for a period of 3 days) and enrofloxacin (30 mg/kg subcutaneously, daily for a period of 4 days) to ensure adequate pain relief and reduce the chance of wound infection. Furthermore, cages were partially placed on a heating pad for a period of two days, and in addition to normal food and water, Solid Drink<sup>®</sup> (Triple A Trading, Tiel, The Netherlands) and soft food (standard food soaked in 3% sucrose water) were provided for a period of four days to decrease the chance of dehydration and extreme weight loss.

#### *Data collection*

From one week prior to surgery until three weeks thereafter, several physiological and behavioural parameters were measured in

the operated mice. Cage mates of LAT mice and SOC mice were weighed, but no other data were collected.

The telemetry device was activated immediately after surgery and measured HR, BT and activity (every 3 min, 24 h a day). Data acquisition was performed using DataQuest ART<sup>®</sup> (Data Science International). Body weight was measured one week prior to surgery and immediately prior to surgery. Post-surgery body weight, corrected for transmitter weight (3.8 g), was measured daily for a period of one week, four times a week in the second week and two times a week in the third week. During weighing sessions, a score was made of wound healing/closing, suture nibbling and ease of handling. Food and water intake was measured twice a week from one week prior to surgery until three weeks post-surgery, starting as soon as soft food and solid drink were removed from the cage. For SOC mice, food and water intake was corrected for cage mate presence by dividing data by 2.

Nest site and complexity were scored one week pre-surgery, immediately pre-surgery and post-surgery during weighing sessions. For LAT mice, the nest site of the cage mate was also registered. Nest complexity was subdivided into four categories: tissue was

not used (1); mouse was sleeping on tissue, tissue was still complete (2); tissue had been formed slightly into nest in which mouse slept (3) and tissue had been formed to complicate nest in which mouse slept (4).

Animals were videotaped in their home cage for 10 min one hour following surgery and after each weighing session to score explorative, rest-related, ingestive and pain behaviours as listed in the ethogram (Table 1). During videotaping, tissues were removed from the cage so that the mouse was visible at all times. Videotapes were analysed with the aid of the Observer Video-Pro (version 5.0 for Windows, Noldus Information Technology, Wageningen, The Netherlands) by focal sampling, enabling scoring of both duration and number of occurrences at the same time. Prior to surgery and on days 1, 3, 7 and 15 post-surgery, behaviour was scored automatically with the aid of LABORAS<sup>®</sup> (Metris BV, Hoofddorp, The Netherlands), an automated behavioural scoring system for small rodents, which translates movements of the mice into behavioural categories (Van de Weerd *et al.* 2001). Four randomly chosen mice were tested simultaneously on four sensing platforms for 60 min per trial between 13:00 and 16:00 h. Each mouse was

**Table 1 Ethogram**

Behaviour	Description
Rest	Movements are absent while the animal is in a lying or sitting position
Grooming head	The mouse is scratching, wiping or licking its snout or ears
Grooming belly	The mouse is scratching, wiping or licking its fur around the site of surgery
Grooming back/tail	The mouse is scratching, wiping or licking the fur on its back or its tail
Eating trough	Gnawing on food particles from the food hopper
Eating ground	Gnawing on food particles from the sawdust or the wet food and water provided for four days post-surgery
Drinking	Licking the nipple of the drinking bottle
Locomotion	Walking, running or jumping
Half-rearing	The animal is standing on its hind feet or toes with its back in a crouched position. The fore paws may lean against the cage wall or food hopper
Rearing	The animal is standing on its hind feet or toes with its back fully stretched. The fore paws may lean against the passage tube, cage wall or food hopper
Climbing	Climbing on or hanging from the bars of the wire cage lid or food hopper, or standing on the drinking nipple
Social	The animal is sniffing or licking its partner or huddling (SOC) or is sniffing its partner through the partition (LAT)
Stagger/fall	Animal loses its footing, this may or may not be followed by a fall on its side
Twitch/writhing/flinching	Any apparently involuntary rapid movement of head, abdomen or back
Stretch	Animal stretches its body completely with abdomen pushed to the ground, hind paws are stretched backwards and tail is kept rigid
Invisible	Not visible, or uncertain what the animal is doing

placed individually in a clean Makrolon<sup>®</sup> type II cage, more or less comparable with their home cages, with wood chips bedding, food and water.

The following behavioural elements could be distinguished: climbing, drinking, eating, grooming, immobility and locomotion. Movements that could not be identified were scored as undefined. In addition, the system determined the following tracking parameters: position, average speed (over periods with animal displacement), maximum speed, distance moved and position distribution.

### *Statistical analysis*

One mouse in the SOC group needed to be euthanized on day 15 due to intestinal torsion and peritonitis. Because of the time between surgery and illness, it is unlikely that this complication was linked to the surgery. Data of this animal were therefore omitted from the analysis.

Three different time points were taken from the data collected by the telemetry device. Daily two 90 min periods in which animals were not disturbed were selected, based on the activity data. One period in which the activity was high (20:15–21:45 h, dark period) and one in which the activity was low (04:30–06:00 h, light period) were selected. Furthermore, the 90 min period after each weighing session was selected. Each 90 min period consisted of 30 data acquisitions of HR and BT that were averaged for use in statistical analysis.

Statistical analyses of the telemetry data on HR and BT and data on body weight, food intake and water intake were performed using a general linear model for repeated measures, with time as a within-subjects factor and housing condition (IND, LAT or SOC) as a between-subjects factor. Because of the presence of a heating pad for two days post-surgery, BT data of those days were not used for the analysis. The behaviours measured by the LABORAS<sup>®</sup> system were compared with the aid of the Wilcoxon matched pairs signed rank test using the pre-surgery data as reference.

Duration or frequency of behaviours immediately following surgery were analysed using a Kruskal–Wallis test, followed by a Mann–Whitney *U*-test where appropriate. Duration of behaviour after weighing sessions was analysed with a linear mixed-effects model with cage as random effect, and days after surgery and housing as fixed effects. When necessary, data were log- or square root-transformed. Descriptive statistics were used for wound healing, suture nibbling, ease of handling, nest site and nest complexity.

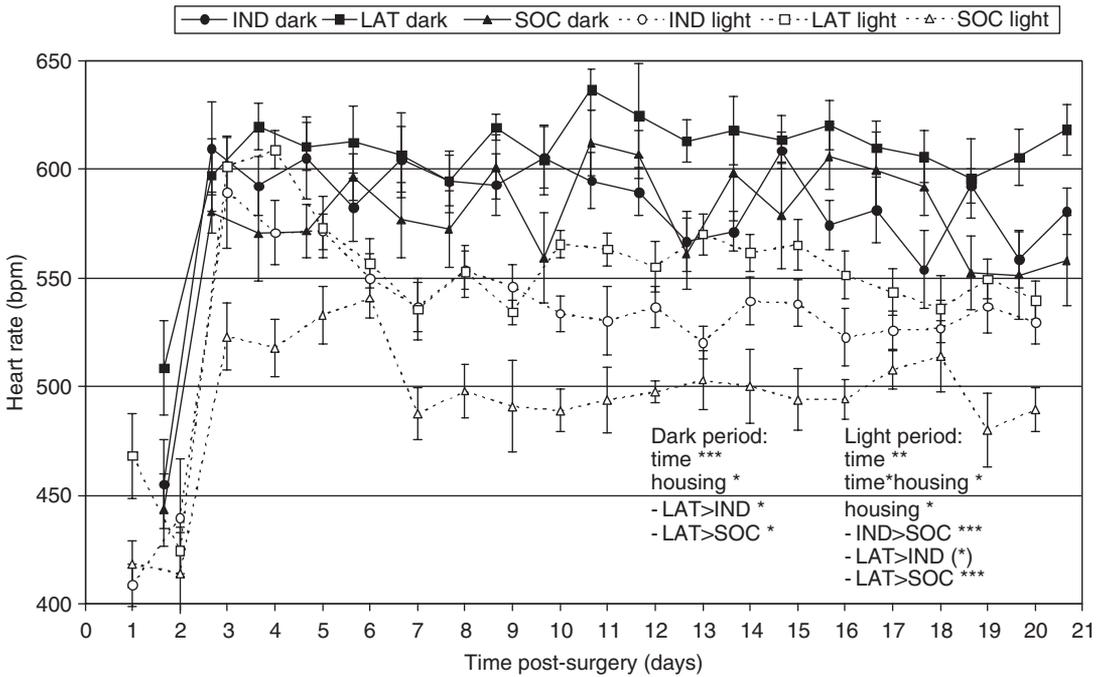
Linear mixed effects analyses were performed using S-plus 2000 Professional Release 2<sup>®</sup> (1988–99, MathSoft, Inc). All other analyses were performed using SPSS for Windows, release 12.0.1 (SPSS Inc, Chicago, IL, USA). Differences were considered significant when  $P < 0.05$ . Data are expressed as mean values  $\pm$  SEM.

## **Results**

For all housing conditions, a rapid healing of the surgery wound was seen. In general, wounds closed within three days after surgery. Although the investigators had the impression that both the LAT mice and their cage mates were generally more agitated prior to being handled, no differences in handling score between housing conditions were found.

### *Heart rate and body temperature*

HR measured during the dark period was higher than in the light period (not statistically analysed), as can be seen in Figure 2. Overall, both in the dark and light period, there was a significant time effect ( $P_{\text{dark}} = 0.001$ ,  $P_{\text{light}} < 0.01$ ), HR increased to stable levels after the light period of day 2. Within subjects, a significant time  $\times$  housing ( $P < 0.05$ ) effect was found in the light period, IND mice and LAT mice reached higher HR levels in the light period of days 3–5 compared with SOC mice and compared with their own HR levels after day 5. Furthermore, an overall significant housing effect was found ( $P_{\text{dark}} < 0.05$ ;  $P_{\text{light}} < 0.001$ ), HR of LAT mice was significantly higher



**Figure 2** Heart rate (mean  $\pm$  SEM) in the dark and light period. IND: individually housed; LAT: living apart together; SOC: socially housed. \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$

compared with both SOC mice ( $P_{\text{dark}} < 0.05$ ;  $P_{\text{light}} < 0.001$ ) and IND mice ( $P_{\text{dark}} < 0.05$ ;  $P_{\text{light}} < 0.1$ ). In the light period, HR of the IND mice was significantly higher compared with the SOC mice ( $P < 0.001$ ).

HR measured 10 min after weighing sessions for a period of 90 min was slightly higher than HR in the undisturbed dark period, but showed a similar pattern in time ( $P < 0.001$ ), ranging from  $517 \pm 11$  bpm on day 1 to  $637 \pm 5$  bpm on day 21 (data not shown). An overall housing trend was found ( $P < 0.1$ ), HR of the LAT mice tended to be higher compared with HR of SOC mice ( $P < 0.1$ ).

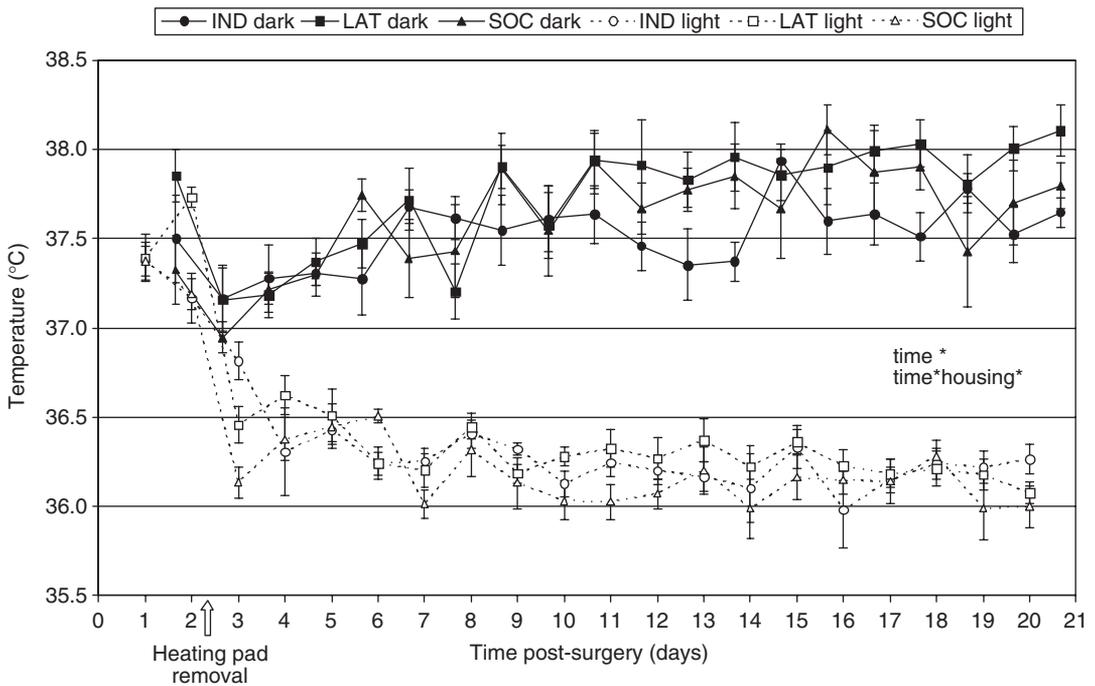
In concordance with HR, BT during the dark period was higher than during the light period (not statistically analysed), as can be seen in Figure 3. An overall significant time effect was found in the dark period ( $P < 0.05$ ) and in the light period ( $P < 0.01$ ) as well as after weighing sessions ( $P < 0.001$ , data not shown). In the dark period and after weighing sessions, BT of the mice increased with time, while in the light period, BT decreased

with time. From day 7 onwards, BT was more or less stable. No significant main housing effects were found.

#### Body weight, food and water intake

Body weight at surgery was  $19.7 \pm 0.2$  g (mean  $\pm$  SEM). A significant time effect was found ( $P < 0.001$ ). Body weight of all animals decreased until two days post-surgery to  $17.6 \pm 0.3$  g. After 12 (IND mice), 14 (SOC mice) or 17 (LAT mice) days, weight of the mice had returned to pre-surgical levels.

Food and water intake are presented in Table 2. Both food and water intake differed in time ( $P < 0.001$ ). Furthermore, there was a significant housing effect and time  $\times$  housing interaction for food intake ( $P < 0.001$ ): IND mice ate significantly more than both LAT mice ( $P < 0.05$ ) and SOC mice ( $P < 0.001$ ) due to a post-surgical rise in food intake of IND mice and a simultaneous drop in food intake of LAT and SOC mice. There were no differences between LAT and SOC



**Figure 3** Body temperature (mean  $\pm$  SEM) in the dark and light period. IND: individually housed; LAT: living apart together; SOC: socially housed. \* $P < 0.05$ .

**Table 2** Food and water consumption for six time periods and three housing conditions (mean  $\pm$  SEM)

Days post-surgery	Food consumption (g/day)			Water consumption (mL/day)		
	Individual	LAT	Social	Individual	LAT	Social
-14 to -7	3.28 $\pm$ 0.06	3.24 $\pm$ 0.08	3.33 $\pm$ 0.06	4.75 $\pm$ 0.13	4.34 $\pm$ 0.17	4.64 $\pm$ 0.14
4-7	3.40 $\pm$ 0.09	3.10 $\pm$ 0.09	2.67 $\pm$ 0.25	4.61 $\pm$ 0.18	5.20 $\pm$ 0.37	4.30 $\pm$ 0.35
7-10	3.00 $\pm$ 0.10	2.51 $\pm$ 0.17	1.88 $\pm$ 0.23	4.30 $\pm$ 0.48	4.47 $\pm$ 0.27	3.92 $\pm$ 0.57
10-14	3.73 $\pm$ 0.14	2.49 $\pm$ 0.11	2.75 $\pm$ 0.23	4.25 $\pm$ 0.22	4.33 $\pm$ 0.33	4.05 $\pm$ 0.26
14-17	3.36 $\pm$ 0.10	2.67 $\pm$ 0.27	2.09 $\pm$ 0.28	4.39 $\pm$ 0.19	5.35 $\pm$ 0.26	3.81 $\pm$ 0.30
17-21	3.50 $\pm$ 0.09	2.26 $\pm$ 0.20	2.29 $\pm$ 0.37	4.58 $\pm$ 0.34	4.46 $\pm$ 0.31	3.91 $\pm$ 0.54
Significance	A***, H***, AxH***			A***		

A: age effect, H: housing effect; \*\*\* $P < 0.001$

LAT: living apart together; SEM: standard error of the mean

mice, nor any group differences in water intake.

### Behaviour

**Nest site and complexity** Almost all nests were built in the corners of the cages. Pre-surgery, all nests were shared, after surgery SOC mice always shared their nests. LAT mice preferably built nests in the corner of

the cage wall with the grid partition. One to four LAT mice out of nine slept near the cage mate on the other side of the grid partition. Prior to surgery, almost all nests were complex (category 4). Nest complexity showed a decrease at day 1 post-surgery for all three housing conditions. The largest decrease was seen in the IND mice (mean complexity score 1.6), followed by the LAT mice (mean complexity score 2.1), while

SOC mice built a nest with a mean complexity score of 2.5. After day 1, nest complexity increased for three to four days. From day 5 onwards, the nest complexity of the IND mice and SOC mice was comparable with pre-surgery nest complexity. The mean nest complexity score of the LAT mice was lower and more variable compared with IND and SOC mice.

*Behaviour measured by LABORAS<sup>®</sup>* An overview of several LABORAS<sup>®</sup> results is presented in Table 3. After surgery, immobility and grooming increased, while climbing, locomotion, average speed and distance moved decreased. Eating, drinking and position in the cage did not reveal any significant differences. For most behavioural parameters, differences from pre-surgery levels had disappeared on day 15. Furthermore, in SOC mice, for most behavioural parameters, a smaller difference was apparent between pre-surgery levels and day 1 as compared with IND mice and LAT mice. Above that,

in SOC mice, the differences between pre- and post-surgery behaviour were of shorter duration compared with the IND and LAT mice. Overall, average speed and distance moved differed between housing conditions ( $P < 0.01$ ) with a significantly higher speed in SOC mice compared with LAT mice ( $P < 0.01$ ) and a trend for IND mice to have a higher speed compared with LAT mice ( $P < 0.1$ ).

*Behaviour immediately following surgery and after weighing sessions* One hour post-surgery, most animals rested the majority of the time. No significant differences in behaviour were found between animals of different housing conditions, except for climbing. Whereas none of the IND mice climbed, and only one LAT mice climbed (about 9 out of 600 s), four out of eight SOC mice were observed climbing (ranging from 16 to 22 out of 600 s).

For most behaviours after weighing sessions, a clear time effect was visible

**Table 3** Results of LABORAS<sup>®</sup> (mean  $\pm$  SEM)

Behavioural parameter	Housing	Day -7	Day 1	Day 3	Day 7	Day 15	P <sub>H</sub>
Climbing (min/h)	IND	22.3 $\pm$ 2.0	0.7 $\pm$ 0.3*	9.5 $\pm$ 1.6*	15.3 $\pm$ 1.7 <sup>†</sup>	17.0 $\pm$ 1.6	
	LAT	19.1 $\pm$ 0.8	0.6 $\pm$ 0.3*	6.7 $\pm$ 1.3*	12.1 $\pm$ 1.6 <sup>†</sup>	17.5 $\pm$ 2.3	
	SOC	17.3 $\pm$ 2.2	2.8 $\pm$ 1.0 <sup>†</sup>	12.2 $\pm$ 1.6 <sup>†</sup>	13.6 $\pm$ 1.3 <sup>‡</sup>	16.3 $\pm$ 1.7	
Locomotion (min/h)	IND	11.5 $\pm$ 0.6	2.5 $\pm$ 0.5*	6.4 $\pm$ 0.4*	8.6 $\pm$ 0.6*	10.8 $\pm$ 0.7	
	LAT	12.6 $\pm$ 0.4	1.7 $\pm$ 0.4*	5.7 $\pm$ 0.4*	9.7 $\pm$ 0.5*	10.9 $\pm$ 0.7 <sup>†</sup>	
	SOC	11.2 $\pm$ 0.5	3.3 $\pm$ 0.9 <sup>†</sup>	6.1 $\pm$ 0.5 <sup>†</sup>	8.3 $\pm$ 0.3 <sup>†</sup>	9.3 $\pm$ 0.3 <sup>†</sup>	
Immobility (min/h)	IND	0.9 $\pm$ 0.5	6.2 $\pm$ 2.1 <sup>†</sup>	2.0 $\pm$ 0.3 <sup>‡</sup>	2.3 $\pm$ 1.0 <sup>†</sup>	0.7 $\pm$ 0.2	
	LAT	0.5 $\pm$ 0.2	9.6 $\pm$ 3.2*	3.1 $\pm$ 0.7*	2.8 $\pm$ 1.6 <sup>†</sup>	1.6 $\pm$ 0.8	
	SOC	1.8 $\pm$ 1.1	9.8 $\pm$ 3.0 <sup>†</sup>	2.5 $\pm$ 0.4	0.8 $\pm$ 0.2	0.5 $\pm$ 0.1	
Grooming (min/h)	IND	1.9 $\pm$ 0.3	12.5 $\pm$ 1.0*	5.5 $\pm$ 0.9*	2.5 $\pm$ 0.3	2.2 $\pm$ 0.4	
	LAT	2.6 $\pm$ 0.4	12.6 $\pm$ 1.7*	4.8 $\pm$ 0.7 <sup>†</sup>	2.9 $\pm$ 0.5	2.1 $\pm$ 0.4	
	SOC	2.2 $\pm$ 0.2	8.5 $\pm$ 1.1 <sup>†</sup>	2.5 $\pm$ 0.5	2.5 $\pm$ 0.3	3.1 $\pm$ 0.7	
Average speed (mm/s)	IND	76.1 $\pm$ 0.8	48.4 $\pm$ 4.6*	68.2 $\pm$ 0.7*	69.4 $\pm$ 0.7*	73.0 $\pm$ 1.1 <sup>†</sup>	§
	LAT	75.7 $\pm$ 0.7	37.0 $\pm$ 4.6*	65.2 $\pm$ 1.6*	68.1 $\pm$ 0.8*	72.7 $\pm$ 0.7 <sup>†</sup>	§ ¶
	SOC	76.2 $\pm$ 0.8	57.6 $\pm$ 2.5 <sup>†</sup>	70.9 $\pm$ 1.2 <sup>†</sup>	68.6 $\pm$ 0.4 <sup>†</sup>	71.3 $\pm$ 0.8 <sup>†</sup>	¶
Distance moved (m)	IND	30.9 $\pm$ 1.8	5.1 $\pm$ 1.2*	16.6 $\pm$ 1.3*	22.4 $\pm$ 1.9*	29.7 $\pm$ 1.9	
	LAT	33.7 $\pm$ 1.3	2.3 $\pm$ 0.7*	15.5 $\pm$ 1.5*	23.1 $\pm$ 1.2*	29.3 $\pm$ 1.6 <sup>†</sup>	
	SOC	30.2 $\pm$ 1.2	7.0 $\pm$ 2.5 <sup>†</sup>	17.9 $\pm$ 1.4 <sup>†</sup>	22.4 $\pm$ 1.1 <sup>†</sup>	25.5 $\pm$ 1.4 <sup>†</sup>	

\*Significantly different from day -7 ( $P < 0.01$ )

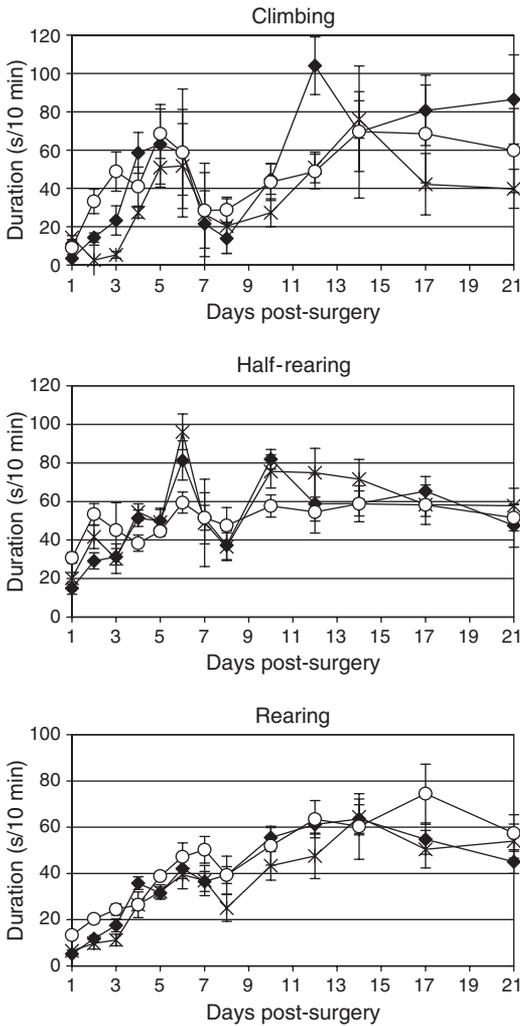
<sup>†</sup>Significantly different from day -7 ( $P < 0.05$ )

<sup>‡</sup>Trend for difference with day -7 ( $P < 0.1$ )

§Overall trend for difference between housing conditions, IND > LAT ( $P < 0.1$ )

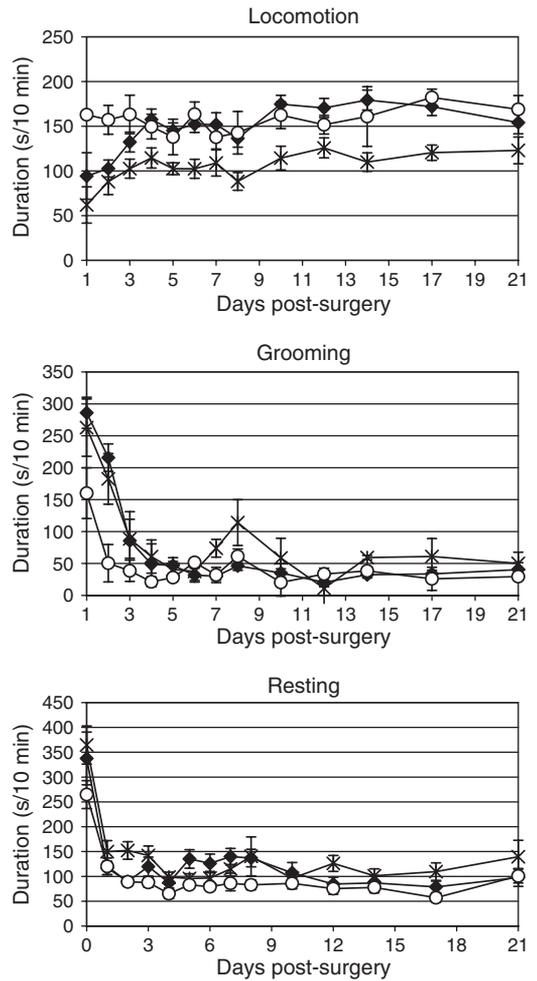
¶Significant overall difference between housing conditions, SOC > LAT ( $P < 0.01$ )

LAT: living apart together; IND: individually housed; SOC: socially housed; SEM: standard error of the mean



**Figure 4** Duration of climbing (top), half-rearing (middle) and rearing (bottom) during 10 min weighing sessions between one and 21 days after surgery. —○—, SOC; —◆—, IND; —×—, LAT

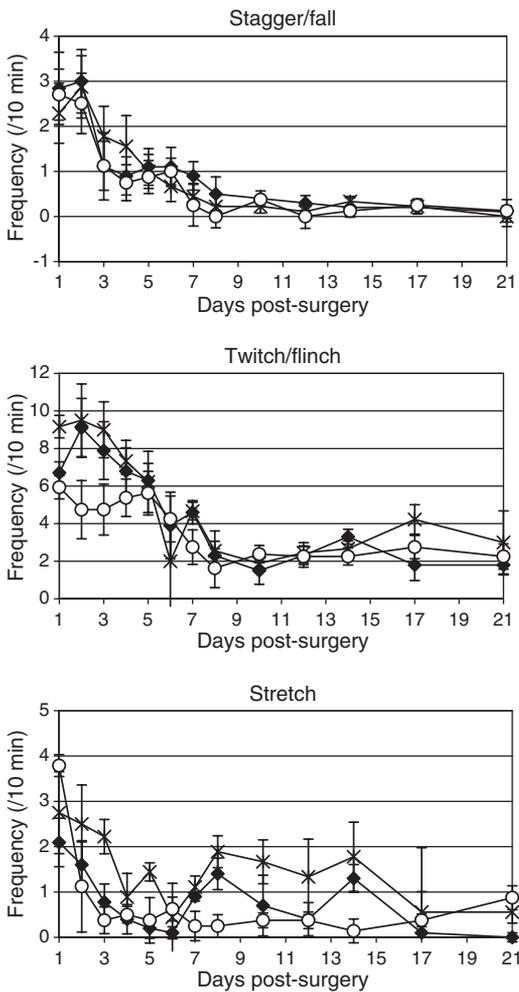
(Figures 4–5). Grooming and resting were high in the first days after surgery, after which levels declined ( $P < 0.001$ ). Half-rearing, rearing, climbing and locomotion all increased in time ( $P < 0.001$ ). Overall, housing differences were present for locomotion ( $P < 0.001$ ), rearing ( $P < 0.05$ ) and resting ( $P < 0.01$ ). LAT animals showed less locomotion and rearing ( $P < 0.05$ ) and more resting ( $P < 0.001$ ) than SOC mice. IND mice showed more locomotion than LAT mice ( $P < 0.05$ ), and rested more than SOC mice



**Figure 5** Duration of locomotion (top), grooming (middle) and resting (bottom) during 10 min weighing sessions between one and 21 days after surgery. —○—, SOC; —◆—, IND; —×—, LAT

( $P < 0.05$ ). Finally, for locomotion and grooming, a significant day  $\times$  housing interaction was present ( $P < 0.01$ ): the decrease in locomotion and increase in grooming that marked the first 2–3 days was less pronounced in SOC mice. Around 6–9 days post-surgery, many behaviours showed either dips (e.g. climbing and half-rearing and rearing) or peaks (resting and grooming). This effect was less pronounced in SOC animals.

Specific pain behaviours (twitch, stagger and stretch) also showed a significant decrease over time ( $P < 0.01$ ) (Figure 6).



**Figure 6** Frequency of three different behaviours associated with pain during 10 min weighing sessions between one and 21 days after surgery.

—○—, SOC; —◆—, IND; —×—, LAT

Furthermore, SOC mice showed less twitches overall ( $P < 0.05$ ), especially during the first days after surgery.

## Discussion

### *Use of anaesthetics, analgesics and antibiotics*

All animals showed a fast physical recovery without problems after surgery, except one SOC mouse which died on day 15 (see Materials and methods, statistical analysis).

Therefore, the suggestion of lower survival after spinal cord injury in IND rats (Olson L, personal communication) does not count for mice after telemetry implantation.

This study was set out to investigate the effects of different housing conditions on postoperative recovery in mice. The first and foremost factors affecting a prosperous recovery, however, are perioperative conditions such as anaesthesia and analgesia. In this study, inhalation anaesthesia was used which is easy and safe to administer. The depth of inhalation anaesthesia can be rapidly and predictably altered, and recovery is rapid.

Preventing pain and providing supportive therapy such as fluid, warmth, analgesia and nutrition might decrease morbidity and, in some cases, mortality. Meijer M ('Influence of analgesic agents on body weight after transmitter implantation', in preparation) has shown that mice that receive carprofen after transmitter implantation lose less weight compared with mice that receive buprenorphin, an opioid-like analgesic. All animals in our experiment lost weight until only two days post-surgery. Even so, behavioural data have shown an interesting phenomenon that between six and nine days post-surgery, animals appear to experience a dip in recovery. Active behaviours that require the use of the abdominal muscles such as climbing and rearing show a clear dip in recovery, whereas grooming, a behaviour that was performed frequently in the first days after surgery, peaks again in this period. Inflammatory processes accompanying tissue trauma can lead to peripheral sensitization for several days, which can be adequately suppressed by analgesia. A foreign body in the traumatized area (i.e. the telemetry transmitter) may extend this period of sensitization (Hellebrekers 2000 and personal communication). The dip in recovery we found could thus be the result of a postponed sensitization of the surgery wound, initially suppressed by carprofen, and building up within 1–2 days after the last carprofen administration. We therefore suggest that when mice are subjected to abdominal surgery including the implantation of a foreign body,

perioperative care should include a period of at least 7–8 days analgesia.

### Physiology

**Initial recovery** Mice show a clear circadian rhythm in their behaviour. They are active during the night, while they sleep most of the day (Van de Weerd *et al.* 2001). This circadian rhythm is also apparent in HR and BT. Stable biological rhythms are a reliable sign of good health and wellbeing. It has been argued that disruption of these rhythms could be a sensitive indicator of impaired welfare (Kant *et al.* 1995). In our experiment, HR sharply increased after two days. Both in the dark period (Figure 2) and after weighing sessions, HR remained stable thereafter. In the light period, however, HR was relatively high from days 3 to 6 for all mice, decreasing to stable levels from day 7 onwards. In the dark period, BT of the mice increased with time post-surgery, while BT in the light period decreased. BT was more or less stable from day 7 onwards. These results are in concordance with Butz and Davisson (2001) who found that IND female mice did not fully recover from anaesthesia and surgery after transmitter implantation until five to seven days post-surgery, as indicated by a return of normal circadian HR rhythms and with Sharp and Lawson (2003), who evaluated HR, body weight, food intake, water intake and visual examination, found that rats recovered in 7–10 days after telemetry implantation. In our experiment, body weight returned to baseline levels after 12 (IND mice), 14 (SOC mice) or 17 (LAT mice) days. This is comparable with the results of Baumans *et al.* (2001), who found that mice reached initial body weight at day 14 after telemetry implantation. Kramer *et al.* (1993) found a stable body weight after 18–20 days.

### Differences between housing conditions

Overall, SOC mice had lower HRs than both IND and LAT mice, and the increased HR levels between days 2 and 7 were less pronounced in SOC mice. This may be due to the presence of a cage mate with a normal circadian rhythm or due to a faster recovery

after surgery. No previous studies were done on effects of LAT-housed cage mates on physiological and behavioural parameters. On the contrary, much is known about the effects of individual housing. It is generally accepted that individual housing is a stressful situation for social animals (Brain 1975). More recently, it is shown for example that IND rats (Ruis *et al.* 1999) and pigs (Ruis *et al.* 2001) developed long-lasting, adverse behavioural and physiological changes after social defeat, in contrast to SOC animals. Several investigators have reported elevated levels of basal HR and BT in IND mice compared with SOC mice (Einstein *et al.* 2000, Späni *et al.* 2003, Meijer *et al.* 2006). Furthermore, individual housing has been reported to decrease food intake (Brown & Grunberg 1996) and body weight (Ruis *et al.* 1999, Vöikar *et al.* 2005) in rodents. Although we did not find any differences in body weight between the different housing conditions, IND mice ate significantly more post-surgery than pre-surgery, and more than both LAT mice and SOC mice. It was difficult to establish the food intake of the LAT mice and SOC mice precisely, since LAT mice were sometimes accidentally able to get food from the cage mate across the grid partition, and for SOC mice the proportion of food the cage mate ate was unknown. This complicates the interpretation of these results.

The higher HR of IND mice and LAT mice, and increased food intake of IND mice could indicate a higher metabolic rate to maintain a stable BT, especially in periods of rest. IND mice and LAT mice could not sleep together to keep each other warm and save energy. Besides that, a long-term increase in HR may indicate that the particular environment, if chronically stressful, has overtaxed the animal's adaptive capacity (Späni *et al.* 2003). Since the LAT mice had the highest HR and SOC mice the lowest, this may indicate that LAT housing is stressful for mice, whereas social housing appears to be the best in terms of animal welfare. At this point, it should be noted that LAT mice had less space as compared with SOC or IND mice. Their cages were, after all, divided in two.

The higher HR found in LAT mice could thus be the result of differences in cage space. A recently performed pilot test in which animals were housed in three different cage sizes, however, indicated that HR actually *decreased* with decreasing cage size (unpublished results).

### *Behaviour*

*Initial recovery* During the first few days after surgery, behaviour of the mice as measured by nest-building, behaviour on LABORAS and behaviour after weighing was clearly impaired. Nest building has been used as a reliable indicator for 'sickness'. Arras *et al.* (2007), for example, found that mice that were not treated with analgesics after surgery destroyed their nests and needed three days to rebuild a proper nest and structure their cage area while mice that did receive pain relief came out with well-established nests within the first day. Both nesting behaviour and burrowing have been reported to be reliable early indicators of sickness in mice (Felton *et al.* 2005, Hawkins *et al.* 2006). In our experiment, the complexity of the nest decreased for all three housing conditions on day 1 post-surgery. The largest decrease in nest complexity was seen in the IND mice, followed by the LAT mice. The nest complexity of the SOC mice was also decreased, indicating that the cage mate did not completely take over the nest building. From day 5 post-surgery, the nest complexity of the IND mice and SOC mice was comparable with pre-surgery complexity. The lower and more variable complexity score of the LAT mice was most likely due to the fact that the two LAT mice sometimes 'stole' each other's nesting material across the grid partition, leading to either no nest or a very complex nest. Although we previously found that mice show a strong preference for sleeping together (Van Loo *et al.* 2004), only 1–4 out of nine LAT mice slept near the cage mate on the other side of the grid partition, as compared with all SOC mice sharing a nest. The stealing of nesting material in LAT mice may have influenced their choice of sleeping site.

Exploratory behaviour, resting and grooming were affected by surgery as well. As could be expected, immobility and grooming increased both in the LABORAS test and after weighing sessions until about four days after surgery. Active behaviours such as climbing, locomotion and rearing were decreased shortly after surgery for a period of 5–10 days. Baumans *et al.* (2001) found similar results after transmitter implantation in mice. In this study, differences with controls existed for about four days after transmitter implantation. This is rather short compared with our results. The use of smaller mice compared with previous research and thus a relatively heavier transmitter may account for these differences. In concordance with our results, Martin *et al.* (2004) found that an abdominal incision in rats selectively suppressed exploratory locomotor activity for one to two days. The different types of exploratory behaviour were differentially affected, with ambulatory and rearing activity being the most sensitive to disruption by abdominal surgery. Small movements such as grooming were less affected and affected for a shorter duration after surgery.

*Differences between housing conditions* Difference in behaviour due to differential housing has been the subject of several studies. Zhu *et al.* (2006) described a strong impact on motor activity and neurotrophins in mice after individual housing. Distance moved, average speed and locomotion increased. Vöikar *et al.* (2005) also found that IND mice displayed significantly higher locomotor activity. Meanwhile, the duration of grooming was shorter in the singly-housed mice. Brain (1975) described that IND mice are more active in a novel situation than group housed animals but it has been reported that such animals are less active in their home cages without disturbance. On the contrary, Bartolomucci *et al.* (2003) found that IND mice showed increased latency to explore the novel environment and reduced movement in the open area. Palanza *et al.* (2001) also proved that IND female mice showed less exploration and a

higher level of anxiety compared with group housed female mice, as indicated by reduced rearing and locomotion.

In our study, only a few small overall housing differences could be detected in behaviour. In LABORAS, average speed of LAT mice was lower than SOC and IND mice, and after weighing, LAT mice spent significantly less time on locomotion. The latter could be due to the fact that cage space of these animals was lower due to insertion of the grid partition.

However, in the first days after surgery, we could find some remarkable differences between housing conditions. LABORAS data showed that in the SOC mice the difference between pre-surgery levels and day 1 was smaller for most behavioural parameters and the differences for some behavioural parameters had a shorter duration compared with the IND mice and LAT mice. Furthermore, four out of eight SOC mice climbed immediately following surgery, compared with none of the LAT or IND mice.

Pain behaviour such as identified by Roughan and Flecknell (2003a,b, 2004) proved to be a useful parameter for scoring pain in our animals. Despite the provision of analgesics during the first days after surgery, animals clearly showed an increased amount of pain behaviours until 5–7 days after surgery. This indicates that not only that the dosage of analgesics provided during the first days may have been inadequate, but also that pain relief possibly should have been administered for a longer period of time. It is, however, noteworthy that the SOC animals perform less twitching and flinching during the first few days after surgery compared with LAT and IND mice. Above that, SOC mice show less grooming and more locomotion, rearing and climbing. This may indicate that SOC mice experienced less pain as a result of the surgery than IND and LAT mice.

#### *General considerations*

In summary, most parameters measured returned to pre-surgical levels within five to seven days, while body weight and some

behavioural parameters needed almost two weeks to fully recover. SOC animals appeared to cope best with the postoperative recovery. These animals showed less pain behaviour, grooming and resting, and more climbing, rearing and locomotion during the first few days after surgery, and HR levels were lower during the course of the entire experiment. Contrary to expectations however, LAT did not promote animal welfare in this sense. Behavioural data did not differ from individual housing, and HR levels were even higher than those of IND mice. Apparently, living within sight, sound, smell and limited touch with each other was not enough to induce the positive effects of social housing on postoperative recovery. D'Amato and Pavone (1993, 1996) and D'Amato (1997) conducted an interesting series of experiments supporting this theory. They found that male sibling mice that are reunited after a period of separation show increased huddling behaviour and an opioid-dependent increase in pain threshold compared with unfamiliar mice. Similar results were found for familiar, but unrelated female mice. In a consequent experiment with male mice they found that neither the brother's scent, nor contact with the brother beyond a partition could induce this increase in pain threshold, indicating that huddling was necessary to induce analgesia. Interestingly, behavioural changes and display of pain behaviours following surgery were less pronounced in SOC mice than in IND or LAT mice, indicating that the activation of the endogenous system through social contact may indeed lead to more adequate and longer lasting pain relief in these animals.

## **Conclusions**

The importance of social interaction for mice has again been proven in this experiment. Our results show that social housing has a positive impact on postoperative recovery. However, when social housing is not possible due to experimental conditions, LAT housing is apparently not a better option than individual housing.

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## References

- Arras M, Rettich A, Cinelli P, Kaisermann HP, Burki K (2007) Assessment of post-laparotomy pain in laboratory mice by telemetric recording of heart rate and heart rate variability. *BMC Veterinary Research* **3**, 16
- Bartolomucci A, Palanza P, Sacerdote P, et al. (2003) Individual housing induces altered immunoendocrine responses to psychological stress in male mice. *Psychoneuroendocrinology* **28**, 540–58
- Baumans V, Bouwknecht JA, Boere H, et al. (2001) Intra-abdominal transmitter implantation in mice: effects on behaviour and body weight. *Animal Welfare* **10**, 291–302
- Brain PF (1975) What does individual housing mean to a mouse? *Life Sciences* **16**, 187–200
- Brain PF, Benton D (1979) The interpretation of physiological correlates of differential housing in laboratory rats. *Life Sciences* **24**, 99–115
- Brown KJ, Grunberg NE (1996) Effects of environmental conditions on food consumption in female and male rats. *Physiology & Behavior* **60**, 293–7
- Butz GM, Davisson RL (2001) Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. *Physiological Genomics* **5**, 89–97
- Council of Europe (2006) *Revised Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123)*. <http://conventions.coe.int/treaty/en/treaties/html/123.htm>
- D'Amato FR (1997) Neurobiological and behavioral aspects of recognition in female mice. *Physiology & Behavior* **62**, 1311–17
- D'Amato FR, Pavone F (1993) Endogenous opioids: a proximate reward mechanism for kin selection? *Behavioral and Neural Biology* **60**, 79–83
- D'Amato FR, Pavone F (1996) Reunion of separated sibling mice: neurobiological and behavioral aspects. *Neurobiology of Learning and Memory* **65**, 9–16
- Einstein R, Rowan C, Billing R, Lavidis N (2000) The use of telemetry to refine experimental technique. In: *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* (Balls M, Van Zeller AM, Halder ME, eds). Amsterdam: Elsevier Science BV, 1187–97
- Felton LM, Cunningham C, Rankine EL, Waters S, Boche D, Perry VH (2005) MCP-1 and murine prion disease: separation of early behavioural dysfunction from overt clinical disease. *Neurobiology of Disease* **20**, 283–95
- Giral M, Armario A (1989) Individual housing does not influence the adaptation of the pituitary–adrenal axis and other physiological variables to chronic stress in adult male rats. *Physiology & Behavior* **45**, 477–81
- Hawkins P, Felton LM, Van Loo PLP, et al. (2006) Report of the 2005 RSPCA/UFWA rodent welfare group meeting. *Lab Animal* **35**, 29–38
- Hellebrekers LJ (2000) Pathophysiology of pain in animals and its consequence for analgesic therapy. In: *Animal Pain* (Hellebrekers LJ, ed). Utrecht: Van der Wees, 71–83
- Jennings M, Batchelor GR, Brain PF, et al. (1998) Refining rodent husbandry: the mouse – Report of the Rodent Refinement Working Party. *Laboratory Animals* **32**, 233–59
- Kant GJ, Pastel RH, Bauman RA, et al. (1995) Effects of chronic stress on sleep in rats. *Physiology & Behavior* **57**, 359–65
- Kramer K, Van Acker SA, Voss HP, Grimbergen JA, Van der Vijgh WJ, Bast A (1993) Use of telemetry to record electrocardiogram and heart rate in freely moving mice. *Journal of Pharmacological and Toxicological Methods* **30**, 209–15
- Kramer K, Kinter LB (2003) Evaluation and applications of radiotelemetry in small laboratory animals. *Physiological Genomics* **13**, 197–205
- Kudryavtseva NN (2003) Use of the 'partition' test in behavioral and pharmacological experiments. *Neuroscience and Behavioral Physiology* **33**, 461–71
- Martin TJ, Buechler NL, Kahn W, Crews JC, Eisenach JC (2004) Effects of laparotomy on spontaneous exploratory activity and conditioned of operant responding in the rat – a model for postoperative pain. *Anesthesiology* **101**, 191–203
- Meijer MK, Spruijt BM, Van Zutphen LFM, Baumans V (2006) Effect of restraint and injection methods on heart rate and body temperature in mice. *Laboratory Animals* **40**, 382–91
- Palanza P, Gioiosa L, Parmigiani S (2001) Social stress in mice: gender differences and effects of estrous cycle and social dominance. *Physiology & Behavior* **73**, 411–20
- Roughan JV, Flecknell PA (2003a) Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *European Journal of Pain* **7**, 397–406
- Roughan JV, Flecknell PA (2003b) Pain assessment and control in laboratory animals. *Laboratory Animals* **37**, 172
- Roughan JV, Flecknell PA (2004) Behaviour-based assessment of the duration of laparotomy-induced abdominal pain and the analgesic effects of carprofen and buprenorphine in rats. *Behavioural Pharmacology* **15**, 461–72
- Ruis MAW, de Groot J, te Brake JHA, et al. (2001) Behavioural and physiological consequences of acute social defeat in growing gilts: effects of the

- social environment. *Applied Animal Behaviour Science* **70**, 201–25
- Ruis MAW, te Brake JHA, Buwalda B, *et al.* (1999) Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. *Psychoneuroendocrinology* **24**, 285–300
- Sharp J, Lawson D (2003) Does cage size affect heart rate and blood pressure of male rats at rest or after procedures that induce stress-like responses? *Contemporary Topics in Laboratory Animal Science* **42**, 8–12
- Späni D, Arras M, König B, Rulicke T (2003) Higher heart rate of laboratory mice housed individually vs in pairs. *Laboratory Animals* **37**, 54–62
- Van de Weerd HA, Bulthuis RJA, Bergman AF, *et al.* (2001) Validation of a new system for the automatic registration of behaviour in mice and rats. *Behavioural Processes* **53**, 11–20
- Van Loo PLP, Van de Weerd HA, Van Zutphen LFM, Baumans V (2004) Preference for social contact versus environmental enrichment in male laboratory mice. *Laboratory Animals* **38**, 178–88
- Võikar V, Polus A, Vasar E, Rauvala H (2005) Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. *Genes, Brain and Behavior* **4**, 240–52
- Zhu SW, Pham TM, Aberg E, *et al.* (2006) Neurotrophin levels and behaviour in BALB/c mice: impact of intermittent exposure to individual housing and wheel running. *Behavioural Brain Research* **167**, 1–8