The effect of routine experimental procedures on physiological parameters in mice kept under different husbandry conditions

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Abstract

Laboratory animals are frequently subjected to routine procedures, such as injections or the withdrawal of blood samples. Acute stress caused by such procedures is associated with physiological changes that can have a strong impact on experimental results. This study investigated the integrated effects of cage enrichment, social housing and handling on the acute stress response of animals subjected to routine experimental procedures. Female mice of two inbred strains (BALB/c and C57BL/6) were housed under either minimal husbandry conditions (MH: no cage enrichment, infrequent handling and a period of individual housing) or enriched husbandry conditions (EH: with cage enrichment, frequent handling and social housing at all times). One mouse in each cage was implanted with a radio-telemetry transmitter for measuring heart rate (HR) and body temperature (BT). The animals were subjected to intraperitoneal injections or short periods of restraint. In addition to telemetry measurements, thymus weight and tyrosine hydroxylase (TH) activity were assessed. It was found that individual housing under MH conditions, as compared with social housing under EH conditions, elevated both basal HR and BT, and significantly elevated the relative recovery time following routine experimental procedures. Thymus weight and TH activity suggested a long-term stress response under MH conditions following individual housing, although the influence of transmitter implantation and (repeated) acute stress remains to be investigated. The results emphasise that husbandry conditions should be taken into account when evaluating physiological measures after routine procedures.

Keywords: animal welfare, husbandry conditions, mice, radio-telemetry, routine procedures, stress response

Introduction

Animal experiments often require routine procedures, such as injections, blood sampling and handling. These procedures are known to act as acute stressors, which result in a physiological stress response. For example, haematological parameters and stress hormone levels in the blood of rats have been found to change after moving cages (Gärtner et al 1980), and increased levels of corticosterone have been found in mice after transport and tail bleeding (Tuli et al 1995a,b). In addition, a depressive-like state in rats (as measured by activity levels) was reported to be induced by repeated saline injections, although this varied between strains (Izumi et al 1997). Furthermore, studies using radio-telemetry showed an increase in heart rate (HR) and body temperature (BT) in mice after weighing, handling or injections with saline (Clement et al 1989; Kramer et al 1993; Harkin et al 2002). Environmental factors (eg cage size and group size) have also been shown to influence the response of HR and mean arterial blood pressure in rats after a single routine procedure (Sharp et al 2002; Sharp et al 2003).

Stress-induced physiological changes are part of the normal adaptive response. As long as an animal can regain homeostasis within a certain period of time, it is said to be able to cope with the stressful situation. Nevertheless, even responses within the normal adaptive range are likely to influence experimental results and have to be considered when designing experiments.

Having a better insight into the reactions of laboratory animals to routine procedures will lead to a refinement of animal experiments and help researchers to determine exactly what is being measured. Furthermore, allowing the animals to adapt to routine procedures can reduce the variability of results, thereby decreasing the number of animals required (Poole 1997).

The present study was designed to investigate to what extent husbandry conditions affect the physiological stress response caused by routine procedures in laboratory mice (*Mus musculus*). Two contrasting husbandry conditions were designed that differed in enrichment, social housing and handling regime. In order to test for differences between strains, two commonly used inbred strains of mice (BALB/c

and C57BL/6) were used, which are known for their differences in emotionality (see Mouse Genome Database 2001a,b). HR and BT were measured using radio-telemetry, which was considered to be an appropriate tool to measure changes in baseline values and the acute physiological response after routine procedures. The possible long-term effects of husbandry conditions and/or the procedures were examined by measuring thymus weight and tyrosine hydroxylase (TH) activity at post mortem. Thymus weight is known to decrease under the influence of stress, caused by increased apoptosis of thymus cells, whereas TH activity is known to rise under these conditions, reflecting an increased activity of the hypothalamic-pituitary-adrenal (HPA) axis (Manser 1992).

Materials and methods

Animals

Eighty-four female mice of two inbred strains (BALB/cByJIco: n = 42; C57BL/6Jico: n = 42; Charles River, Maastricht, The Netherlands) arrived at the facility at the age of three weeks. The mice were housed in a conventional animal room (temperature 18–24ºC) with a 12h:12h light:dark cycle (lights on: 0700h, light intensity at shelf level approximately 100 lux; lights off: 1900h, light intensity approximately 2 lux); a radio was on during the light-period. The animals were provided with food pellets (CRM: SDS, Witham Essex, UK) and tap water *ad libitum*. All procedures were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University.

Husbandry conditions

On the day of arrival, the mice were randomly assigned to 28 cages (elongated Macrolon® type II [floor area 530 cm²] with wire tops: Tecniplast, Milan, Italy), housing three mice of the same strain per cage. Two of the three mice were marked by clipping either the left or right ear. For each strain of mice, half were housed under 'minimal husbandry' (MH) conditions and the other half were housed under 'enriched husbandry' (EH) conditions; cages were placed randomly on shelves. MH conditions had sawdust bedding (Lignocel® ¾: Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany) without cage enrichment, mice were handled only when necessary (eg during cage cleaning) and were individually housed in Macrolon® type II cages (floor area 370 cm²) from week 16 of the experiment onwards (see Table 1 for a timeline). Under EH conditions, the mice were additionally provided with cage enrichment, including a Shepherd Shack (a paper-based mini-mouse house, 15 × 9 × 6 cm, length × width × height; Shepherd Specialty Papers: Kalamazoo, MI, USA), two Kleenex® tissues (Kimberly Clark, Ede, The Netherlands), approximately 15 g of EnviroDry® (folded paper strips; BMI, Helmond, The Netherlands), an opaque grey PVC tube (15 × 5 cm, length × width) and two aspen wood chew sticks (5 × 1 × 1 cm in size; Finn Tapvei, Finland). In addition, the EH mice were handled gently each weekday for 30–60 s, at variable times of the day during specific weeks (see Table 1), but always by the same person; these animals were group-housed throughout the experiment.

The MH conditions imitated the husbandry conditions used in many laboratories: cage enrichment is not yet standard and infrequent handling is becoming more common with the increased use of individually ventilated cage systems. Although individual housing is not standard practice for female mice, it is well accepted when social interaction has to be avoided (eg after surgery or in metabolic studies), or when in the course of an experiment cage mates are removed or die. Although these two contrasting husbandry conditions have the limitation that they do not allow discrimination between all variables, it is a well considered choice: if no differences could be detected in this set-up, it would be unlikely to find any if the conditions were less different.

Transmitter implantation and post-operational care

Seven weeks after the start of the experiment (see Table 1), at the age of 10 weeks (mean body weight C57BL/6–MH: 21.2 ± 0.2 g; C57BL/6–EH: 21.2 ± 0.5 g; BALB/c–MH: 24.5 ± 0.5 g; BALB/c–EH: 24.3 ± 0.6 g, see Figure 1), 28 mice (1 mouse per cage, randomly chosen at the beginning of the experiment) were implanted with a radio-telemetry transmitter (TA10ETA-F20: DataSciences International, St Paul, MN, USA). The mice were anaesthetised using isofluorane (ISOFLO: Schering-Plough, Maarssen, The Netherlands), N_2O and O_2 (induction: isofluorane 5%, $N_2O:O_2$ 1:1, 2 l; maintenance: isofluorane 1.4–1.6%, $N_2O:O_2$ 1:1, 0.5–0.8 l). During surgery, the eyes were protected from the airflow with eye ointment (Chloramphenicol: Cevasanté, Naaldwijk, The Netherlands). The implantation procedure was carried out as described in detail by Kramer *et al* (1993), but with minor modifications. The abdomen was opened and the transmitter was placed in the peritoneal cavity with the negative lead placed subcutaneously at the right shoulder and the positive lead at the left lower chest. Both transmitter and leads were sutured to the muscle layer (by non-absorbable Prolene® 4-0 and absorbable Vicryl® 4-0 respectively: Johnson & Johnson, Amersfoort, The Netherlands); before closure, the peritoneal cavity was filled with warm, sterile saline (0.9%, Braun Melsungen AG, Melsungen, Germany). The mean duration of each operation was 45 min. After surgery, the mice were placed in an incubator, at 32°C, for 1 h. Together with their two non-implanted cage mates, they were then returned to a clean home cage that was partially placed on a heating pad for at least 24 h after surgery. To prevent harassment of the implanted animals by their cage mates, 70% ethanol was gently rubbed on to the abdomen of each non-implanted animal using a gauze so that all animals carried a novel, unfamiliar odour for a short period of time. In addition to normal food and water, Solid Drink® (Triple A Trading, Otterloo, The Netherlands), moistened food pellets and food 'porridge', made with 3% glucose solution, were provided for four days. The mice were administered with buprenorphine (0.5 mg kg⁻¹; TEMGESIC®: Schering-Plough, Brussels, Belgium), by intraperitoneal (ip) injection, for two days after surgery and were allowed four weeks of recovery. Handling of the EH animals was resumed in the second week after surgery (Table 1).

Table 1   Time-line of the experiment.

Week		Week	
1–6	Habituation, handling of EH groups	17	Ip injections
7	Transmitter implantations	18	Handling of EH groups
8–11	Recovery of transmitter implantation, handling of EH groups	19	Ip injections
12	Ip injections	20	Handling of EH groups
13	Handling of EH groups	21	Ip injections
14	Ip injections	22	Restraint by hand
15	Restraint by hand	23	Euthanasia and post mortem
16	Individual housing MH groups, handling of EH groups		

From transmitter implantation onwards, HR and BT measurements were collected every 3 min, 24 h per day. All transmitters functioned correctly, except for one in the BALB/c–EH group; therefore, the results of this group are based on a total of six rather than seven animals. Transmitter-signals were sent to a PC and saved to disk; data acquisition and analysis were performed using DataQuest A.R.T. (DSI, St Paul, MN, USA).

Routine experimental procedures

Stress response measurements after routine experimental procedures began in the fifth week after surgery, in week 12 of the experiment. No routine experimental procedures were performed in weeks 13, 16, 18 and 20 (see Table 1 for a time-line).

Cages were cleaned on Mondays, at which time the animals were weighed and food consumption per cage was measured. With the exception of the resting-weeks and week 16, when the MH mice were individually housed (see Table 1), experimental procedures were performed from Tuesday until Friday, between 1100h and 1200h, once per week for each cage. It was chosen to perform the procedures during the light period because this is the common working method in the majority of animal facilities. Cage order was randomly chosen and changed each week. In each cage, the implanted animal ('transmitter + procedures') and one of the two cage mates ('only procedures') were subjected to the procedures. The third cage mate ('control') was not subjected to either surgery or procedures. The implanted animals were always subjected to the procedures second, in order to start collecting telemetry data as soon as possible after completion of the procedures. Ip injections consisted of 0.5 ml of sterile saline (0.9%; Braun Melsungen AG, Melzungen, Germany) administered at room temperature, which is a volume and temperature regularly used for administering substances to laboratory mice (Pekow & Baumans 2003).

The animal was lifted out of the cage by the tail and placed on the cage lid. It was then restrained by holding the scruff, the base of the tail and the left hind leg. The animal was held with its head tilted downwards and the abdomen was gently palpated to assess the location of the transmitter (if present). The saline was gradually injected in the lower quadrant of the abdomen, off midline, using a 26 gauge needle. The restraint procedure consisted of the same protocol; however, no saline was injected nor was a needle inserted into the abdomen. All experimental procedures were performed by the same experimenter who also handled the EH groups, which took place in the room housing the animals. Baseline levels of HR and BT were assessed during the 15–20 min period before the procedures started, after the experimenter had just entered the animal room. HR and BT were also assessed on Sundays (weeks 11–22) between 1100h and 1200h and between 2300h and 2400h, to inspect the baseline levels when no one was present in the room.

Euthanasia and post mortem

All animals but one (a transmitter-implanted C57BL/6 mouse in the MH group which died of unknown causes) survived until the end of the experiment. In week 23 of the experiment, between 0900h and 1200h, all animals were euthanased by decapitation and dissected for post mortem examination and analysis. Euthanasia and dissection were performed in a separate room to where the mice were housed. Each group of three animals housed together under EH conditions, or three individually housed mice under MH conditions, were transported to the room and immediately euthanased simultaneously by three animal technicians. The thymus was dissected and weighed for each mouse. The adrenal glands from each mouse were also dissected, individually stored in 5 mM Tris-HCl-buffer (pH 7.2), shock-frozen in liquid nitrogen and stored at –70ºC for further analysis. In the adrenal glands, TH activity was measured using a tyrosine–^{14}C assay. In short, the adrenal glands were thawed, homogenised and incubated with tyrosine–^{14}C. TH then synthesised the tyrosine into dopa which was, in turn, converted into dopamine. The remaining tyrosine–^{14}C was then separated from the dopamine–^{14}C by elution over a column with aluminium oxide and counted in a liquid scintillation counter. The converted tyrosine was a measure of the TH activity in the adrenal gland (modification of the method described by Witte & Matthaei 1980).

Figure 1
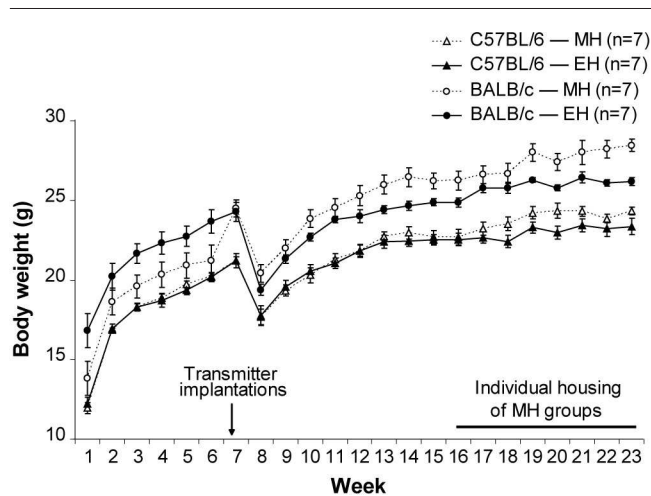
Mean (± SEM) body weight (g) of the 'transmitter + procedures' mice, measured once per week throughout the experiment. Transmitter implantations and individual housing of the MH groups are indicated; see Table 1 for more detailed information of the weeks.

Statistics

HR levels were compared at 0–9, 18–27, 36–45 and 54–63 min after ip injection. BT levels were compared as with the HR levels, but only after the restraint procedure (weeks 15 and 22; Table 1), because, as expected, the injection of saline in the abdomen influenced the BT measurements.

Each analysed time-point was the mean of three data acquisitions. This method was chosen in order to avoid missing values, which can occur when a mouse is very active and the position of the transmitter is out of range of the receiver. To correct for baseline differences, baseline values were subtracted from the subsequent post-procedure measurements. Statistical analysis was performed on the resulting data. However, as visual comparison of baseline and post-procedure values provides relevant information and a better insight, data are presented as the actual (mean) values. Analysis was performed using a general linear model for repeated measures, with time as a within-subject factor, and strain and husbandry condition as between-subject factors; unless otherwise stated, data fulfilled the requirements for repeated measures.

Thymus weight and TH concentration were analysed for all mice by univariate analysis of variance with strain, husbandry condition and treatment ('transmitter + procedures', 'only procedures' and 'control') as fixed between-subject factors. Dunnett *post hoc* tests (treating the 'only procedures' animals as control) were performed where appropriate. All analyses were performed using SPSS for Windows (version 10.1.0). Differences were considered statistically significant when $P < 0.05$. Data are expressed as mean values ± standard error of the mean (SEM).

Figure 2
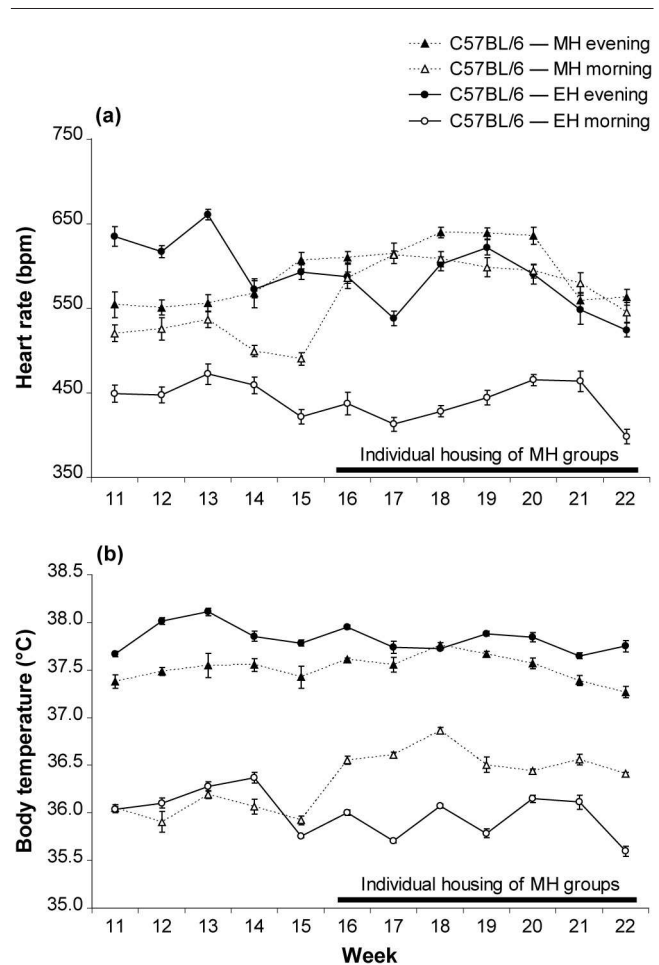
Mean (± SEM) baseline levels of **(a)** heart rate (bpm) and **(b)** body temperature (°C) of C57BL/6 mice (both MH and EH groups) in the morning (1100h–1200h) and evening (2300h–2400h) on each Sunday of the experiment (n = 7 per group, except for the C57BL/6 — MH group in weeks 21 and 22, n = 6).

Results

Body weight and food intake

During the first four days after surgery, all implanted animals lost weight; however, body weight was back to pre-surgery levels at the end of the four-week recovery period (corrected for the weight of the transmitter; Figure 1). Food intake by the MH groups increased considerably in the weeks during individual housing (approximately 40% and approximately 60% increase in the C57BL/6 and BALB/c strains respectively), but the body weight gain was not proportional (Figure 1).

Heart rate and body temperature after intraperitoneal injection and restraint

Mean baseline HR and BT levels during the morning period (1100h–1200h) on each Sunday of the study, with no one present in the animal room, showed an increase for the MH groups after the animals had been individually housed (see Figure 2 for C57BL/6 strain; a comparable pattern over the
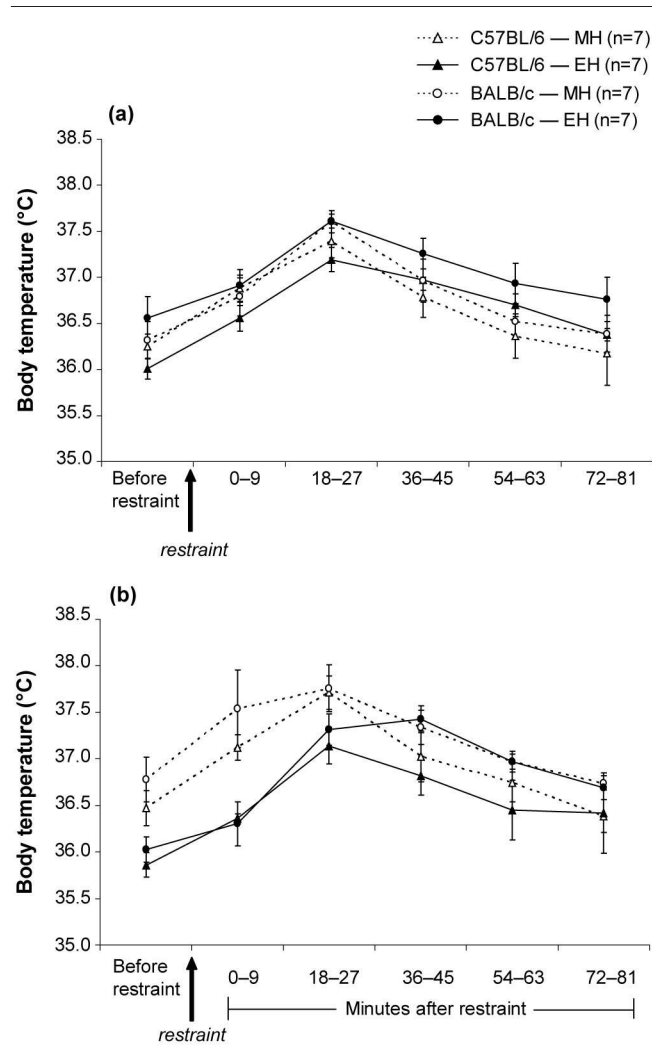
Figure 3

Legend:
···△··· C57BL/6 — MH (n=7)
—▲— C57BL/6 — EH (n=7)
···○··· BALB/c — MH (n=7)
—●— BALB/c — EH (n=7)
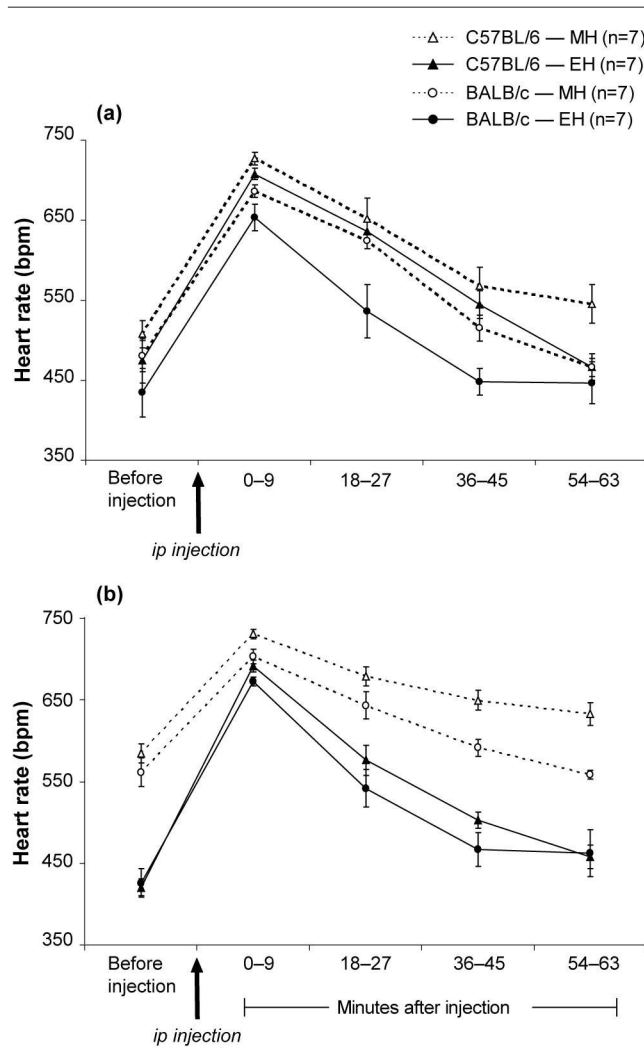
Mean (± SEM) heart rate (bpm) for **(a)** weeks 12 and 14 (no significant differences) and **(b)** weeks 17, 19 and 21 (time*husbandry effect: $P < 0.001$; overall husbandry effect: $P = 0.007$).

Figure 4

Legend:
···△··· C57BL/6 — MH (n=7)
—▲— C57BL/6 — EH (n=7)
···○··· BALB/c — MH (n=7)
—●— BALB/c — EH (n=7)

Mean (± SEM) body temperature (°C) for **(a)** week 15 (time*husbandry effect: $P = 0.05$) and **(b)** week 22 (time*husbandry effect: $P = 0.007$; time*strain effect: $P = 0.044$; not all requirements for repeated measures were fulfilled).

weeks was found for the BALB/c strain). This is consistent with baseline levels prior to the procedures (with the experimenter present in the animal room, see Figures 3 and 4).

In general, the HR of the mice increased during the first 10 min, and BT during the first 20 min, after a procedure and returned to baseline levels during the following hour (Figures 3 and 4).

As the results of the separate weeks were found to be the same, the data from weeks 12 and 14 and the data from of weeks 17, 19 and 21 were pooled and analysed. Figure 3a shows the average HR results of weeks 12 and 14, the period before individual housing of the MH groups. After individual housing of these groups (see Figure 3b) HR recovery of both EH groups was relatively faster than that of the MH groups (time*husbandry effect: $P < 0.001$). Overall, HR levels of the MH groups were increased (husbandry effect: $P = 0.007$); no strain effects were found.

The response of HR to the restraint procedures in weeks 15 and 22 of the experiment was similar to that observed after ip injections in the other weeks (data not shown).

In week 15 (before individual housing of the MH groups), restraint revealed a significant interaction for time*husbandry on BT ($P = 0.05$; Figure 4a). In week 22 (Figure 4b), the baseline BT of the MH groups was more than 1ºC higher than the EH groups. Analysis of the data from week 22 showed a faster increase in BT in the MH groups (within the first 20 min after restraint; time*husbandry effect: $P = 0.007$) and a longer time to reach maximum BT of the BALB/c–EH group (time*strain effect: $P = 0.044$). Although the results of this week did not fulfil all requirements for repeated measures, visual inspection of the data equally suggests the same interactions. No overall husbandry or strain effects were found in either period.

Figure 5
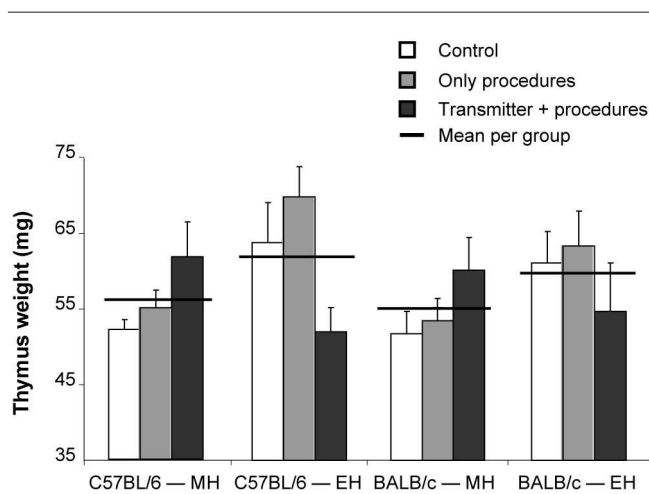
Mean (± SEM) thymus weight (mg) (n = 7 per group, except for C57BL/6 — MH where n = 6). Overall husbandry effect $P = 0.034$; husbandry*treatment effect: $P = 0.001$ (see text for *post hoc* test results).

Thymus weight and tyrosine hydroxylase activity

Overall, absolute thymus weight was lower in MH animals compared with EH animals ($P = 0.034$), as can be seen in Figure 5 (solid black lines). When comparing the impact of different treatments, an interaction between husbandry condition and treatment was found ($P = 0.001$). The Dunnett *post hoc* test proved that the thymus weight of the 'transmitter + procedures' animals was significantly lower in the EH groups ($P = 0.015$). Analysis of the thymus weight as a proportion of the body weight of the mice revealed no differences from the analysis of the absolute values.

Overall TH activity was lower in the EH animals than in the MH animals ($P < 0.001$; Figure 6, solid black lines). Furthermore, a significant interaction was found between strain and treatment ($P < 0.001$). Dunnett *post hoc* analysis showed that in the C57BL/6 strain, the 'transmitter + procedures' animals had a lower TH activity compared with the other animals ($P = 0.004$), while in the BALB/c strain, the 'only procedures' animals had a lower TH activity compared with their cage mates ('transmitter + procedures': $P = 0.006$; 'control': $P = 0.007$).

Discussion

In this experiment, the effect of different husbandry conditions on the stress response following routine experimental procedures was studied in two inbred strains of mice. Different husbandry conditions resulted in increased baseline values of HR and BT during the light-period following individual housing of the MH groups. This was true not only for the period just prior to the procedures (Figures 3b and 4b) but also during the weekend (Figure 2). This was in accordance with other published studies reporting elevated levels of basal HR in individually housed mice (Einstein *et al* 2000; Späni *et al* 2003).

Notably, individual housing also elevated basal BT. It has been shown that female rats can maintain a stable BT when exposed to an ambient room temperature of <12–29.5ºC (Yang & Gordon 1996); therefore, it is unlikely that ambient room or cage temperature in this study (with a much smaller temperature range) were the cause of the increased baseline BT. If at all, housing conditions would have been more likely to reduce BT because the individually housed animals had no cage mates or nesting material to keep them warm. Therefore, the increased BT baseline values are more likely to be explained by the increased food intake that was found during individual housing of the MH groups, resulting in a rise in basal metabolism and, accordingly, a rise in basal BT. Several hypotheses exist for this phenomenon. For example, the increased food intake may have been the result of the increased body heat loss caused by individual housing or of boredom attributable to the impoverished environment without stimuli (compare with the increased sucrose intake in play-deprived juvenile rats: van den Berg *et al* 2000). Alternatively, a possible increase of catecholamines (adrenaline and nor-adrenaline) could have increased the metabolic rate. However, none of these hypotheses can be supported or rejected by the currently available data. The elevated baseline levels will, however, have to be taken into account when analysing the acute stress response after routine procedures.

Heart rate and body temperature after intraperitoneal injection and restraint

The first study to use radio-telemetry for cardiovascular measurements in mice showed a maximum HR of approximately 750–800 bpm, which was found during restraint by hand and after cage change (Kramer *et al* 1993). In the present study, all four groups reached approximately the same maximum HR after the routine experimental procedures, with only a slight increase of the MH groups compared with the EH groups (Figure 3). Therefore, the HR response was analysed over time and was found to change significantly following individual housing of the MH groups compared with the EH groups (Figure 3b). Although the HR recovery of both MH and EH groups took approximately 1 h, HR recovery in the MH groups was relatively slower. It is possible that after a stressful event HR recovery follows a general pattern, independent of basal levels, always recovering within approximately 1 h. However, in rats, moving cages during the resting period was found to cause a tachycardia that persisted for only 10 min (Gärtner *et al* 1980); and also other studies have shown that the duration of the response differs according to the procedure (Harkin *et al* 2002; Sharp *et al* 2002; Sharp *et al* 2003). This suggests that the recovery of HR is influenced by the intensity of stress an animal perceives; therefore, the present findings indicate the need for further research in which other variables, such as cage enrichment, are studied in more detail.

Although based on the results of only two trials (weeks 15 and 22), the BT data suggests that it is the development of Stress Induced Hyperthermia (SIH; an increase in BT following a stressful event), rather than the recovery period, that might

give an indication of the intensity of the stress response. The increase in BT found in the current study strongly resembles the results of other studies, which used a rectal probe to measure BT in mice (Zethof *et al* 1994); although, in the current study, the maximum BT level in week 22 occurred later: at 18–27 min compared with 8 min in the study by Borsini *et al* 1989 and van der Heyden *et al* 1997. However, the use of a rectal probe requires the animal to be restrained for more than 20 s, in addition to the insertion of the probe into the rectum to a length of 2 cm. This procedure can be considered more invasive than the handling procedure used in the current study and may explain the faster increase in BT. If the development of SIH is positively correlated with the amount of stress perceived by an animal, this would suggest that, in the current experiment, the animals in the MH groups were experiencing more stress following individual housing than the animals in the EH groups that remained socially housed, because the development of SIH was significantly slower in the EH group (Figure 4b).

Only in week 22 was a significant interaction found for time*strain, and no overall effects of strain were found for HR or BT in any week; therefore, it should be concluded that the acute stress of the routine procedures was perceived equally by the two strains, despite their known differences in emotionality. However, it is also possible that HR and BT were not sufficiently sensitive to measure the influence of strain characteristics on the stress response in this study.

Thymus weight and tyrosine hydroxylase activity

Thymus weight and TH activity are both known indicators of long-term stress. Under the influence of stress, atrophy of thymus cells (a normal physiological process initiated by circulating sex steroids at the onset of sexual maturity) occurs more rapidly than usual (Manser 1992), resulting in a more pronounced reduction in thymus weight. In the present study it was therefore expected to find an overall lower thymus weight in the MH groups, which indeed was the case. However, an interaction between husbandry and treatment was also found: thymus weights under EH conditions were relatively lower in the 'transmitter + procedures' animals, whereas under MH conditions 'transmitter + procedures' animals showed higher thymus weights than their cage mates (Figure 5). The fact that thymus weight can only be measured after euthanasia means that possible effects of the (repeated) acute stress of the routine procedures cannot be determined, nor can the effects of transmitter implantation and its possible interaction with husbandry conditions, which might have significantly affected the immune system and therefore thymus weight.

The same problem arose when assessing TH activity, which could also only be measured once in the adrenal glands after euthanasia. In contrast to thymus weight, TH levels were expected to be higher in the MH groups. TH is an enzyme involved in the synthesis of (nor-)adrenaline in the adrenal medulla, reflecting long-term activity of the adrenal gland, provoked by an increased activity of the HPA-axis, which can be the result of chronic stress

Figure 6
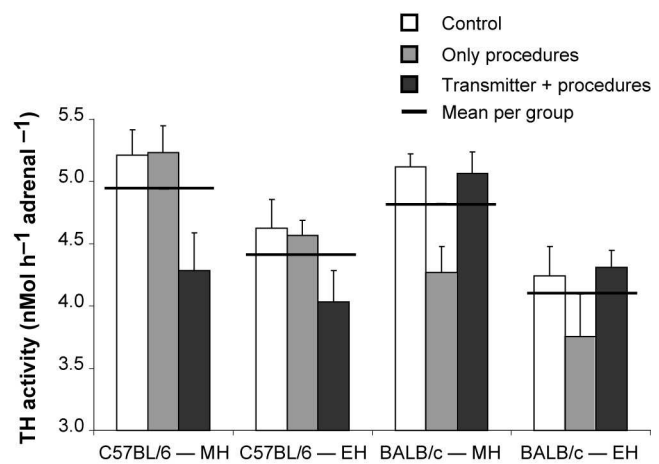
Mean (± SEM) TH activity (nMol h⁻¹ adrenal⁻¹) in the adrenal glands (n = 7 per group, except for C57BL/6 — MH where n = 6). Overall husbandry effect: $P < 0.001$; strain*treatment effect: $P < 0.001$ (see text for *post hoc* test results).

(Manser 1992). Indeed, overall TH activity proved to be highest in the MH groups, indicating that these animals may have experienced long-term stress caused by their husbandry conditions. However, the different effects of treatment found for the two strains were not expected and cannot be explained with the current information. Further studies on TH activity and thymus weight will be needed in order to explore the exact effect of implanted radio-telemetry transmitters, husbandry conditions and routine procedures on these stress parameters in mice.

Conclusions

In this study, individual housing under MH conditions was found to have a considerable impact on several physiological parameters. Baseline values of HR, BT, thymus weight and TH activity were significantly influenced, and routine experimental procedures were found to influence the acute stress response as measured by radio-telemetry. We hypothesise that individual housing under MH conditions changes the animals' ability to cope, which might lead to an imbalance of the physiological systems and a possible loss of homeostasis. At which moment the normal adaptive process becomes maladaptive, and if this is further influenced by the repeated acute stress of routine procedures, remains to be answered. Further research including environment, frequency of procedures and genetic background is therefore required.

Animal welfare implications

The present study has shown physiological effects caused by routine experimental procedures in mice housed under different conditions. Therefore, experimenters should realise that husbandry conditions can have a considerable effect on test results obtained after relatively simple routine procedures, such as handling or injections, and this should be taken into account when designing experiments.

Acknowledgements

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