

Effect of Conditioning on the Increase of Heart Rate and Body Temperature Provoked by Handling in the Mouse

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Summary — To assess the effect of procedures on animal welfare, various physiological parameters, such as body weight, hormone levels in plasma and/or urine, heart rate (HR), blood pressure and body temperature (BT), can be used. When measuring physiological parameters with techniques involving restraint of the animals, the results must be interpreted with caution, since restraint itself may have an effect on those parameters. Radio-telemetry, using an implantable transmitter, provides a way to obtain more accurate and reliable physiological measurements from freely moving animals in their own environment. In this study, we have used radio-telemetry to investigate the influence of conditioning on the increase of HR and BT as provoked by handling of mice. It was found that, after a conditioning period of 12 days, the increase of HR due to handling was significantly reduced.

Key words: *body temperature, conditioning, handling, heart rate, mouse, telemetry.*

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Introduction

Animals subjected to various laboratory procedures and environmental changes will react to the new situation with a change in their physiological response. Radio-telemetry with an implantable transmitter provides a way to obtain accurate and reliable physiological measurements from awake and freely moving animals in their own environment without the need for (stressful) handling (1, 2).

Handling of mice or offering them a new environment, such as a new cage with clean bedding, results in an acute increase in body temperature (BT; 3–5) and heart rate (HR; 6, 7). It was found that body temperature increased by 1.5°C after handling (3). The heart rate of freely moving mice increased from 400–450 beats per minute (bpm) to 750–800bpm after handling or placing the animals in a new cage (6, 7). These data are obtained without prior conditioning of the animals.

HR has been reported as an indicator for measuring animal welfare (8), or stress (9). HR has also been used as a physiological parameter in testing the conditioned response in rats (10–13). Iwata & LeDoux (10) showed an HR increase, triggered by the presentation of an auditory stimulus that was previously paired with footshock. Investigations on conditioned response in C57BL/6 mice revealed that when a single acoustic stimulus was paired with footshock, the

stimulus could provoke defensive behaviour (freezing) in a memory test (14). Moreover, the results from Stiedl & Spiess (15) on HR effects during a tone-dependent retention test after a one-trial classical fear conditioning, indicated that HR reflects associative learning in C57BL/6N mice. It was shown that in mice subjected to a conditioning stimulus (tone), paired with footshock, the tone onset triggered an immediate HR increase. Both studies indicate that mice can be used in learning and memory tests. Learning abilities of different strains and sub-strains of mice may differ (16, 17). It seems that C57BL/6N mice are fast learners, whereas Balb/c mice have been reported to be slow learners (17).

Handling and restraint of mice are common procedures in the laboratory animal house. The purpose of the present study was to investigate whether conditioning of mice with an acoustic stimulus (10kHz, 60dB), can reduce the stress effects of these routine procedures on HR and BT.

Materials, Methods and Procedures

Animals

Male SPF mice (C57BL/6N, 8–10 weeks old, body weight 20–24g), purchased through the Central

Laboratory Animal Institute (CLAI), Utrecht University, Utrecht, The Netherlands, were allowed to adapt to laboratory housing conditions during 1 week before implantation of the transmitter.

After the implantation of the transmitter, the animals were housed individually in wire-topped Macrolon Type II cages (375cm²; UNO Roestvaststaal BV, Zevenaar, The Netherlands) provided with 50g sawdust bedding (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany) and Kleenex tissues (Kimberly-Clark Corporation®, Veenendaal, The Netherlands) as nesting material in a clean, conventional animal room. Temperature 21–23°C, relative humidity 45–60%, ventilation 15 air changes per hour and light–dark periodicity (L:D 12:12: lights on from 07:00–19:00). Throughout the experiment, food (RMH-B®; Hope Farms BV, Woerden, The Netherlands) and tap water were provided *ad libitum*.

The animals were treated in cohorts (six animals per cohort) according to a random block design. During the experiments, the mice were individually housed.

Transmitter implantation

After 1 week of acclimatisation a transmitter for measuring HR and BT (TA 10ETA-F20-L20; Data Sciences International [DSI], St Paul, MN, USA) was placed into the abdominal cavity of each individual mouse. Before surgery, enrofloxacin (Baytril® 2.5%; Bayer, Mijdrecht, The Netherlands; 25µl/mouse s.c.) was given. Surgery was performed as previously described (6, 18). In brief, the transmitter was implanted in the abdominal cavity, under anaesthesia (intraperitoneal [i.p.] injection of a mixture [1:1:2] of fentanyl/fluanisone [Hypnorm®; Jansen Pharmaceutical, Beerse, Belgium], midazolam [Dormicum®; Roche Nederland BV, Mijdrecht, The Netherlands] and aquadest [0.1ml/10g body weight]). Artificial tears (Duodrops®; Apharmo BV, Arnhem, The Netherlands) protected the eyes. After shaving and disinfection, the abdomen was opened and the transmitter was placed into the abdominal cavity, fixed to the abdominal wall with three non-absorbable Ethilon® 7–0 (Johnson & Johnson, Amersfoort, The Netherlands) stitches, and the muscle layer and skin were closed in two separate layers with PDS® 5–0 (Johnson & Johnson). Both leads of the transmitter were sutured subcutaneously in lead II position, the negative lead at the right shoulder, the positive at the lower left chest. After surgery the animals were wrapped in aluminium foil and/or paper tissues, and placed back in their home cage (placed by half on a heating pad for 24 hours). The animals received buprenorphin (Temgesic®; Reckitt & Colman Products Ltd, Kingston-upon-Hull, UK, 0.5mg/kg body weight i.p.) as an analgesic for 2 days, twice a day. In addition to standard food pellets and tap water, the operated animals received Solid

Drink® (Triple A Trading, Otterlo, The Netherlands) for 4 days and moistened food pellets for 7 days in their home cage.

Experimental design

Two weeks after surgery the mice were randomly allocated to one of three groups (Groups 1–3; n = 6). The animals of Group 1 (control) were not handled for 2 weeks. The animals of Group 2 (no sound) were handled three times per day, 6 days per week, for 2 weeks, at arbitrary times. The animals of Group 3 (sound) were housed in another animal room, under the same conditions as Groups 1 and 2. The handling procedure was executed as in Group 2; however, it was executed after an acoustic stimulus (conditioning) of 10kHz and 60dB (15).

Handling procedure

For the purpose of this study, the procedure of handling was accurately standardised. The mice were picked up at the tail base and held in the palm of the hand for 3 seconds, three times per day, at arbitrary times. Handling was performed by one person throughout the experiment. Following handling, they were returned to their home cage. Entering the animal room prior to handling may disturb the animals in an unspecific way and may also cause an increase in HR and BT. In order to distinguish the HR and BT response of handling from the response of entering the animal room, both animal rooms were entered five (instead of three) times per day (two times without handling).

HR and BT were measured before, during and after the different experimental conditions, using the radio-telemetry technique, as published before in detail (2, 6). Baseline data were collected for each animal during the periods between handling.

Statistics

All parameters were expressed as mean of six mice \pm SD and evaluated using analysis of variance (ANOVA), with day and time as within-subjects factors and stimulus and handling as between-subjects factors. The statistical tests were carried out with the aid of SPSS for Windows, Release 9.0 (SPSS Inc., Chicago, USA).

Results

General

We successfully operated on 18 mice. After surgery, recovery criteria included weight gain after an ini-

tial loss, and changes in behaviour, such as building nests with available tissues. Body weight returned to normal levels within 2 weeks of surgery, as described previously (6, 18).

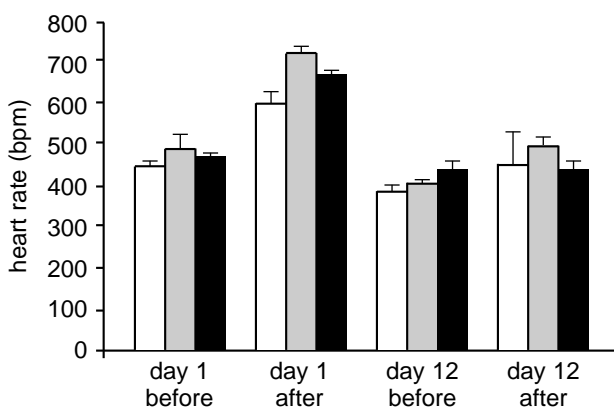
Heart rate

Data on HR were recorded during the whole experimental period. For the presentation of results, we have selected 2 days (days 1 and 12) and two time points on each of these days (2:30pm [Figure 1] and 9:30am [Figure 2]). These are representative for the effects of handling procedures and the influence of acoustic conditioning.

All groups

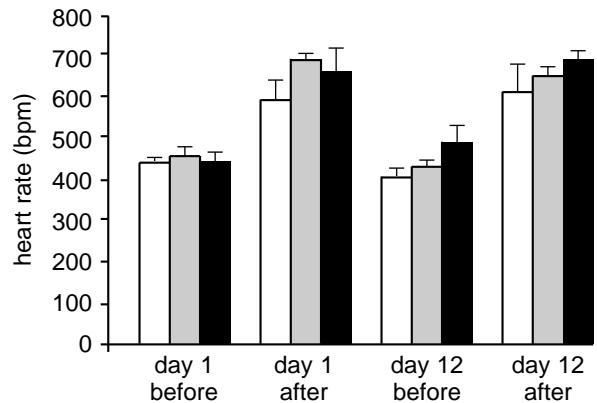
Entering the animal room by the technician, without performing the handling and/or the acoustic stimulus increased HR significantly ($p < 0.005$; Figures 1 and 2 [white bars]: day 1, “after” vs. day 1, “before”). After 12 days, the HR values after entering the animal room at the first time point (Figure 2 [white bar] 9:30am: day 12 “after”) were still significantly increased ($p < 0.005$) compared with the HR values before entering the room (Figure 2 [white bar] 9:30am: day 12 “before”).

Figure 1: Heart rate (beats per minute [bpm], mean \pm SD) measured in freely moving mice, before and after entering the room



□ = control animals, ▒ = entering the room and restraint, ■ = entering the room, acoustic stimulus and restraint, on day 1 and day 12 at a fixed time point (2:30pm). In all groups day 1, “after” is significantly different from day 1, “before” ($p < 0.005$). In all groups day 12, “after” is significantly different from day 1, “after” ($p < 0.005$).

Figure 2: Heart rate (beats per minute [bpm], mean \pm SD) measured in freely moving mice, before and after entering the room



□ = entering the room (control animals), ▒ = entering the room and restraint, ■ = entering the room, acoustic stimulus and restraint, on day 1 and day 12 at a fixed time point (9:30am). In all groups, day 1 and 12, “after” are significantly different from day 1 and 12, “before”, respectively ($p < 0.005$).

In the afternoon, HR increased significantly ($p < 0.005$; Figure 1 [2:30pm] day 1 “after” vs. “before”); however, there was no significant increase in HR after 12 days (Figure 1 [2:30pm] day 12 “after” vs. “before”). Handling further increased the HR (Figures 1 and 2 [grey bars] “after” vs. “before”).

Group 1 (control)

After repeatedly entering the animal room at a fixed time point (2:30pm), HR was significantly reduced after 12 days ($p < 0.005$; Figure 1 [white bar]: day 12, “after” compared with day 1, “after”). However the sole entering of the animal room at 9:30 a.m. still increased HR significantly even after 12 days ($p < 0.005$; Figure 2 [white bar]: day 12, “after” compared with day 12, “before”).

Group 2 (no sound)

Repeated handling reduced the increase in HR significantly after 12 days ($p < 0.005$; Figure 1 [grey bar], 2:30pm: day 12, “after” compared with day 1, “after”). However, repeated handling did not reduce the increase in HR on the first time point of the day after 12 days (Figure 2 [grey bar], 9:30am: day 12, “after” compared with day 1, “after”).

Group 3 (sound)

Repeated handling preceded by an acoustic stimulus ("conditioning") reduced the increase in HR significantly after 12 days ($p < 0.005$; Figure 1 [black bar], 2:30pm: day 12, "after" compared with day 1, "after"). However, there was no reduction in HR observed during this procedure for 12 days on the first time point of the day (Figure 2 [black bar], 9:30am: day 12, "after" compared with day 1, "after").

Body temperature

Data on BT were recorded during the whole experimental period. In general, handling increased BT. Although the same effects of handling and/or conditioning were observed for BT, the results were not always as obvious as they were for HR, because of the minimal differences in BT values after each procedure. For BT values, there was a slight, though not significant, increase and/or decrease observed (data not shown). There was only one clear significant effect observed: entering the animal room by the technician, without performing the handling and/or the acoustic stimulus increased BT significantly ($p < 0.005$). After 12 days the BT after entering the animal room at the first time point still increased significantly ($p < 0.005$). Effects of handling and/or conditioning could not be observed.

Discussion

Broom & Johnson (8) have defined animal welfare as "the state of an individual as regards its attempts to cope with its environment", thus, the ability of an animal to cope with or adapt to internal and external stressors. To assess animal welfare, the impact of stress on physiological parameters, such as body weight, hormone levels in plasma and/or urine, HR, blood pressure (BP) and BT can be measured (8, 19–23). When measurements of the physiological parameters are performed using conventional techniques, the results must be interpreted with caution, since the technique itself may have an effect on the animals. This has been found for body weight (24), catecholamine levels (25) and BP (26). Intra-arterial catheterisation of rats has been shown to cause decreased food intake as well as body weight loss (27). BP measurements in mice via the tail-cuff technique caused increases in both HR and BP (28). Recently, Duke *et al.* (22) described the effects of routine cage changing on cardiovascular and behavioural parameters in male Sprague-Dawley rats. They concluded that standard animal husbandry procedures such as moving rats to a clean cage can induce transient, but significant, cardiovascular and behavioural changes.

The present study was performed in order to investigate the possibility of reducing the stress response of mice to routine handling procedures. Tabata *et al.* (29) have shown that procedures such as handling, cage transport and anaesthesia have less effect on physiological parameters in rats than in mice. Male Wistar rats adapt within 1 week to the stress of being placed on a swimming platform (30), whereas in the mouse (male Balb/c), after several weeks of handling, no adaptation was observed (2). However, in the present study, it has been shown that conditioning of mice with an acoustic stimulus can reduce the stress response, as measured by change of HR.

Conclusions

It can be concluded that entering the animal room, even without handling of the animals, increases HR and BT. After 2 weeks, the animals seem to habituate to this type of disturbance, with the exception of the first entrance in the morning (9:30am). In order to mimic standard procedures as much as possible, the day–night cycle was not reversed. Thus, the first entrance is in the beginning of the animal's rest period. Disturbance at this time point may cause more stress than later on.

Repeated handling with or without conditioning by an acoustic stimulus reduces the increase in HR after 12 days especially in the afternoon. Although we did not find a significant effect in BT (probably due to the small effects), we observed a trend similar to HR. Thus, it can be concluded that conditioning of mice can be effective in attenuating the stress effects of routine procedures.

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