INTRA-ABDOMINAL TRANSMITTER IMPLANTATION IN MICE: EFFECTS ON BEHAVIOUR AND BODY WEIGHT

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Abstract *Animal Welfare 2001,* ***10:*** 291-302

Biotelemetry is a useful tool for the simultaneous measurement of several physiological and behavioural parameters in non-restrained, freely moving animals. However, the weight and volume of the implanted intra-abdominal transmitter may cause discomfort. The aim of this study was to assess body weight and behaviour of BALB/c and 129/Sv mice after implantation of an intra-abdominal transmitter. In order to measure more detailed behaviour, the automated behaviour observation analysis system (LABORAS™) was used. During the first days after surgery, body weight and the behaviours of climbing, locomotion and eating were found to decrease in both strains, whereas grooming and immobility increased. These changes were more pronounced in the transmitter animals than in the sham operated animals, however, indicating a temporary impairment in well-being. Within two weeks after surgery, the animals seemed to have fully recovered.

Keywords: *mice, telemetry, transmitter implantation*

Introduction

Biotelemetry is a useful tool for measuring several physiological and behavioural parameters such as heart rate, blood pressure, body temperature, electrocardiogram (ECG) and locomotory activity simultaneously in non-restrained animals. The advantages of implantable wireless telemetry transmitters, as claimed by various authors, include:
i) Providing a humane method for monitoring conscious, freely moving laboratory animals (Brockway & Hassler 1993);
ii) Eliminating stress related to the use of restraint, which can alleviate a potential source of experimental artifact and interanimal variability (Schnell & Gerber 1997; Stokstad 1999);
iii)Reducing animal use by 70 per cent in single studies (Van Acker *et al* 1996), and by more than 90 per cent in multiple studies (Kinter 1996);
iv)Allowing 24-hour data collection via computer, for days, weeks, or months, without any special animal care (Brockway *et al* 1998).

Biotelemetry can be defined as "the remote detection and measurement of a human or animal function, activity, or condition". Wireless biotelemetry is used in infra-red, ultrasound

and radio-frequency transmission in several animal species. During the 1950s, telemetry was used to study the physiological effects of space travel in the first 'spacedog', Laika. The early telemetry systems were attached outside the body in backpacks. Later on, during the 1960s, implantable devices were used in several animal species and in several fields of research. Recently (Brockway *et al* 1998), advanced technology has allowed the development of miniature devices to monitor various behavioural and physiological parameters in small animal species such as rats and mice.

Although implantable transmitters may reduce stress resulting from handling and restraint, the procedure requires invasive surgery. The transmitter is placed in the abdominal cavity of the mouse through a ventral laparotomy (Kramer *et al* 1993). The weight of the transmitter without leads (about 3 g) is quite high for a mouse with a body weight of about 25 g. Also, the volume of the implant (1.6 ml) has to be taken into account. It can be expected that a mouse would experience discomfort from such a device. Superficial observations of transmitter-implanted mice during experiments do not reveal abnormal behaviour. However, Kramer *et al* (1996) found, after implantation, an abnormal diurnal rhythm of body temperature and weight loss for four days in mice. The aim of the present study was to assess in an objective way the behaviour of mice carrying an intra-abdominal transmitter. Using an automated Laboratory Animal Behaviour Observation Registration and Analysis System (LABORAS™; Metris System Engineering, Hoofddorp, the Netherlands), several behavioural parameters can be monitored without disturbing the animal (Bulthuis *et al* 1996; Schlingmann *et al* 1998).

LABORAS™ is a system for automated behaviour classification for individually housed mice and rats. With a specially designed sensing platform, the position and the behavioural categories of climbing, locomotion, immobility, grooming, eating and drinking can be deduced from the movements of the animal in the cage. Other behaviour is classified as 'undefined'. Each of the behavioural categories is characterised by a specific and unique movement pattern, which is detected by the sensors under the platform. The signals from the sensors are filtered, amplified, converted and processed by the computer into the behavioural categories. The recorded behaviours are quantified as relative duration (seconds per hour, s h^{-1}) and frequency (number h^{-1}).

During the experiments, the mice were individually housed for the following reasons:
i) By placing back an implanted BALB/c male after recovering from surgery in a group of counterparts, severe fighting may occur;
ii) LABORAS™ is a system for automated behaviour classification in individual mice.

Materials and Methods

Animals

Male BALB/cAnNCr/BR (BALB/c) (n = 19) and 129/Sv (n = 17) mice aged 12 weeks, body weight 24–28 g, purchased SPF (specified pathogen free) from Central Laboratory Animal Institute, Utrecht, were used in three different treatment groups:
i) Control animals (BALB/c, n = 6; 129/Sv, n = 6);
ii) Sham operated animals (BALB/c, n = 7; 129/Sv, n = 5);
iii)Transmitter implanted animals (BALB/c, n = 6; 129/Sv, n = 6).

The BALB/c strain is a widely used inbred strain. The 129/Sv strain is frequently used to create knockouts, using recombinant DNA technologies. The animals were housed individually in wire-topped Macrolon type II cages (375 cm^2, UNO Roestvaststaal BV, Zevenaar, The Netherlands) in a clean, conventional animal room, and were provided with

50 g of sawdust (Lignocel ¾, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany) and Kleenex tissues (Kimberley-Clark Corporation®, Veenendaal, the Netherlands) as nesting material. Temperature was 21–23°C; relative humidity was 45–60 per cent; ventilation rate 15 air changes per hour. Tap water and food pellets (RMH-B®, Hope Farms BV, Woerden, the Netherlands) were provided *ad libitum*.

After one week of acclimatisation to a shifted light–dark schedule (light 2300h–1100h at approximately 200 lux at 1 m above the floor; dark 1100h–2300h with a 30-minute dimming period), the animals were treated in cohorts (four animals per cohort) according to a random block design.

Surgery

The transmitter implantation was carried out by placing a dummy without leads (weight ~3.0 g; volume 1.6 ml) (Data Sciences International, St Paul, MN, USA) into the abdominal cavity.

Before surgery, the antibiotic enrofloxacin, (Baytril® 2.5%, Bayer, Mijdrecht, the Netherlands, 25 µl mouse^{-1}) was given subcutaneously. The mouse was anaesthetised with an intraperitoneal (i.p.) injection of a 1 : 1 : 2 mixture of fentanyl/fluanisone (Hypnorm®, Jansen Pharmaceutica, Beerse, Belgium), midazolam (Dormicum®, Roche Nederland BV, Mijdrecht, The Netherlands) and aquadest (0.1 ml mixture per 10 g body weight). The eyes were protected by artificial tears (Duodrops®, Apharmo BV, Arnhem, the Netherlands). After shaving, the abdomen was opened, the transmitter dummy was placed into the abdominal cavity, and was fixed to the abdominal wall with three non-absorbable Ethilon® 7-0 (Johnson & Johnson, Amersfoort, the Netherlands) stitches. The muscle layer and skin were closed in two separate layers with PDS® 5-0 (Johnson & Johnson, Amersfoort, the Netherlands) stitches. The procedure was carried out under aseptic conditions. The sham operation was performed in a similar way, except that the dummy transmitter was removed from the abdominal cavity after having first being fixed to the abdominal wall.

Much attention was paid to the post-operative care. After surgery, the animals were wrapped in aluminium foil and placed back into their home cage, half of which was placed on a heating pad for 24 hours. They received buprenorphin (Temgesic®, Reckitts Colman Products Ltd, Kingston-upon-Hull, UK; 1 mg kg^{-1} body weight, i.p.) as an analgesic for two days, twice a day. In addition to standard food pellets and water, the operated animals received Solid Drink® (Triple A Trading, Otterlo, The Netherlands) for four days, moistened food pellets and water containing 10 per cent (w/v) glucose for seven days in their home cage. The animal's home cages and the LABORAS™ system were in the same animal room.

During the experiment, four animals died — three 129/Sv (sham operated) mice and one BALB/c (transmitter) mouse. These animals died 2–4 days after recovery from the anaesthesia, with no clear cause of death. In order to give sufficient power to the statistical analysis, these four animals were replaced by four other animals.

Behavioural assessment

Behaviour of each animal was measured on LABORAS™ twice per week (days 2 and 4) for one hour directly after the start of the dark/dimming period during the first week, and three times per week (days 9, 11 and 13) for one hour during the second week. In this study, four platforms were used to sample four animals simultaneously.

Details of LABORAS™ are described by Bulthuis *et al* (1996). LABORAS™ registrations were validated by comparing them with data from observations of videotapes by human observers (Van de Weerd *et al* 2001). Body weight was determined each day at the same time in the morning. At the end of the experiment, the animals were killed and post mortem macroscopic inspection was carried out.

Statistical evaluation

The Kolmogorov-Smirnov one-sample test was used to check normality of the data. All results within groups were found to be normally distributed. The body-weight results were subjected to a multiple analysis of variance (MANOVA; repeated measures) with strain and treatment as main between-subject factors and day as main within-subject factor. For the behavioural measurements per day, the significance of the differences between groups was calculated using a two-way analysis of variance (ANOVA) with strain and treatment as the main factors. Homogeneity of the variances was tested using Bartlett's test. When necessary, the variances were equalised by a ranking transformation (Hora & Conover 1984). After transformation, the variances were similar and the transformed within-group data were still normally distributed. Thus, application of an analysis of variance on the (transformed) data is then straightforward. If the analyses of variance showed significant effects with respect to the behavioural measurements, the group means were further compared using the unpaired Student's *t*-test. These tests were performed with pooled (for equal variances) or separate (for unequal variances) variance estimates. The equality of variances was then tested using an *F*-test. To take into account the greater probability of a type I error due to multiple comparisons, the level of significance for the Student's *t*-test was pre-set at $P < 0.05$ / number of meaningful comparisons (i.e. $P < 0.05 / 3 = 0.0167$) instead of $P < 0.05$, according to Bonferroni's adaptation. In all other cases, the probability of a type I error < 0.05 was taken as the criterion of significance. Two-sided probabilities were estimated throughout. All statistical analyses were carried out according to Steel and Torrie (1981), using a SPSS PC$^+$ computer programme (SPSS, 1990).

Results

MANOVA repeated measurements: S, T, D, T x S and T x D.
T = main effect of the between-subject factor 'treatment'
S = main effect of the between-subject factor 'strain'
D = main effect of the between-subject factor 'day'
T x S = interaction effect between the factors 'treatment' and 'strain'
T x D = interaction effect between the factors 'treatment' and 'day'

Body weight

Body weight (corrected for transmitter weight) showed a significant decrease during the first four days after operation in the sham and transmitter animals, the decrease being more pronounced in the transmitter animals. From day four onwards, an increase was seen until the initial body weight was reached at day 14. There was no difference between the two strains, except that the BALB/c mice had a higher initial body weight (Figure 1).

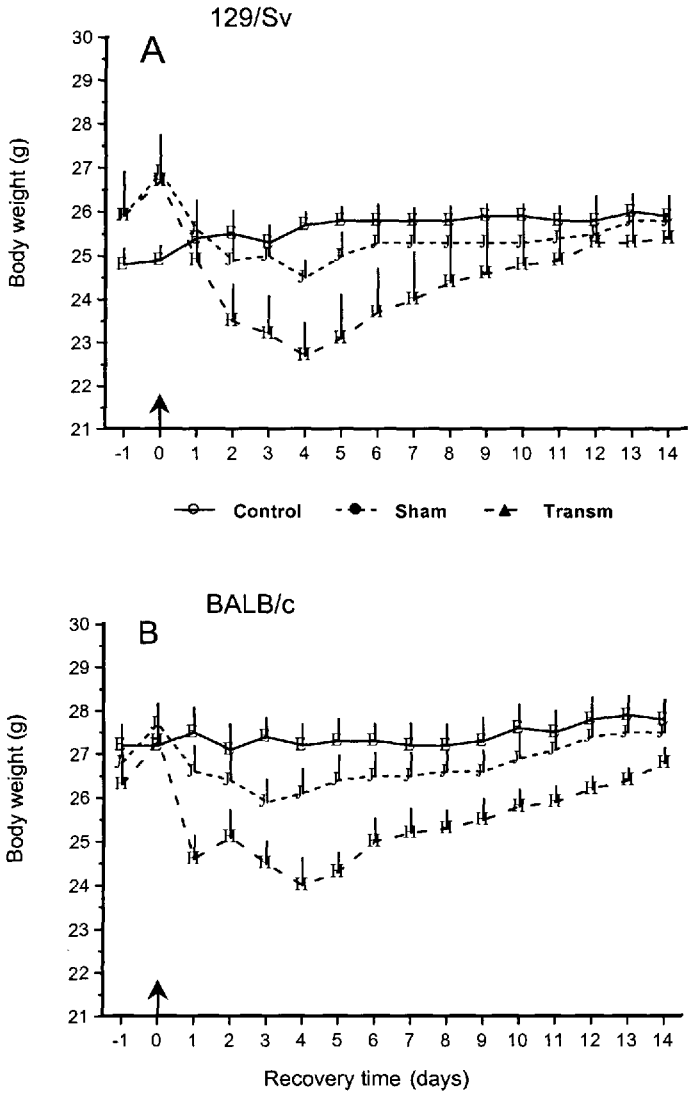

Figure 1 **Body weight recovery after surgery in (a) 129/Sv and (b) BALB/c mice in control, sham and transmitter (Transm) groups. The arrow indicates day of surgery (day 0). Results are expressed as means ± SEM.**

Climbing

(Two-way ANOVA. Frequency of climbing: day 2, T and S [after transformation]; day 4, T and S [after transformation]; day 9, S and T x S; day 11, S; day 13, S. Duration of climbing: day 2, T and S [after transformation]; day 4, T and S; day 9, S; day 11, S; day 13, S.)

On each day, a clear strain effect was found: in BALB/c mice, the duration and frequency of climbing were significantly higher than in 129/Sv animals. On days 2 and 4, the duration and frequency of climbing were significantly affected by treatment in both strains (Figure 2). There was a significant decrease in the duration of climbing behaviour on day 2 in the 129/Sv

strain in both sham and transmitter animals when compared with control animals. On day 2, the duration of climbing was higher in BALB/c control mice than in their transmitter counterparts. With respect to the duration of climbing on day 4, the only significant difference was between sham and transmitter BALB/c mice. For each strain on day 2, control mice climbed more frequently than did transmitter animals. In addition, on day 2, control BALB/c mice had a significantly higher frequency of climbing than sham BALB/c mice. On day 4, for only the BALB/c strain, transmitter mice climbed less frequently than did control mice. The other days did not show significant treatment differences. This was true of duration as well as frequency (see Immobility behaviour section, below).

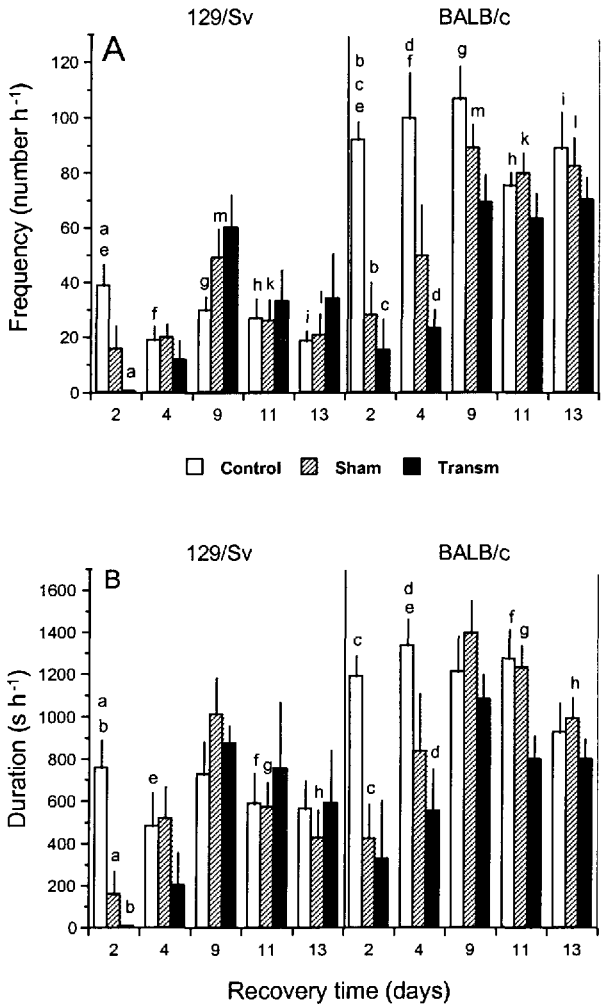

Figure 2 **Climbing behaviour: (a) frequency and (b) duration of climbing in 129/Sv and BALB/c mice in control, sham and transmitter groups. Within each diagram, groups bearing the same letter are significantly different (Student's *t*-test; $P < 0.0167$). Results are expressed as means ± SEM.**

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Locomotion

(Two-way ANOVA. Frequency of locomotion: day 2, T and S; day 4, T and S; day 9, S; day 11, S; day 13, S. Duration of locomotion: day 2, T and S; day 4, T and S; day 9, S and T x S; day 11, S; day 13, S.)

Again, there was a clear difference in locomotion (duration and frequency) between the two strains: BALB/c mice showed more locomotion. In addition, a decrease in frequency and duration of locomotion was seen on day 2 for transmitter animals of both strains when compared to control animals (Figure 3). On day 2, sham BALB/c mice showed less frequent locomotion than control BALB/c mice. On days 4 and 9, control BALB/c mice showed more frequent locomotion than their transmitter counterparts.

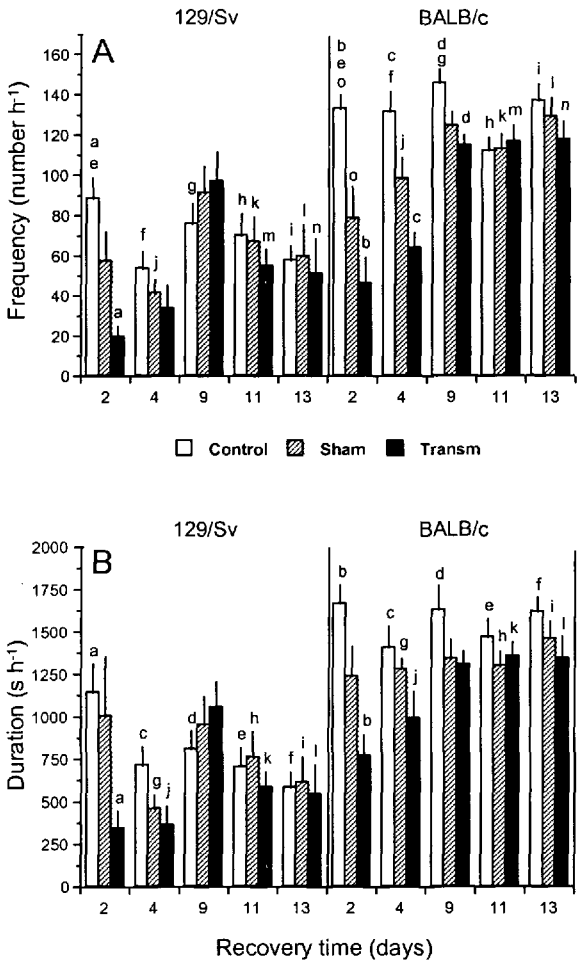

Figure 3 **Locomotory behaviour: (a) frequency and (b) duration of locomotion in 129/Sv and BALB/c mice in control, sham and transmitter groups. Within each diagram, groups bearing the same letter are significantly different (Student's *t*-test; $P < 0.0167$). Results are expressed as means ± SEM.**

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Immobility behaviour

(Two-way ANOVA. Frequency of immobility: day 2, T [after transformation]; day 4, S [after transformation]; day 9, S [after transformation]; day 11, S [after transformation]; day 13, S [after transformation]. Duration of immobility: day 2, T [after transformation]; day 4, S [after tranformation]; day 9, S [after transformation]; day 11, S [after transformation]; day 13, S [after transformation].)

129/Sv mice showed more immobility than BALB/c mice on days 4, 9, 11 and 13 (Figure 4). More frequent immobility was shown on day 2 in the transmitter animals when compared with control animals. In terms of duration of immobility, only a tendency towards more immobility was shown in transmitter and sham animals, due to large individual variation (Figure 4).

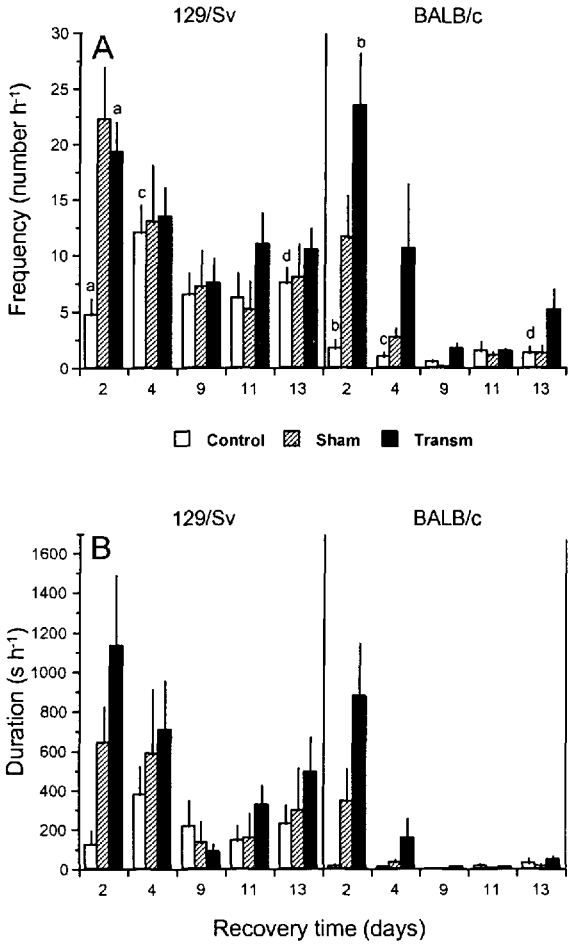

Figure 4 **Immobility behaviour: (a) frequency and (b) duration of immobility in 129/Sv and BALB/c mice in control, sham and transmitter groups. Within each diagram, groups bearing the same letter are significantly different (Student's *t*-test; $P < 0.0167$). Results are expressed as means ± SEM.**

Grooming

(Two-way ANOVA. Frequency of grooming: day 2, T and S [after transformation]; day 4, T and S; day 9, S; day 11, S; day 13, S [after transformation]. Duration of grooming: day 2, T and S [after transformation]; day 4, T and S [after transformation]; day 9, S [after transformation]; day 11, S [after transformation]; day 13, S [after transformation].)

129/Sv mice groomed far more than did BALB/c mice (Figure 5). On day 2, the duration of grooming of sham BALB/c animals was higher than that of their control counterparts. Transmitter animals of the BALB/c strain groomed more frequently than did the control animals on day 4 (Figure 5).

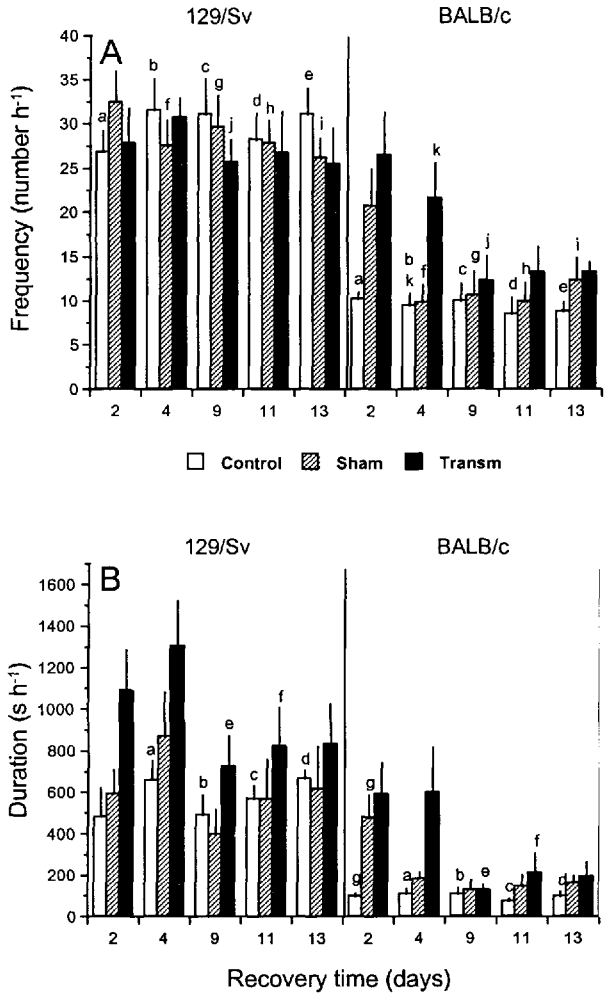

Figure 5 **Grooming behaviour: (a) frequency and (b) duration of grooming in 129/Sv and BALB/c mice in control, sham and transmitter groups. Within each diagram, groups bearing the same letter are significantly different (Student's *t*-test; $P < 0.0167$). Results are expressed as means ± SEM.**

Eating

(Two-way ANOVA. Frequency of eating: day 2, T and S; day 4, T and S; day 9, S; day 11, S; day 13, S [after transformation]. Duration of eating: day 2, T; day 4, no effects; day 9, T x S; day 11, T x S; day 13, no effects.)

On day 2, control animals ate more frequently than did the transmitter animals in both strains (Figure 6).

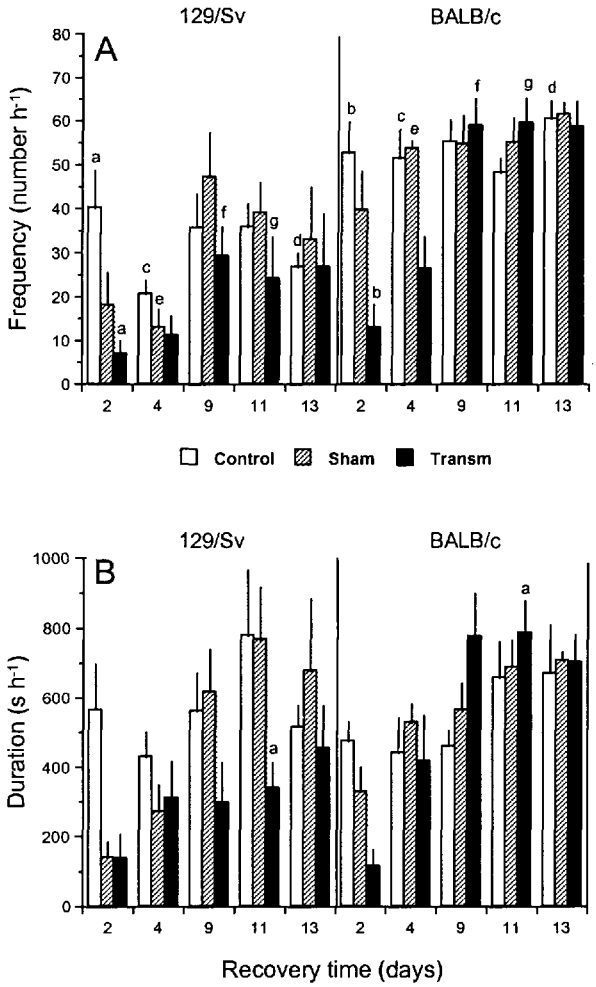

Figure 6 **Eating behaviour: (a) frequency and (b) duration of eating in 129/Sv and BALB/c mice in control, sham and transmitter groups. Within each diagram, groups bearing the same letter are significantly different (Student's *t*-test; $P < 0.0167$). Results are expressed as means ± SEM.**

Drinking

(Two-way ANOVA. Frequency of drinking: days 2, 4, 9 and 11, no effects; day 13, S [after transformation]. Duration of drinking: days 2, 4, 9 and 11, no effects; day 13, T and S [after transformation].)

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No clear strain differences or treatment effects for drinking behaviour were found on days 2, 4, 9 or 11. On day 13, control 129/Sv mice showed more drinking behaviour than control BALB/c mice. On this day, transmitter 129/Sv mice also drank more frequently than transmitter BALB/c animals. Note: the total amount of time spent on, and the frequency of, drinking was low.

In summary, differences in behaviour between treatment groups per strain were mainly found during the first week after surgery.

Post mortem

The majority of the transmitter animals had epididymal fat between the transmitter and the abdominal wall attached to the suture-tab on the transmitter body, which did not seem to cause trouble. No other pathology was found.

Discussion

During the first four days after surgery, differences in body weight and behaviour between treated animals and controls were shown; these effects were more pronounced in transmitter animals of both strains. The body weight recordings are in accordance with Kramer *et al* (1996), who also found a decrease in body weight during the first four days.

Climbing, locomotion and eating showed a decrease in transmitter animals as compared to control animals in both strains, whereas grooming and immobility increased in the first two days in transmitter animals. These findings may indicate an impairment in the well-being of the animals (Büttner 1991; Van de Weerd *et al* 1994; Baumans *et al* 1996). Considerable strain effects were found between BALB/c and 129/Sv mice. Sham operated animals, compared with controls, also showed effects on body weight and behaviour, but these effects were less conspicuous than in the transmitter animals.

Although the cause of death of the 129/Sv animals could not be determined, one might argue that 129/Sv mice are more sensitive to stress, surgery and possibly analgesics (this would be in line with previous observations [J A Bouwknecht, personal communication 1999]). No major pathology was found during the post mortem examination. Thus, the choice of strain may be important in this type of experiment. Moreover, adequate peri- and post-operative care seems to be of the utmost importance.

The LABORAS™ system allows faster and less time-consuming scoring of behaviour compared with a video system, and separates the behavioural elements of activity such as grooming and climbing, which may decrease or increase in opposite directions. Other recording systems measure overall activity, which can appear unchanged. A limitation of the system is that currently only individual animals can be tested.

Animal welfare implications

In conclusion, it can be stated that the well-being of the animals is compromised during the first week after surgery. However, when body weight has normalised after 14 days, the animals seem to have recovered completely.

This implies that biotelemetry provides a humane method for monitoring conscious, freely moving animals, without the need for restraint, which complies with the R of Refinement. Moreover, animal use can be considerably reduced as long-term data can be collected, which meets the R of Reduction.

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