A BALANCE DEVICE FOR THE ANALYSIS OF BEHAVIOURAL PATTERNS OF THE MOUSE

F Schlingmann[1†], H A Van de Weerd[2], V Baumans[2], R Remie[1] and L F M Van Zutphen[2]

[1] Solvay Duphar, Department of Laboratory Animal Science, Weesp, The Netherlands

[2] Utrecht University, Department of Laboratory Animal Science, Utrecht, The Netherlands

[†] Contact for correspondence and requests for reprints

Final acceptance: 4 April 1997

Abstract *Animal Welfare 1998, 7:* 177-188

A device for analysing mouse behaviour which is based on recording the movements of the animal in a balance test cage has been developed. The amplitude patterns provoked by the movements of the animal correlate with behavioural patterns. The system not only allows differentiation between five behavioural categories: resting, grooming, eating, locomotion and climbing – but also indicates the location of the animal in its cage. Upon validation, the system has proven to be a reliable and time-saving device for the non-invasive recording of behavioural patterns in the mouse.

Keywords: animal welfare, balance device, behavioural analysis, behavioural patterns, circadian rhythm, mice.

Introduction

Analysis of behaviour is an important tool in the assessment of the well-being of laboratory animals. When using behaviour as a parameter, a detailed knowledge of normal animal behaviour is necessary (Barclay *et al* 1988). Experimental procedures or discomfort due to insufficient housing and care conditions can change normal behavioural patterns (Spruijt & Gispen 1984; Van de Weerd *et al* 1994; Badiani *et al* 1995).

Behavioural patterns such as locomotion, eating, climbing and drinking, as well as body temperature and hormone concentrations in the blood, show a circadian rhythm (Vincent *et al* 1983; Clement *et al* 1989; Matthew *et al* 1990; Büttner 1991). This implies that, in studies of the impact of housing or experimental procedures on the behaviour of an animal, observations should be rather detailed and preferably cover a period of at least one circadian cycle. Information on behavioural patterns can be obtained by analysis of video recordings of animals, but this is rather time-consuming. To reduce observation time, a device, previously designed for recording the position of a singly housed mouse in its cage (Schlingmann *et al* 1993), has been modified so that both the position of the mouse and specific behavioural categories can be detected. The movements of the animal provoke characteristic amplitude patterns which reflect specific behaviours. Five different categories of behaviour can be distinguished. This paper describes the design of the balance system and its validation. For validation (experiment 1), the recorded amplitudes caused by the movements of the mouse were compared with a visual analysis of the behaviour as recorded by a video system. The recorded amplitudes were scored by two observers. Experiment 2 describes an application of the device.

Animals, materials and methods

Experiment 1: Validation

Animals

For the validation study, data obtained from both male and female mice (*Mus musculus* n = 6, age 7 weeks, weight 20-25g) of the C57BL/6JIcoU strain were used. Prior to their use the test animals were housed individually for acclimatization in Macrolon type II cages, ($375cm^2$; UNO Roestvaststaal, Zevenaar, The Netherlands) with a stainless steel cover which had a food-hopper at the front. The animals were conventionally housed with a controlled light/dark cycle (light: 0700-1900h), light intensity in the cage of 60-90lux, relative humidity 50-70 per cent, temperature 20-22°C and a ventilation rate of 12 air changes h^{-1}. Bedding material consisted of sawdust (Lignocel™ 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Tap water and pellets (RMH-B™, diameter 10mm, Hope Farms, Woerden, The Netherlands) were provided *ad libitum*.

Equipment

The design for recording amplitudes is shown schematically in Figure 1. The cage is placed on a balance platform, with the left side of the platform resting on an isometric displacement transducer (Force transducer FT-03C, Grass) and a spring on the other side holding the balance platform in zero position. The spring prevents interruption of the contact between the platform and the transducer. The transducer detects the forces generated by the various movements of the mouse, and converts these into an electrical signal. The force sensor signal is transmitted via an amplifier (Kyowa DPM 310 A) to a 6-channel pen recorder (BBC Goerz Metrawatt SE 460/61/62/63, paper speed 1cm min^{-1}), which reproduces the signal on a paper roll. Video recordings of a mouse were made by an infrared video camera (Panasonic, WV-1510) and a time-lapse video recorder (Panasonic, AG-6700). The time-lapse images were taken at 1/9 normal speed.

The experiment was carried out in a room under conditions similar to the acclimatization period. The test cage was divided into two equal compartments by a stainless steel plate with a 3.5cm diameter hole, through which the animal could move freely from one half to the other. This facilitated clear distinction of the position of the animal in its cage. Each compartment was provided with 50g of sawdust. The food-hopper (filled with 100g of food pellets) was placed in the middle of the galvanized wire cover to make eating and drinking in both cage compartments possible and could be approached from either side. Each compartment was provided with a water bottle. The cage lid did not touch the cage (Figure 2).

Procedure

Before the start of each test period, the balance platform with the test cage containing bedding was calibrated with an object comparable in length and weight to a mouse. By calibrating the amplifier, the minimum and maximum amplitudes and the baseline (midline position) were set.

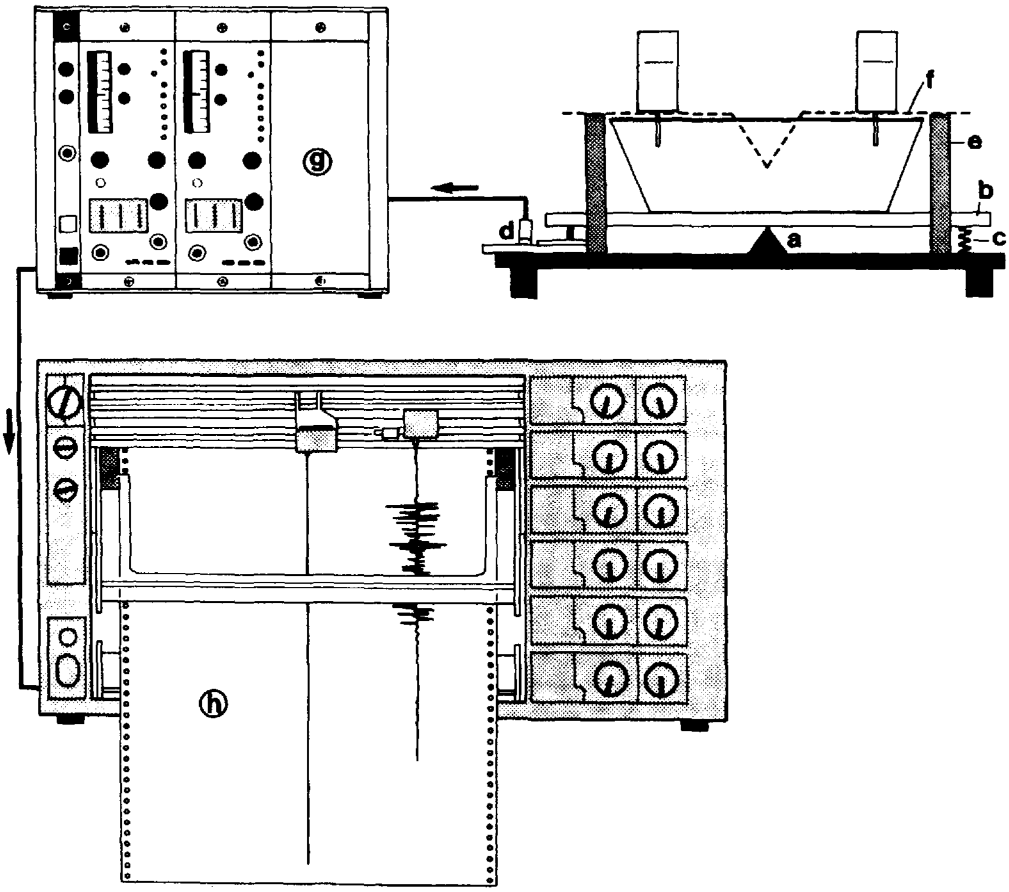

Figure 1 **The test design for recording the force sensor signal: (a) knife-edge; (b) balance platform; (c) spring; (d) transducer; (e) PVC bar; (f) wire lid with water bottle and food-hopper. The force sensor signals are reproduced by the pen recorder on a paper roll (h) via the amplifier (g) as behaviour-characteristic amplitudes.**

After the acclimatization period, a mouse was placed in the test cage. Each test started at 1500h (4h before the onset of the dark period). The amplitudes provoked by the behaviour and position of the mouse in the cage were registered by the pen recorder during the following 24h. Specific behavioural elements (eating, grooming, etc) have characteristic patterns in frequency and shape of amplitudes. The amplitude pattern also reflects the position of the animal in the cage (right or left compartment).

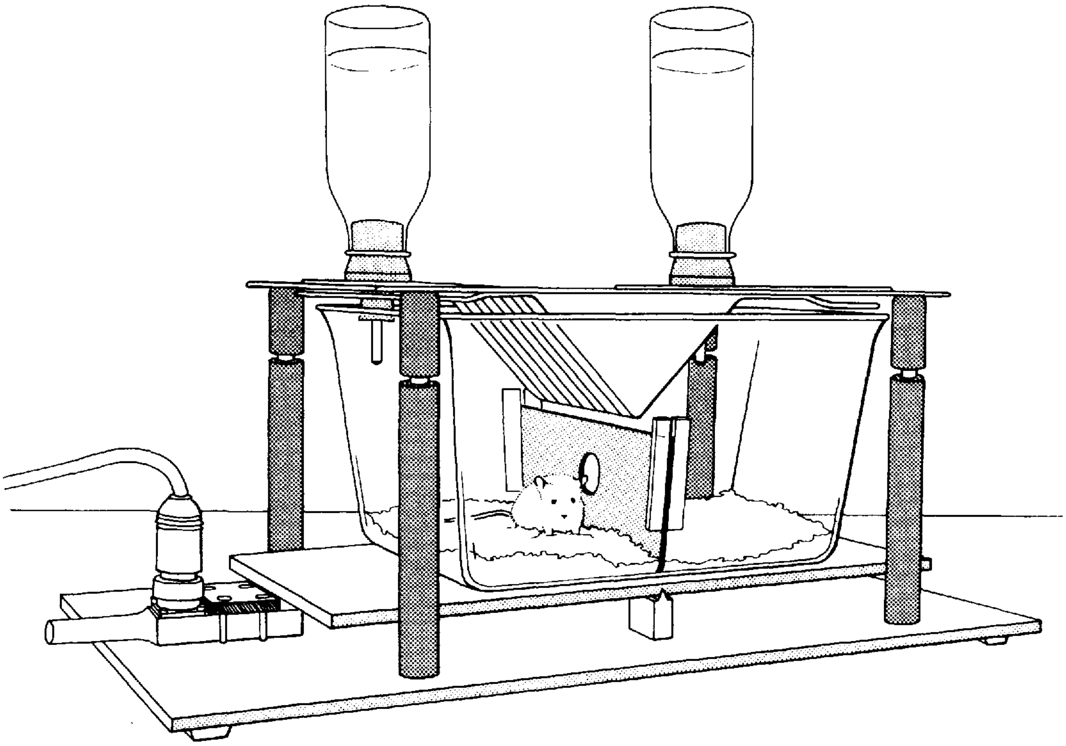

Figure 2 **Test cage on the balance platform; the position of the wire cover on the four vertical bars is clearly visible. The turnable section at the top of the bars can be used to accurately adjust the distance between the wire cover and the cage wall.**

The system was validated in two ways. First the amplitude patterns recorded by the balance plateau were translated into behavioural categories by interpretation of the recordings collected over 144h (six different animals) by two observers, A and B, who had prior experience of interpretation from analysis of preliminary testings. The behaviour as deduced from the characteristic amplitude pattern, was registered at 1min intervals. Then, simultaneous video recordings (one observation each 45s) were analysed and the resulting behavioural patterns compared with the results of the interpretation of the amplitude recordings.

The ethogram used for the validation was based upon Blom (Blom *et al* 1992; Blom 1993) and is shown in Table 1.

In order to detect climbing behaviour, the cage lid was positioned on four PVC bars (Figure 2) leaving a 2mm space between lid and cage. The bars were not connected to the balance platform, thus the cage was recorded 'empty' and there was no amplitude recording when the animal was grasping the cage lid.

Table 1 Ethogram used for validation in experiment 1.

Resting Movements are absent while the animal is in a sitting or lying position. Very short or minor movements (eg turning over while sleeping) are not considered as an interruption. *Grooming* Shaking, scratching, wiping or licking fur, snout, ears, tail or genitals. Usually carried out before and after sleeping. *Eating* Eating food pellets while standing upright, gripping the bars of the food-hopper with the front paws, and gnawing the food between the bars using two incisors. Also covers gnawing a particle of food clasped between the front paws. *Locomotion* This mainly covers exploratory behaviour such as running, sitting upright, digging, sniffing and jumping against the cage wall. During locomotion the animal may carry food or sawdust for nestbuilding. It also includes short periods of drinking behaviour which normally follow eating. *Climbing* Climbing and hanging on the bars of the wire cage lid or food-hopper. While the animal is climbing or hanging, the hind legs or the tail may be positioned against the floor or side walls of the cage.

Statistical analysis

The balance device scores from both observers A and B (a total of 8640 observations, 60 observations h^{-1} over 144h) were compared by calculating the ratio of both observers' scores for each behavioural category. Observer A was taken as the standard (100%).

A similar comparison was made between the video recordings (11 520 observations, 80 observations^{-1}, over 144h^{-1}) and the balance device scores. For each behavioural category and sex, the ratio between video and amplitude was calculated. Video recordings were taken as the standard (100%).

Experiment 2: Application

Animals

Six female C57Bl/6JIcoU mice were used. Materials and methods were similar to experiment 1, except that the behaviour of the mice was recorded with the balance system over 48h and no video recordings were made.

Results

Experiment 1: Validation

The characteristic amplitude patterns as recorded by the pen recorder, and the corresponding categories of behaviour are shown in Figure 3 (clear, representative traces from actual recordings are shown). Line F is the baseline indicating the middle of the cage.

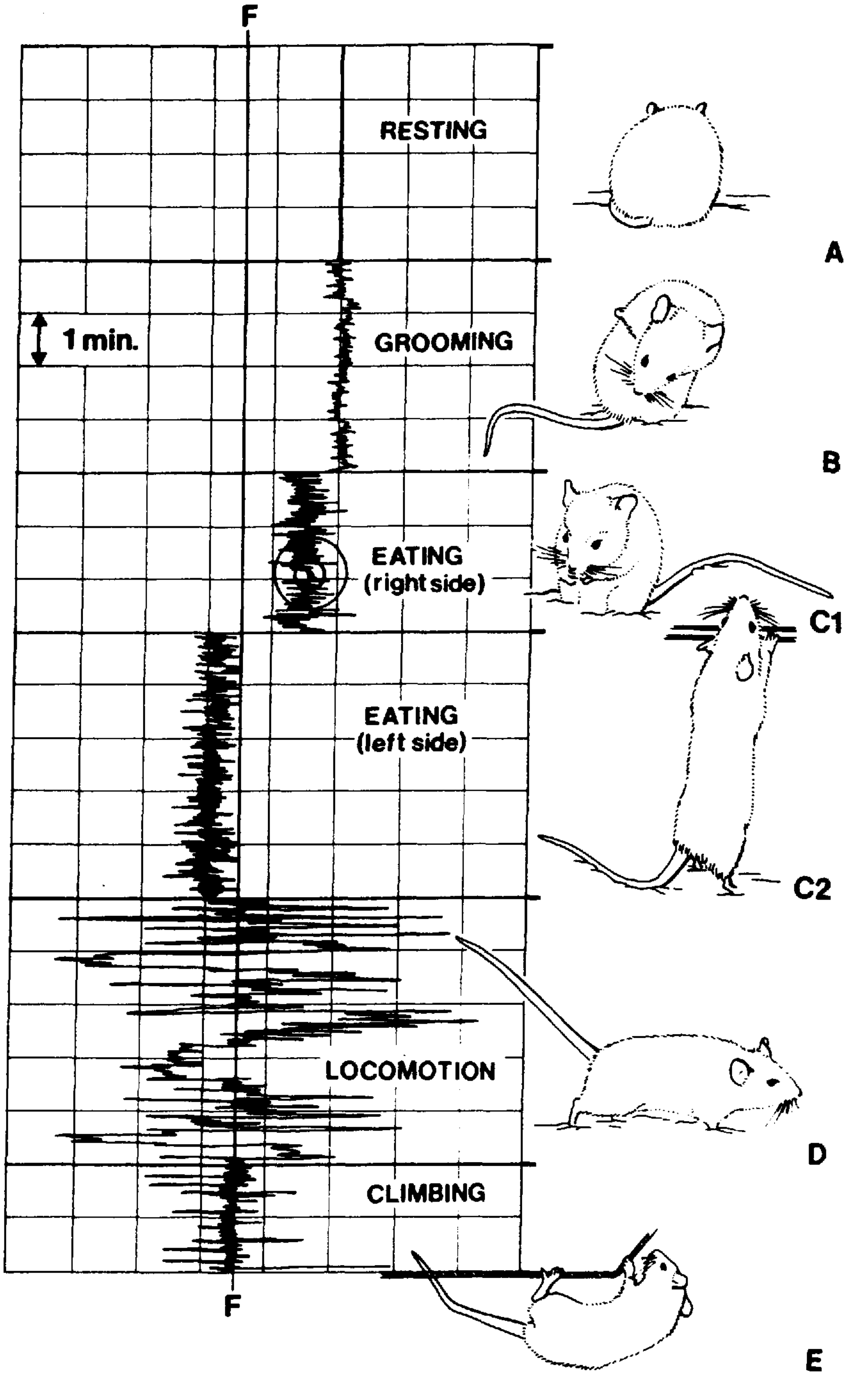

Figure 3 **The different amplitude characteristics which reflect the five different behavioural categories. In addition to behaviour, the position of the animal in either side of the cage can also be determined. See text for description of abbreviations and categories.**

Resting behaviour (A) is characterized by a minimum amplitude, which may occasionally be interrupted by a larger amplitude, due to movement of the animal during rest. Movements associated with grooming behaviour (B), give rise to small but frequent amplitudes which are rarely interrupted and generally occur before and after the resting period.

Movements associated with standing at the food-hopper and eating (C_2) record lower frequencies and less regular amplitudes than those associated with grooming, which are characterized by marked peaks. Periods of smaller amplitudes indicate the animal was sitting and eating a small piece of food clasped between its forepaws (C_1).

Locomotion (D) involving mainly exploratory behaviour, is characterized by amplitudes which extend to either side of the baseline, indicating that the animal is active on the entire surface of the cage. It also includes short interruptions during which the animal sniffs, sits up or jumps against the cage wall. Other behaviour which was not covered by the ethogram is also included in this category. Climbing behaviour (E) produces small amplitudes around the baseline. During climbing, the hind legs or tail may touch the side walls of the cage, leading to characteristic deviations from the baseline recordings.

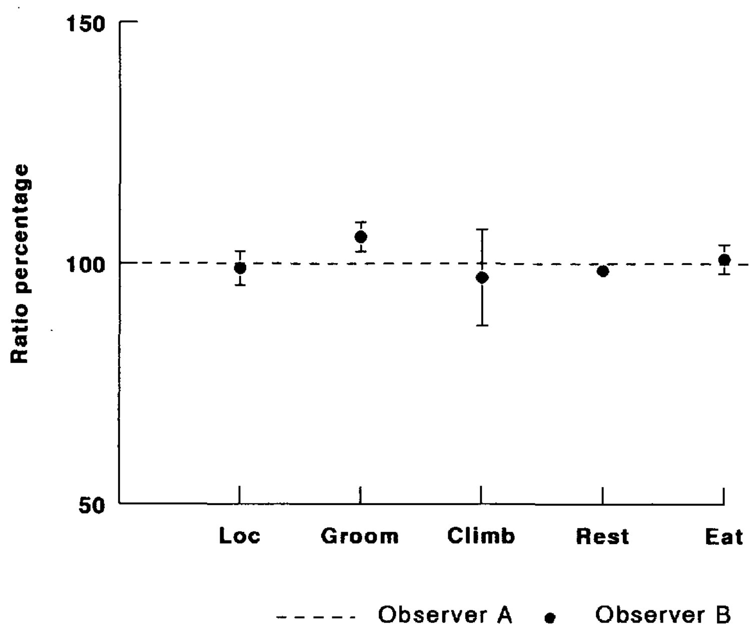

Figure 4 **The ratio of the balance device scores of observers A and B for 144h of observations (6 tests of 24h each) with standard deviations (all < 5%). Observer A was taken as the standard (100%).**

Figure 4 illustrates the degree of concordance between the two observers A and B, which did not differ by more than five per cent. Figure 5 illustrates the degree of concordance between the amplitudes and video recordings. Again, all deviations were less than five per cent.

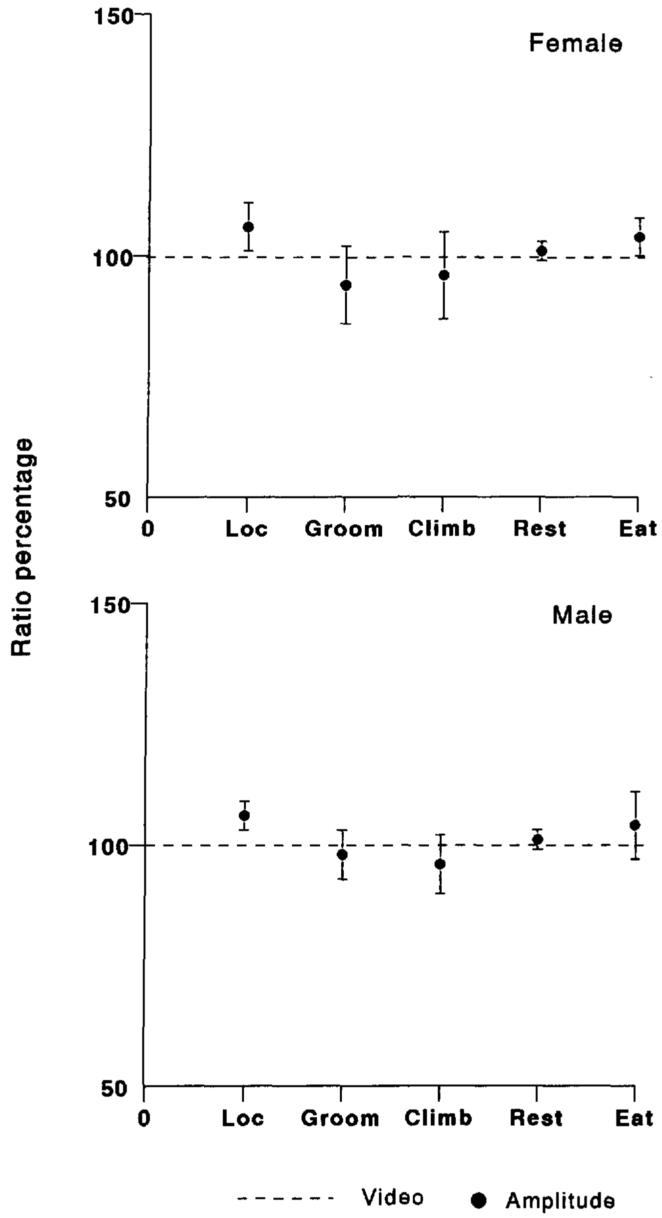

Figure 5 **The ratio of the balance device scores and the video scores for 144h of observations (6 tests of 24h each) with standard deviations (all < 5%) for female (n = 3) and male (n = 3) C57BL/6JIcoU mice.**

Experiment 2: Application

Figure 6 (overleaf) shows the mean time spent in the five behavioural categories as measured by amplitude evaluation over 48h for six female mice. A circadian rhythm can be recognized for each of the five behavioural categories. Also, an increase in exploratory behaviour (locomotion) can clearly be recognized during the first few hours after the animal was placed in its new environment. The level of grooming behaviour appears to be fairly constant.

Discussion

The validation of the balance cage as a device for measuring the behaviour of singly housed mice revealed that this approach yields data comparable to that obtained from the analysis of video recordings. The maximum deviation in concordance observed for locomotion and grooming, was less than five percent. Thus, it may be concluded that this system provides a reliable tool for the analysis of behavioural patterns in mice. An animal may move bedding from one side of the cage to the other, or it may defecate more on one side of the cage. However, the cage does not become unbalanced by these small changes, which only cause the baseline and the amplitude patterns to shift to a slightly different position on the paper roll.

Other recording systems usually collect data on only one aspect of behaviour (such as eating or locomotion) or measure overall activity over a fixed period. Yet overall activity may remain unchanged – even though separate behavioural elements (such as grooming or climbing) increase or decrease in opposite directions (Crawley *et al* 1982; Ikeda *et al* 1987; Barclay *et al* 1988; Heckl-Ensslin & Van Butler 1988; Minematsu *et al* 1991; Badiani *et al* 1995). The present device is also able to recognize stereotypic behaviour eg circling: continuously repeated climbing movements (Schlingmann unpublished data).

One limitation of the present device might be that only individual animals can be tested. However, with some adaptations it will be possible to analyse social interactions between group-housed animals, because behaviours such as fighting or mating also have characteristic amplitude patterns (Schlingmann unpublished data).

The behavioural patterns quantified by this system (experiment 2) are in line with the observations of various researchers (Vincent *et al* 1983; Clement *et al* 1989; Matthew *et al* 1990; Büttner 1991). After an acclimatization period of 24h, all animals showed a circadian rhythm for each of the behavioural categories.

The present device allows faster scoring of behaviour than with a video system (reading the amplitudes was about three times faster than the analysis of the video recordings). This allows for the performance of long-term studies, eg on the long-term effects of pharmaceutical or toxicological agents, which are too time-consuming with existing observational methods. Since the reading of the amplitude recordings still consumes some time, a computerized system for the automatic analysis of the amplitude recordings is presently under development. This will not only substantially reduce the observation time but will also standardize the interpretation of the data and allow further discrimination of behavioural patterns obtained over a long period.

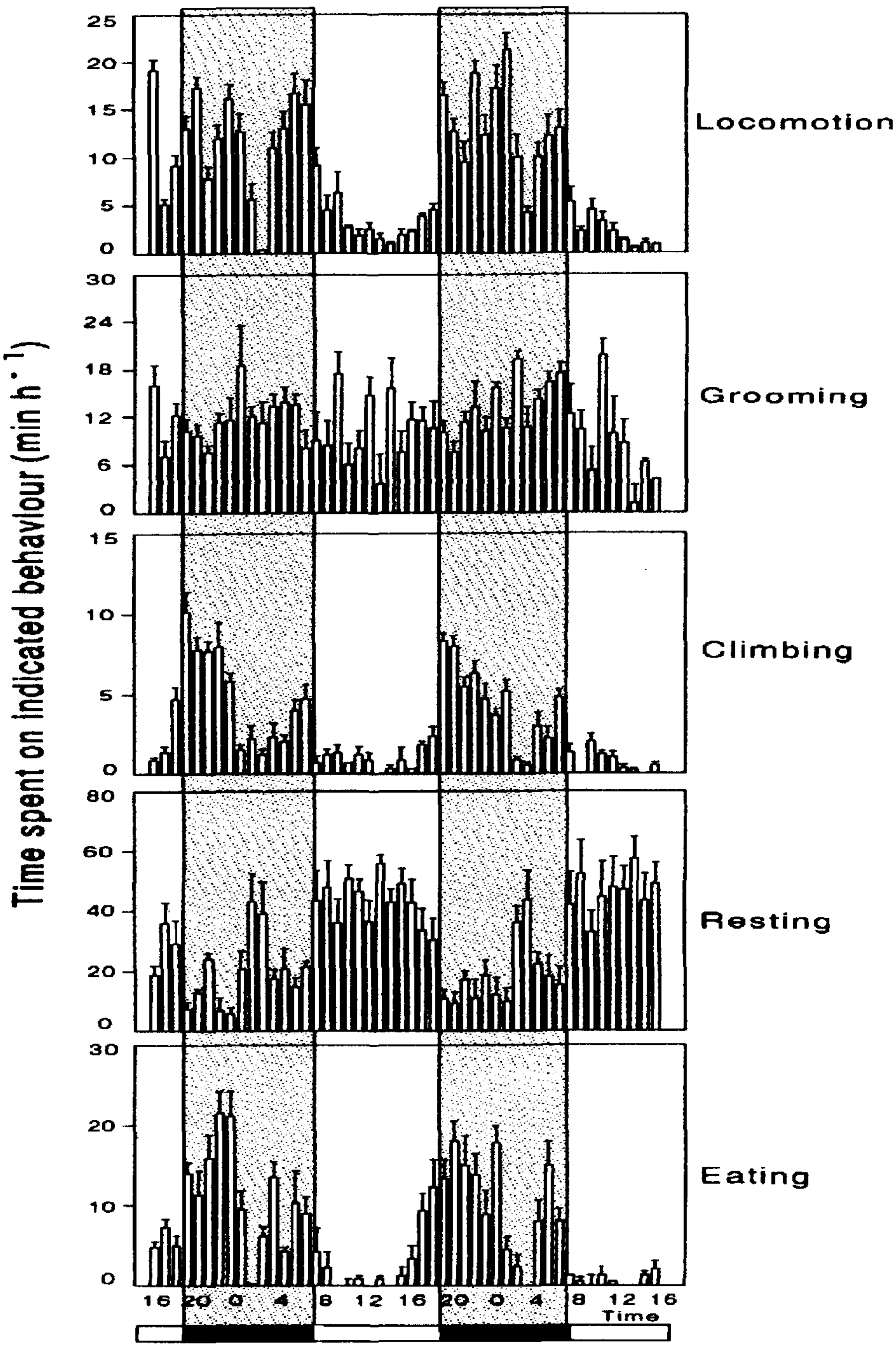

Figure 6 Hourly representation of the mean (and SEM) time spent on five different behavioural categories by female C57BL/6JIcoU mice (n = 6) in a 48h period, as registered by the balance device. The two horizontal dark bars and vertical stippled bands represent the 12h periods of darkness.

Animal welfare implications

Major advantages of the present device, when compared with existing non-video systems for measuring behavioural activity, seem to be the possibility of discriminating between at least five different behavioural categories, recognizing stereotypic behaviour and detecting circadian rhythms in a non-invasive way.

Acknowledgements

The authors would like to thank M Clarijs of the Laboratory Instrumentation Department of Solvay Duphar for his help in developing and producing the balance platform; H J Rensema for preparing the drawings of the Figures 1, 2 and 3; J Tolboom for statistical advice, and P L P van Loo for analysing the amplitude recordings.

A part of this study was presented as an abstract at the 5th FELASA Symposium in Brighton, UK (1993).

References

Badiani A, Mundl W J and Cabilio S 1995 A computerized system for the continuous recording and analysis of feeding, drinking, diuresis and locomotor activity. *Physiology & Behavior 57:* 973-981

Barclay R J, Herbert W J and Poole T B 1988 *The disturbance index. A behavioural method of assessing the severity of common laboratory procedures on rodents.* UFAW Animal Welfare Research Report No 2. Universities Federation of Animal Welfare: Potters Bar, UK

Blom H J M 1993 *Evaluation of housing conditions for laboratory mice and rats. The use of preference tests for studying choice behaviour.* PhD thesis, Utrecht University, The Netherlands

Blom H J M, Van Vorstenbosch C J A H V, Baumans V, Hoogervorst M J C, Beynen A C and Van Zutphen L F M 1992 Description and validation of a preference test system to evaluate housing conditions for laboratory mice. *Applied Animal Behaviour Science 35:* 67-82

Büttner D 1991 Climbing on the cage lid, a regular component of locomotor activity in the mouse. *Journal of Experimental Animal Science 34:* 165-169

Clement J G, Mills P and Brockway B 1989 Use of telemetry to record body temperature and activity in mice. *Journal of Pharmacological Methods 21:* 129-140

Crawley J N, Szara S, Pryor G T, Creveling C R and Bernard B K 1982 Development and evaluation of a computer-automated color TV tracking system for automatic recording of social and exploratory behavior of small animals. *Journal of Neuroscience Methods 5:* 235-247

Heckl-Ensslin C and Van Butler I 1988 Locomotor activity of a behaviour mutant in NMRI mice. In: Beynen A C and Solleveld H A (eds) *New Developments in Biosciences: Their Implications for Laboratory Animal Science. Proceedings of the third FELASA symposium* pp 373-374. Martinus Nijhoff Publishers: Dordrecht

Ikeda T, Takeyuki A B E and Tashiro K 1987 An actograph for small laboratory animals controlled by a personal computer. *Experimental Animals 36:* 453-457

Matthew J, Kluger C A, Conn B F, Freter R and Abrams G D 1990 Effect of gastrointestinal flora on body temperature of rats and mice. *Regulatory Integrative Comparative Physiology 27:* 552-557

Minematsu S, Hiruta M, Taki M, Fujii Y and Aburada M 1991 Automatic monitoring system for the measurement of body weight, food and water consumption and spontaneous activity of a mouse. *The Journal of Toxicological Sciences 16:* 61-73

Schlingmann F, De Rijk S H L M, Pereboom W and Remie R 1993 Avoidance as a behavioural parameter in the determination of distress amongst albino and pigmented rats at various light intensities. *Animal Technology 44:* 2-8

Spruijt B M and Gispen W H 1984 Behavioral sequences as an easily quantifiable parameter in experimental studies. *Physiology & Behavior 32:* 707-710

Vincent P, Dole A H and Gentry R T 1983 An improved technique for monitoring the drinking behaviour of mice. *Physiology & Behavior 30:* 971-974

Van de Weerd H A, Baumans V, Koolhaas J M and Van Zutphen L F M 1994 Strain specific behavioural response to environmental enrichment in the mouse. *Journal of Experimental Animal Science 36:* 117-127