ELSEVIER

Behavioural Processes 53 (2001) 11–20

BEHAVIOURAL PROCESSES

www.elsevier.com/locate/behavproc

Validation of a new system for the automatic registration of behaviour in mice and rats

H.A. Van de Weerd [a,1], R.J.A. Bulthuis [b,*], A.F. Bergman [b], F. Schlingmann [c], J. Tolboom [c], P.L.P. Van Loo [a], R. Remie [c], V. Baumans [a], L.F.M. Van Zutphen [a]

[a] *Department of Laboratory Animal Science, Utrecht University, PO Box 80.166, 3508 TD Utrecht, The Netherlands*
[b] *Metris B.V., Saturnusstraat 12, PO Box 3023, 2130 KA Hoofddorp, The Netherlands*
[c] *Department of Laboratory Animal Science, Solvay Pharmaceuticals, PO Box 900, 1380 DA Weesp, The Netherlands*

Received 23 March 2000; received in revised form 21 September 2000; accepted 22 September 2000

Abstract

A newly developed behaviour registration system, Laboratory Animal Behaviour Observation, Registration and Analysis System (LABORAS) for the automatic registration of different behavioural elements of mice and rats was validated. The LABORAS sensor platform records vibrations evoked by animal movements and the LABORAS software translates these into the corresponding behaviours. Data obtained by using LABORAS were compared with data from conventional observation methods (observations of videotapes by human observers). The results indicate that LABORAS is a reliable system for the automated registration of eating, drinking, grooming, climbing, resting and locomotion of mice during a prolonged period of time. In rats, grooming, locomotion and resting also met the pre-defined validation criteria. The system can reduce observation labour and time considerably. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Animal welfare; Automatic behaviour registration; Validation; Behavioural analysis; Mice; Rats

1. Introduction

Behavioural studies are performed not only to gain more insight into the behaviour of animals, but are also used to investigate the effects of environmental changes (e.g. Brain, 1975; Van de Weerd et al., 1994; Saibaba et al., 1995; Van de Weerd et al., 1997), the effects of drugs (pharmaco-ethology, e.g. Minematsu et al., 1991; Griebel et al., 1993; Young et al., 1993), or to gain insight in the neural regulation of behaviour (neuro-ethology, e.g. Van Rijzingen, 1995). In most of these research areas short-term behavioural tests are performed in which the behavioural changes of an animal are observed

* Corresponding author. Tel.: +31-23-5542250; fax: +31-23-5571069.
E-mail address: info@metris.nl (R.J.A. Bulthuis).
[1] ADAS Terrington, Terrington St. Clement, Norfolk, PE34 4PW, UK.

PII: S0376-6357(00)00135-2

before and after certain experimental interventions, such as drug administration. Long-term studies are less often performed because they are very time-consuming and labour intensive, or there are technical limitations (Scheibe et al., 1998). The advantages of long-term behavioural observations however, can be major. The present methods for long-term behavioural observations are to make video recordings at regular intervals or to use time-lapse methods. In both cases important information might be missed, because time periods are skipped or, with the use of time-lapse methods, behavioural details cannot be observed. Furthermore, rare behaviour patterns may be missed altogether if observation sessions are not long enough (Martin and Bateson, 1986).

Most animals have a clear circadian rhythm in their behaviour, e.g. mice are active during the night, with high levels of activity after the beginning of darkness. Thereafter periods of rest and activity alternate. Before dawn they have another (lower) activity peak. During the day they mostly sleep (Van Oortmerssen, 1971; Weinert, 1994; Schlingmann et al., 1998). When using interval recordings or time-lapse recordings, changes in behaviour due to circadian rhythms can be missed.

1.1. Automatic registration of behaviour

Obtaining and analysing behavioural data can be rather time consuming and this can be reduced with the use of automated behaviour registration systems. Most behaviour registration systems however, measure only a few specific behaviours such as eating (e.g. Hulsey and Martin, 1991; Lax et al., 1996), others only measure overall activity over a fixed period of time (e.g. Minematsu et al., 1991), whereas it might be important to register a larger part of the time budget, to detect possible (subtle) differences. Over a fixed period of time, some behaviours might increase while at the same time others might decrease, in which case overall activity remains the same (Baumans et al., 1998).

In order to be able to collect behavioural data over longer periods of time, without visual observations, an automatic balance platform with one sensor was developed by Schlingmann et al. (1998). This system is based on transposing the movements of an experimental animal into electrical signals, which are sent to a pen recorder. The characteristic vibration pattern for each behavioural element can be scored visually by an observer, thus reducing experimental time by one third. Advantage of such a system is not only experimental time reduction, but also the collection of behavioural data of an animal in an undisturbed environment, e.g. in its own home cage without the presence of an observer. This balance system, which was the predecessor of LABORAS, has been described and validated in Schlingmann et al. (1998).

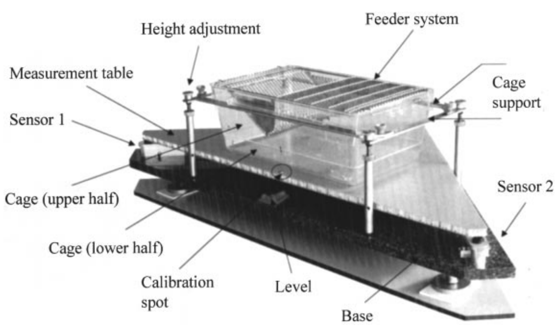

Fig. 1. The LABORAS behaviour registration system. Sensor platform with cage.

1.2. The LABORAS system

In order to further reduce observation time, the balance device was further developed and the role of the human observer was taken over by a computer program, which automatically recognises behavioural categories from the signals caused by the movements of the animals. The newly developed, triangular shaped, Laboratory Animal Behaviour Observation, Registration and Analysis System system (LABORAS™, Bulthuis et al., 1998, Fig. 1) has two sensors and can automatically register the occurrence of six different behavioural categories. An advantage of such a system is that data collection is standard, followed by standard classification into behavioural elements. Therefore, there will be no variations over time (observer's fatigue) or between different observers or different laboratories (always some degree of judgement errors or differences in interpretation). This implies that the intrarater- and interrater variability, that trouble the method of human observation (Martin and Bateson, 1986), are entirely eliminated. Because of the automated procedure, data can be obtained during prolonged periods of time. Since the data acquisition is based on weight displacement, and not on visual monitoring, it is independent of the illumination condition (measures in light as well as dark).

This paper describes the validation process of LABORAS by comparing the results of the behavioural analysis from the computerised device (LABORAS) with the visual scorings of three human observers in order to assess the degree of concordance. A large data set of 24-h behaviour recordings were collected of mice and rats in order to establish the reliability of the system.

2. Animals, materials and methods

2.1. Animals and housing conditions

For the validation study one male and one female mouse of each of the strains BALB/cAnNCrlBR and C57BL/6NCrlBR, and also one male and one female rat of each of the strains Wistar BR:WU and BR:Sprague Dawley were used ($N = 8$). At the start of the experiment the animals were 6 weeks of age.

Prior to the experiment the animals were housed in a room with controlled photo-period (lights on 07:00–19:00 h), relative humidity ($55 \pm 5\%$) and temperature ($22 \pm 1°C$). The mice were housed individually in wire topped Makrolon type II (375 cm^2) cages, the rats in Makrolon type III (840 cm^2) cages (UNO Roestvaststaal, Zevenaar, The Netherlands) provided with sawdust. Tap water and food pellets (RMH, Hope Farms, Woerden, The Netherlands) were provided ad libitum.

2.2. The LABORAS test system

The LABORAS system (Metris B.V., Hoofddorp, The Netherlands) is a fully automated device for the recording of the behaviour of individually housed mice and rats (see also Bulthuis et al., 1998). The system (Fig. 1) consists of a triangular shaped sensor platform ($690 \times 690 \times 976 \times 13$ mm, Aeroweb Honeycomb material, CIBA-Geigy, Switzerland), which is positioned on two orthogonally placed sensors (SPS Load cell, 1 kg, AE-sensors BV, Dordrecht, The Netherlands) and a third fixed point, attached on a bottom plate. This whole structure is standing on three poles, which have rubber feet for absorption of external vibrations and which are adjustable in height to level it. A Makrolon type II cage (375 cm^2) or type III (840 cm^2) can be placed on this sensor platform.

Each sensor transforms the mechanical vibrations caused by the movements of the animal into electrical signals, which are amplified and filtered to eliminate noise and then stored on a computer. Each movement pattern has its own unique frequency and amplitude pattern and thus separate behavioural categories can be distinguished and classified by the computer. The upper part of the cage (with the cage lid) is separated from the lower part, so that climbing behaviour on the cage lid can be detected by the disappearance of the signals. Up to eight platforms can be connected to the hardware and computer.

The LABORAS software (Metris B.V., Hoofddorp, The Netherlands) consists of an administration module that registers information on the experiment and test conditions. The data acquisition and storage module controls the hardware and handles the storage of the sampled signals. The analysis and classification module processes the stored data and compares the signals with the predetermined characteristic patterns and thus classifies the data into the behavioural categories. LABORAS follows the movements continuously but — as yet — classifies these signals for long-term behavioural observations every 10 s only (the behaviour, which dominates the 10-s period, is scored). A LABORAS upgrade version with a higher resolution is under development.

2.3. Procedure

2.3.1. Validation

Four animals (rats or mice) were tested at the

Table 1
Ethogram used by the observers and LABORAS

Behaviour	Description for human observations	LABORAS category (mice)	LABORAS category (rats)
Drinking	The animal stands upright to lick water from the water bottle	Drinking	Drinking
Eating	The animal eats food pellets while standing upright, gripping the bars of the food hopper with its front paws and gnaw the food between the bars. It also includes gnawing a particle of food clasped between the front paws	Eating	Eating
Climbing	Climbing and hanging on the bars of the wire cage lid or food hopper with two or four feet. While the animal is climbing or hanging, the hind legs or tail may touch the floor or side wall of the cage	Climbing	Climbing (did not occur)
Grooming	Shaking, scratching, wiping or licking its fur, snout, ears, tail or genitals	Grooming	Grooming
Resting	Movements are absent while the animal is in a lying position. Very short movements (e.g. turning over while sleeping) are not considered as an interruption	Resting	Resting
Motionless	Movements are absent while the animal is in a sitting position	Resting	Resting
Locomotion	Activities such as walking, running, or jumping. This also includes rearing, i.e. standing on the hind legs without touching the cage walls with the forepaws	Locomotion	Locomotion
Leaning	Standing on hind legs with forepaws leaning against the cage wall for support (sniffing at the slit between cage halves is included)	Locomotion	Lean/dig
Digging	Bedding material is pushed forwards or backwards with nose, forepaws or hind legs	Locomotion	Lean/dig
Undefined	All behaviours that do not fit in one of the previous categories	Undefined (occurred rarely)	Lean/dig

same time, on four different platforms. Each animal was placed individually in a (clean) Makrolon type III cage (840 cm^2) with bedding. Cardboard shields separated adjacent cages, so that the animals could not see each other.

The tests started between 15:00 and 23:30 h. During 24 h the behaviour of an animal was registered with the sensor platform, at the same time the behaviour of each animal was recorded on videotape. For this purpose time-lapse video recorders (Panasonic NV 8050, Philips HS 5424, Panasonic AG 6124 and Panasonic AG 6024) were used that recorded at 1/9 of normal speed. During dark periods (19:00–07:00 h) red light was present to allow video recordings.

The behaviour of the animals on tape was observed and scored by three independent observers using behavioural observation software (The Observer, v 2.0, Noldus b.v. Wageningen, The Netherlands). Instantaneous sampling was used, meaning that every 5 s it was noted which behaviour the animal was performing according to a pre-defined ethogram. Since the tapes were recorded at time-lapse speed, this meant that in reality the sample interval was 45 s. In order to cover most of the LABORAS samples, behaviour was scored with a 15 s delay between observers: observer A started at time T, observer B at time $T + 15$ s, and observer C at time $T + 30$ s. Thus, three samples were taken during every 45 s time frame.

2.3.2. Ethogram

The ethogram (based on the ethogram in Blom, 1993; Schlingmann et al., 1998) used by the observers is presented in Table 1. For the validation the behavioural categories scored by LABORAS were compared with the behavioural categories as scored by the observers, according to Table 1. Some behaviours scored by the observers were grouped. Due to differences in behavioural patterns of mice and rats, some behavioural categories used by LABORAS differ for mice and rats. Motionless is included in resting, since LAB-

ORAS cannot distinguish if an animal is actually sleeping or just sitting or lying motionless. Mice perform their behaviour much faster than rats, therefore leaning and digging are not registered separately by LABORAS but are included in locomotion. LABORAS puts behaviours that are not recognised by the algorithms of above mentioned behaviours in the category 'undefined'. For the mice, undefined behaviour only occurred very rarely and thus this was not used as a separate behavioural category. For the rats, the undefined behavioural category is combined with leaning and digging (which can be scored separately from locomotion), and therefore this category is renamed as leaning/digging. The rats did not perform climbing.

2.3.3. Acceptance criteria

Prior to the experiments the criteria on which the system would be accepted as a valuable tool for the automated detection of behaviour of laboratory mice and rats, were determined. The acceptance criterion was defined as follows: per animal and behavioural element the mean deviation between LABORAS and the three observers over 24 h should be smaller than the S.D. of the observers plus a 5% margin (comparable with 3 min/h). The criterion was defined as a delta, which should be $\leq 5\%$ for 95% of the equations and $\leq 15\%$ for the remaining 5% of the equations. Per animal and per behavioural element the deltas were calculated with the following formula:

$$\text{Delta} = 1/24 \text{ Sum ABS(LABORASt} - \text{MEANt)} - 1/24 \text{ Sum SDt}$$

Per hour the percentage of time spent on each behavioural element is calculated for LABORAS, resulting in LABORASt, $t = 1, \ldots, 24$.

Per hour the percentage of time spent on each behavioural element is calculated for each observer, resulting in OBS1t, OBS2t, OBS3t, $t = 1, \ldots, 24$.

The mean of the observers is then calculated: MEANt = MEAN(OBS1t, OBS2t, OBS3t), $t = 1, \ldots, 24$.

Per hour the S.D. of the observers is calculated for each behavioural element, resulting in a mean S.D.: SDt = STD(OBS1t, OBS2t, OBS3t), $t = 1, \ldots, 24$.

Per hour the absolute difference between LABORAS and the mean of the observers is calculated.

The absolute difference minus the mean of the S.D. results in a delta, which should comply with the predefined criteria.

In practice this meant that the long-term tests were accepted when from the total of 48 equations that were produced (four mice × six behavioural elements and four rats × six behavioural elements), 46 (= 95%) of the equations should be $\leq 5\%$. The remaining two (= 5%) of the equations should be $\leq 15\%$. This would mean that LABORAS followed the changes in behaviour over 24 h correctly and will be accepted as a reliable system.

3. Results

3.1. Validation

First, the total cumulative time spent on each behaviour per animal, for the three observers and LABORAS was calculated. Then the relative duration per behavioural category per animal for the three observers and LABORAS for the 24-h observation period was calculated. The results of these calculations are presented in Fig. 2 (mice) and Fig. 3 (rats) as the mean of the three observers in comparison with the scores by LABORAS.

The fact that not all behavioural categories as scored by LABORAS add up to 100% can be explained by a low percentage of undefined behaviour. Overall the LABORAS scores over 24 h for rats as well as mice are in good agreement with the mean scores of the three observers. The largest differences between LABORAS and the observers are found in the behaviours, which are performed most by the mice: locomotion, resting and grooming. The largest differences in scorings of the rats between LABORAS and the observers are found in eating and in leaning/digging.

For the acceptance criterion, the deltas were calculated according to the formula. In this calculation the S.D. between the hourly scores of the three observers, averaged over 24 h were also obtained. For both mice and rats the lowest S.D. was found for drinking behaviour (both mice and rats S.D. 0.4%) and the highest S.D. for locomotion (mice S.D. 3.6%, rats: SD: 3.2%). Other data are not shown.

The results in Tables 2 and 3 show the outcome of the 48 equations, calculated with the means per hour and the S.D. and the acceptance criteria formula, and shows whether they fit within the 5% limit. The deltas of the mice are all within the acceptance criteria. Therefore, all behaviours in mice are classified correctly. But the deltas of the rats show that three equations are on the border of acceptance (5.3% for eating, 5.2% for drinking and 5.3% for lean/dig of three different animals) and one equation falls outside it (8.8% for eating of the Wistar female). According to the acceptance criteria, only two equations are allowed to fall outside the 5% limit. In rats, grooming, locomotion and resting are classified adequately, whereas drinking, eating and leaning/digging are not detected sufficiently by LABORAS.

Fig. 4 illustrates, for two mice, the concordance between the observations of LABORAS and the three observers. The LABORAS line should in general fall within the area between the upper and lower bound of the observer's line, which corresponds with the S.D. plus a 5% margin. As can be seen, the line of the BALB/c mouse has a better fit.

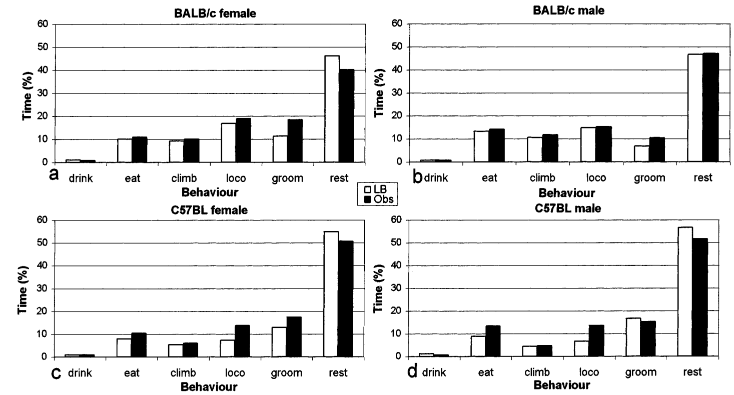

Fig. 2. Relative duration (over 24 h) of mouse behaviour as measured by LABORAS and the mean of the observers. For a, b, c and d LABORAS classified 95.2, 93.7, 89.8 and 95.0% of the behaviour respectively; the observers classified 99.7, 99.9, 100 and 99.8% of the behaviour.

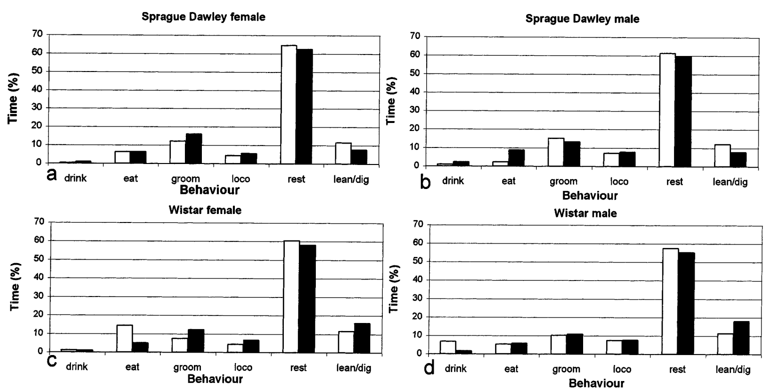

Fig. 3. Relative duration (over 24 h) of rat behaviour as measured by LABORAS and the mean of the observers. For a, b, c and d LABORAS classified 100, 100, 100 and 99.2% of the behaviour respectively; the observers classified 99.8, 99.7, 99.7 and 100% of the behaviour

Table 2
Acceptance criteria (deltas) for mouse behaviour[a]

	Behaviour (%)					
	Drink	Eat	Climb	Loco	Groom	Rest
BALB/c						
Female	0.6	0.9	−1.1	0.6	4.6	4.7
Male	0.3	0.6	−0.7	1.2	2.1	0.0
C57BL						
Female	0.3	2.0	−0.3	4.4	5.0	3.1
Male	0.5	3.7	−0.3	3.2	1.3	3.0

[a] Deviation between LABORAS and the mean of the observers minus their S.D. All values are $\leq 5\%$ and are accepted.

Table 3
Acceptance criteria (deltas) for rat behaviour[a]

	Behaviour (%)					
	Drink	Eat	Groom	Loco	Rest	Lean/dig
Sprague–Dawley						
Female	0.4	1.3	3.9	−0.0	1.5	2.2
Male	0.6	5.3	2.4	−0.8	1.0	1.9
Wistar						
Female	0.2	8.8	3.6	−0.7	2.6	3.7
Male	5.2	2.0	2.2	−0.6	2.8	5.3

[a] Deviation between LABORAS and the mean of the observers minus their S.D. The rat behaviours climbing, locomotion and grooming are accepted (all values $\leq 5\%$). Drinking, eating and resting are not accepted here (one or two values $> 5\%$).

4. Discussion

When developing a new registration system, which can replace human observers, it is essential to validate such a system. Therefore in the present experiment a large set of data obtained with LABORAS was compared with data obtained by human observers.

The most logical way of validating an automated system would be to compare the behavioural registrations made with LABORAS with those made by the regular method (in this case human observations), on a one-to-one base. The best way of doing this would be to compare scorings obtained by exactly similar scoring techniques. This was not possible however, because the techniques differ (time lapse vs. continuous recording) and this is inherent to the fact that an automated system will in general be developed to improve the methods used by humans.

An alternative method for a direct comparison would be to compare the cumulative durations of the behavioural categories as classified by both methods over the total 24 h observation period. However, this method gives no information on whether LABORAS recognises and detects fluctuations and changes in the behaviour of an animal. When LABORAS would only give overall scores, it could be possible that the system does not detect changes, e.g. due to circadian rhythms, by which over a period of time behaviours increase and others decrease (Baumans et al., 1998). Another possible way of validating an automatic behaviour recording system is to investigate whether the system follows induced behavioural changes, e.g. comparing the behaviour of an animal before and after treatment with a drug with a well-known effect. Young et al. (1993) compared the locomotor activity of rats injected with saline with rats injected with amphetamine (known for enhancing locomotion in rats) in an infrared light matrix system. Minematsu et al. (1991) injected mice with 2-deoxy-D-glucose, in order to see whether their automatic monitoring system measured changes in behaviour correctly. The disadvantage of these pharmacological validation methods is that these are based on the assumption that the measurement of the behaviour of the control animal is correct.

The validation method chosen in this study is a compromise between a very detailed comparison of the behavioural scores and considering cumulative durations of the behavioural scores over a longer period of time. For the validation, observations were totalled per h and compared (the tables and figures in 3 give summarised means of the total 24-h period, which can be misleading with regard to the accuracy of the validation process). Three human observers scored alternating (with a 15-s delay) in order to cover as much of the observations made by LABORAS as possible. The validation criterion allowed for a deviation between the observers and LABORAS of 3 min/h, per behavioural element of an animal.

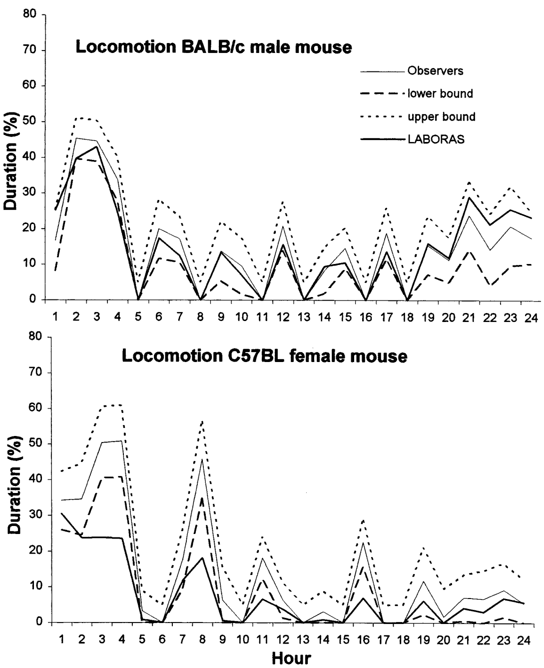

Fig. 4. (Mean) frequencies of locomotion behaviour over 24 h of BALB/c and C57BL mice as scored with the automated behaviour registration system LABORAS and the three human observers (line with upper and lower bound, indicating the margins of the S.D. plus a delta of 5%). Dark period: from 19:00 to 07:00 h.

Getting to know the ‘absolute truth’ about the correct behavioural scores (what is an animal doing at a certain point in time) will always be difficult. In order to approach the ‘absolute truth’ as nearly as possible, the mean of the observations of the three human observers was used in the validation criterion. By using this mean, the S.D. of the observers could also be taken into account in the validation criterion in order to correct for the fact that behavioural observations made by several human observers will never be exactly similar, because the observations will be influenced by some degree of subjective interpretation. ‘Observer drift’ may also occur, as observers become more familiar with the behaviours, and the definitions and criteria tend to drift with the passage of time (Martin and Bateson, 1986). This will happen even if the observers are well trained and the ethogram is precisely defined. The validation criterion must not be too strict, because in such a case the automated system might unjustly be rejected, while in reality it is scoring more accurate than the human observers are. This assumption is not very unlikely because an automated system does not suffer from concentration problems or fatigue and will always be consistent in observing, which is especially advantageous during longer bouts of observations.

Eight animals were observed in order to obtain the data for this validation study. This allowed 5760 human observations for comparison with 8640 observations by LABORAS. Different types of animals were chosen (two species and per species two strains and two sexes) in order to have a wide variety in the performance of the behavioural elements. It is important when studying the impact of experimental procedures on the behaviour of animals that behavioural observations are rather detailed and preferably cover a period of at least one circadian cycle (Schlingmann et al., 1998). The LABORAS system appeared to follow the changes in behaviour over time well as is illustrated by Fig. 4. The circadian rhythm can be seen in the locomotion behaviour of the mice, with higher levels of activity during darkness (19:00–07:00 h). This is in concordance with the literature (e.g. Van Oortmerssen, 1971) and findings of others using automated behaviour registration systems (Büttner, 1991; Minematsu et al., 1991; Schlingmann et al., 1998).

LABORAS appeared to reflect the distribution of behaviour over time well, especially the results for the mice completely fall within the validation criteria. The results of the rats indicated that for this species the system still needs some improvements, especially with respect to the behavioural categories eating and drinking. The low concordance score for eating might be explained by the fact that rats assume a lying posture when eating in Makrolon type III cages, because the food hopper is very low. This may give rise to signals, which are less clearly recognisable for LABORAS in comparison with eating of mice. This problem can be solved by using a higher cage on LABORAS, in which the rats can eat in an upright posture. Such a feeding (and drinking) device is currently in development.

In the future it will be investigated whether the system can be more refined in order to score more detailed behaviours. It might be possible to score behaviours such as stereotypies, which have a very characteristic pattern or social behaviours such as mating or fighting (Schlingmann et al., 1998). Also the X–Y location, the position track, travelled distance, speed, maximum speed etc. of the animal can be derived from the dual sensor input. Combination of the activity of the animal with a certain location in the cage would also seem feasible.

The LABORAS system is a promising system, which can perform behavioural recordings and classifications in a standardised and non-invasive way. One of the main advantages of the system is that the experimental time is reduced so that observations over prolonged periods of time can be made. This can provide information on the impact of all kinds of experimental procedures, but also on the effects of daily laboratory routines, e.g. changes in the environment or housing conditions of laboratory mice and rats.

Acknowledgements

The authors like to thank S. Nijessen and E. Van der Voorden and workers of the Solvay Pharmaceuticals technical workplace for their contribution to the LABORAS project.

References

Baumans, V., Schlingmann, F., Van Herck, H., Boere, H.A.G., Tolboom, J., 1998. Assessment of discomfort in the mouse by means of a balance device. In: O'Donoghue, P.N. (Ed.), Harmonization of Laboratory Animal Husbandry, Proceedings of the Sixth FELASA Symposium, Basel. Royal Society of Medicine Press, UK, pp. 14–16.

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

Brain, P., 1975. What does individual housing mean to a mouse? Life Sci. 16, 187–200.

Bulthuis, R.J.A., Bergman, A.F., Nijessen, S., Schlingmann, F., Tolboom, J., Remie, R., Van de Weerd, H.A., Van Loo, P.L.P., Baumans, V., Van Zutphen, L.F.M., 1998. Automated behaviour classification: the LABORAS project. In: O'Donoghue, P.N. (Ed.), Harmonization of Laboratory Animal Husbandry, Proceedings of the Sixth FELASA Symposium, Basel. Royal Society of Medicine Press, UK, pp. 17–18.

Büttner, D., 1991. Climbing on the cage lid, a regular component of locomotor activity in the mouse. J. Exp. Anim. Sci. 34, 165–169.

Griebel, G., Belzung, C., Misslin, R., Vogel, E., 1993. The free-exploratory paradigm: an effective method for measuring neophobic behaviour in mice and testing potential neophobia-reducing drugs. Behav. Pharmacol. 4, 637–644.

Hulsey, M.G., Martin, R.J., 1991. A system for automated recording and analysis of feeding behavior. Physiol. Behav. 50, 403–408.

Lax, P., Zamora, S., Madrid, J.A., 1996. A contact eatometer suitable for feeding restriction schedules. Physiol. Behav. 59, 1179–1183.

Martin, P., Bateson, P., 1986. Measuring Behavior. An Introductory Guide. Cambridge University Press, Cambridge, UK.

Minematsu, S., Hiruta, M., Taki, M., Fujii, Y., Aburada, M., 1991. Automatic monitoring system for the measurement of body weight, food and water consumption and spontaneous activity of a mouse. J. Toxicol. Sci. 16, 61–73.

Saibaba, P., Sales, G.D., Stodulski, G., Hau, J., 1995. Behaviour of rats in their home cages: daytime variations and effects of routine husbandry procedures analysed by time sampling techniques. Lab. Anim. 30, 13–21.

Scheibe, K.M., Schleusner, Th., Berger, A., Eichhorn, K., Langbein, J., Dal Zotto, L., Streich, W.J., 1998. ETHOSYS®-new system for recording and analysis of behaviour of free-ranging domestic animals and wildlife. Appl. Anim. Behav. Sci. 55, 195–211.

Schlingmann, F., Van de Weerd, H.A., Baumans, V., Remie, R., Van Zutphen, L.F.M., 1998. A balance device for the analysis of behavioural patterns of the mouse. Anim. Welfare 7, 177–188.

Van de Weerd, H.A., Baumans, V., Koolhaas, J.M., Van Zutphen, L.F.M., 1994. Strain specific behavioural response to environmental enrichment in the mouse. J. Exp. Anim. Sci. 36, 117–127.

Van de Weerd, H.A., Van Loo, P.L.P., Van Zutphen, L.F.M., Koolhaas, J.M., Baumans, V., 1997. Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. Physiol. Behav. 62, 1019–1028.

Van Oortmerssen, G.A., 1971. Biological significance, genetics and evolutionary origin of variability in behaviour within and between inbred strains of mice (*Mus musculus*). A behaviour genetic study. Behaviour 38, 1–92.

Van Rijzingen, I., 1995. Functional recovery after brain damage. Effects of environmental enrichment and ORG 2766 treatment. Utrecht University, The Netherlands Ph.D. Thesis.

Weinert, D., 1994. Lower variability in female as compared to male laboratory mice: investigations on circadian rhythms. J. Exp. Anim. Sci. 37, 121–137.

Young, M.S., Li, Y.C., Lin, M.T., 1993. A modularized infrared light matrix system with high resolution for measuring animal behaviors. Physiol. Behav. 53, 545–551.