Genes, Brain and Behavior (2006) **5:** 458–466

Novel approach to the behavioural characterization of inbred mice: automated home cage observations

L. de Visser[*,†,‡], R. van den Bos[†,‡], W. W. Kuurman[†], M. J. H. Kas[‡] and B. M. Spruijt[†,‡]

[†]*Department of Animals Science and Society, Utrecht University and* [‡]*Rudolf Magnus Institute of Neuroscience, UMC, Utrecht, The Netherlands*

**Corresponding author: L. de Visser, Department of Animals, Science and Society, Utrecht University, Yalelaan 2, 3584 CM, Utrecht, The Netherlands. E-mail: l.devisser@vet.uu.nl*

Here we present a newly developed tool for continuous recordings and analysis of novelty-induced and baseline behaviour of mice in a home cage-like environment. Aim of this study was to demonstrate the strength of this method by characterizing four inbred strains of mice, C57BL/6, DBA/2, C3H and 129S2/Sv, on locomotor activity. Strains differed in circadian rhythmicity, novelty-induced activity and the time–course of specific behavioural elements. For instance, C57BL/6 and DBA/2 mice showed a much faster decrease in activity over time than C3H and 129S2/Sv mice. Principal component analysis revealed two major factors within locomotor activity, which were defined as 'level of activity' and 'velocity/stops'. These factors were able to distinguish strains. Interestingly, mice that displayed high levels of activity in the initial phase of the home cage test were also highly active during an open-field test. Velocity and the number of stops during movement correlated positively with anxiety-related behaviour in the elevated plus maze. The use of an automated home cage observation system yields temporal changes in elements of locomotor activity with an advanced level of spatial resolution. Moreover, it avoids the confounding influence of human intervention and saves time-consuming human observations.

Keywords: Automation, behavioural phenotyping, elevated plus maze, exploratory behaviour, home cage, inbred mice, open field

Received 7 April 2005, revised 6 September 2005, accepted for publication 9 September 2005

The increasing number of mouse models provides a major challenge for behavioural neuroscientists. The complex phenotypic alterations that result from genetic mutations demand extensive behavioural assays (Gerlai 2002; Wahlsten *et al*. 2003). Currently, a number of test procedures are used for behavioural screening that measure both spontaneous and conditioned behaviours and drug effects (Crawley 2000; Crawley *et al*. 1997). Standard behavioural tests, such as the open field and elevated plus maze, have proven their usefulness and validity and have contributed much to today's knowledge of the regulation of locomotion and, for example, anxiety-related behaviours. Behavioural assessments using an extensive battery of both neurological assessment and standard tests (SHIRPA protocol, Rogers *et al*. 1999) have elucidated comprehensive behavioural profiles by addressing a broad spectrum of motivational systems. However, with regard to understanding complex behavioural traits and disorders, current methods have some limitations. Relevant dynamic and circadian processes are not included due to the limited time period of testing. Moreover, with the isolation of behavioural categories in specific tasks, important information on both baseline conditions and interactions between motivational systems cannot be obtained (Tecott & Nestler 2004).

Recently, much effort has been put in developing new and refined phenotyping methods. One example is the modified hole board task, which allows for several measures in behavioural categories of locomotion, exploration, anxiety and cognition in a single test (Ohl *et al*. 2001; Ohl *et al*. 2003). It also takes into account the complex interactions between different motivational systems. As an alternative to short-term tests, the home cage of rodents has since long been used as testing environment for a variety of research purposes (e.g. Casadesus *et al*. 2001; Spruijt 1992). Home cage observations yield several advantages for behavioural phenotyping purposes (Kas & Van Ree 2004; Spruijt *et al*. 1983). For example, testing animals in their home cage environment allows for long-term continuous observations. Tang *et al*. (2002) emphasized the relevance of studying baseline activity in the home cage for the interpretation of behaviour in novel environments. Furthermore, minimal human intervention is needed which reduces stress caused by handling (Tecott & Nestler 2004) and saves time-consuming human observations. By designing the home cage environment as an automated, modular system that contains different stimuli (e.g. nesting box, light and sound stimuli, novel objects), a broad range of behaviours as a result of interacting motivational systems can be studied. It allows the distinction of novelty-induced and baseline behaviours and offers the opportunity to study circadian rythmicity in detail.

 doi: 10.1111/j.1601-183X.2005.00181.x

Locomotor activity is of considerable interest when phenotyping mice. This is reflected by the numerous studies using locomotor activity as a read out parameter to describe emotional responses to stimuli under various conditions (e.g. Cabib *et al*. 2002; Griebel *et al*. 2000; Kopp *et al*. 1999; Ohl *et al*. 2003; Rodgers *et al*. 2002; Tang *et al*. 2002; Van Gaalen & Steckler 2000). The majority of these studies focus on a limited number of parameters, such as beam breaks, distance travelled or running wheel revolutions. The reinforcing properties of running wheels make them less suitable to study activity as wheel-running behaviour is not representative for other forms of locomotor activity (Sherwin 1998; de Visser *et al*. 2005). Experiments are usually either short lasting (e.g. open field, elevated plus maze) or long term but with a low temporal resolution (e.g. daily monitoring of home cage behaviour with only a few sampling moments). In the present study, a newly developed system for automated home cage observations was used to demonstrate the potential of this method for behavioural phenotyping of mice (de Visser *et al*. 2005). The aim was to demonstrate that automated home cage observations indeed characterize four well-known inbred strains of mice, C57BL/6, DBA/2, C3H and 129S2/Sv, in a novel way with regard to specific elements of locomotor activity. This approach included the analysis of behaviour under both novelty and baseline conditions. Furthermore, circadian rythmicity was addressed as well as the development of behaviour over time, reflecting the adaptation to the environment. Principal component analysis (PCA) was performed to identify whether different elements of locomotor activity are related and to reveal underlying motivational systems. To extend the validity of our approach for behavioural phenotyping, we quantitatively compared different elements of novelty-induced locomotor activity with behaviour of the mice in the open field and elevated plus maze.

Materials and methods

Subjects

Female mice of the C57BL/6OlaHsd ($n = 12$), DBA/2NHsd ($n = 12$), C3H/HeNHsd ($n = 8$) and 129S2/SvHsd ($n = 8$) strains were purchased from Harlan (Horst, The Netherlands) at 8 weeks of age. Prior to the experiments, animals were housed in pairs and maintained under a reversed light/dark cycle (white light: 1900–0700 h, red light: 0700–1900 h) with food and water available *ad libitum*. Per cage, animals were provided with a shelter, tissues (Kleenex®, Kimberly Clark, Surrey, UK) and paper shreds (EnviroDri®, TecniLab, Someren, The Netherlands) as environmental enrichment. Humidity was kept at a constant level, and room temperature was maintained at 21.0 ± 2.0 °C. The mice were allowed to acclimatize for 2 weeks before the onset of the experiments. The Animal Ethical Committee of Utrecht University approved the experiments.

Experimental design

Mice were tested in the open field, during the second part of the dark period (between 1200 and 1500 h). Ten days later, mice were placed in the PhenoTyper® (Noldus Information Technology, Wageningen, The Netherlands) for the home cage test, one animal per cage for 6 consecutive days. Seven to 10 days later, the elevated plus-maze test was performed during the dark period (between 1200 and 1500 h). In between behavioural tests, mice were housed in pairs in their original home cages.

Open field

The apparatus consisted of a dark-grey PVC cylinder with a diameter of 80 cm and 30 cm in height. The cylinder was placed on a white floor in an experimental room that was illuminated by red light only. The mice were individually transported from the adjacent room to the experimental room and immediately placed near the wall of the open field. Locomotor activity was recorded by videotracking software (ETHOVISION 3.0, Noldus Information Technology) for 15 min. Between trials, the apparatus was thoroughly rinsed with warm water and dried with clean towels.

The following parameters were obtained using the EthoVision analysis module: duration and frequency of movement, total distance travelled and mean velocity. Same criteria for the calculation of parameters were used as described for the home cage test (see below).

Home cage test

Home cage activity was automatically recorded with videotracking in specially designed cages (PhenoTyper®, Noldus Information Technology). Each cage contains a top unit with built-in hardware for videotracking, i.e. a digital infrared-sensitive video camera and infrared lights. These provide constant and even illumination of the cage. An infrared filter placed in front of the camera prevented interference with room illumination. This method allowed continuous behavioural recordings in both dark and light periods. ETHOVISION 3.0 was used as videotracking software (Spink *et al*. 2001).

Four home cages were connected to a single PC. The video images of these cages were converted into a single video image by a Quad unit. Eight home cages in total were used in the present experiment. The cages (30 × 30 × 35 cm) are made of transparent perspex walls with an aluminium floor covered with sawdust and a tissue (Kleenex®, Kimberly Clark) and paper shreds (EnviroDri®, TecniLab). A feeding station and a water bottle are attached to the outside of a cage wall. A shelter (height: 10 cm, diameter: 9 cm; non-transparent material) was fixed in one of the corners. In EthoVision, the shelter could be defined as a 'hidden zone'; the program could distinguish the parameters 'in shelter' and 'on shelter'. Videotracking was performed at a rate of 12.5 samples/second.

The following parameters were used for analysis: duration and frequency of shelter visits (separately for 'in' and 'on' the

shelter), duration and frequency of 'movement', total 'distance moved' and mean 'velocity' (for more information on algorithms used by the program to calculate parameters see the EthoVision 3.0 manual). The system was set to score 'movement' when the centre of gravity of the mouse moved at a velocity of 3.5 cm/second and higher, averaged over 12 samples. This threshold was set to avoid small movements caused by noise being analysed by the program as 'movement'. The number of stops was calculated using the frequency of 'non-movement' fragments, per unit distance travelled. This yielded a measure that is independent of overall amount of activity. Velocity was calculated only of 'movement' episodes, thus excluding periods of non-movement.

All parameters were calculated in 1-h bins and subsequently lumped into 12-h fragments to distinguish dark/light periods. For circadian rhythmicity, hourly values of the parameter duration of 'movement' were used.

Elevated plus maze

The maze (modified from Lister 1987) was made of dark-grey PVC and was elevated 75 cm above the floor. The four arms (50 $\times$ 10 cm) formed a cross with a central platform of 10 $\times$ 10 cm. A 30-cm-high wall of non-transparent material enclosed two arms, located opposite to each other. The maze was illuminated by white light of approximately 500 lux, to enhance the anxiety component of this test. The mice were individually transported from the adjacent room to the experimental room and immediately placed on the centre platform facing one of the open arms. Behaviour was then recorded for 5 min by a video camera placed 150 cm above the maze. Between trials, the apparatus was thoroughly rinsed with warm water and dried with clean towels. The following parameters were scored using The Observer 4.1 (Noldus Information Technology, Wageningen, the Netherlands): time spent on open and closed arms and the centre platform as a percentage of total observation time, open and closed arm entries as a percentage of total arm entries and the number of head dips.

Statistical analysis

Group composition: Three animals, one from the C57BL/6 strain and two from the DBA/2 strain, were discarded from the analysis. They had too many missing samples during the tracking period (>5%) due to technical problems. Surprisingly, a group of five C57BL/6 mice built a nesting place outside the shelter and could therefore not be included in the main analysis in order to maintain the same conditions for all animals. The number of animals per strain used in all statistical tests is $n = 6$ for C57BL/6, $n = 10$ for DBA/2, $n = 8$ for C3H and $n = 8$ for 129S2/Sv.

Statistical analyses were conducted using SPSS 10.0 for Windows. One-way ANOVA was performed to test for the effect of 'strain' on the behavioural parameters recorded in the open field and elevated plus maze. *Posthoc* comparisons were carried out using the Scheffé test. For parameters not following a normal distribution, a Kruskal–Wallis (KW) test was used to detect 'strain' effects, using Mann–Whitney *U*-test for *posthoc* comparisons. Partial correlations were calculated to compare the relevant parameters of the open field and the elevated plus maze with the home cage test. Partial correlations are used to obtain a measure of correlation with the effect of 'strain' (i.e. the strain differences in behaviour displayed in the home cage test, open-field test and elevated plus maze) eliminated (Ferguson 1981). Correlations were calculated for three different time intervals in the home cage test; the first hour after introduction to the home cage (1 h), the dark phase of day 1 (day1) and the dark phase of day 6 (day 6). Following this procedure, possible correlations under both novelty and baseline conditions can be distinguished. Also, correlations within the home cage test between day 1 and day 6 were calculated to further confirm differences between measures under novelty and baseline conditions. For all correlations, a Bonferroni correction was performed to correct for the number of parameters compared.

Cronbach's alpha values were calculated to determine the reliability of changes of home cage parameters over time. The test values indicate the degree of consistency between individuals in the time–course for each parameter. Values range from 0 to 1; a value of 1 represents perfect reliability. Means and SD for each 12-hour bin were used.

To investigate the interrelation of parameters measured in the home cage test and to identify possible independent factors, we performed a PCA with varimax rotation (Ferguson 1981). By varimax rotation, the variance within each extracted factor is maximized to allow an easier interpretation of the factor structure. Prior to factoring, 1s were inserted in the diagonals of the correlation matrix. Factors with eigenvalues >1 were retained for further analysis. Parameters that showed a loading >0.6 were regarded as being relevant for a specific factor. PCA was performed across strains and days under the assumption that the interrelation between parameters is in itself independent of strain or time. However, the relative scores of each individual mouse on the factors extracted were expected to be strain and time dependent. Therefore, factor scores were calculated for each animal by multiplying the mean values of each 12-hour bin with the factor loadings to create new variables. These variables were further analysed with repeated measures ANOVA to detect any strain differences in the course of the factor scores over days. Separate repeated measures ANOVA were performed for dark/light period of the day with within-subjects factor 'day' and between-subjects factor 'strain'. *Posthoc* comparisons between strains were carried out using the Scheffé test. Levels of significance levels were assigned at $P = 0.05$.

Development of behaviour over days in the home cage test was quantified using the factor scores extracted from PCA. Factor scores for each day for either the light or dark phase were saved as independent variables for each animal.

The effect of day on factor scores was assessed using mixed linear regression models:

$$\text{Factor}_{ijday} = \text{intercept}_i + \text{slope}_i \times \text{Ln[day]} + \text{mouse}_j + \varepsilon_{ijday}$$

where Factor_{ijday} is the factor score for each day for mouse j from strain i, intercept_i is the fixed effect of factor on day 1 for strain i, slope_i is the fixed decrease of factor per natural logarithm of day for strain i, mouse_j is the random effect for mouse j, and ε_{ijday} is a random residual for each day for mouse j from strain i. From this model, the rate of changes in behaviour for each factor can be derived by differentiating with respect to day, so that the rate for each strain is slope_i/day. *Posthoc* comparisons between strains for intercept and slope were conducted using a Bonferroni correction of 3, i.e. strains were considered significantly different if $P < 0.017$.

Results

Descriptives and reliability of home cage parameters

Mean values per strain for each day on the parameters measured in the home cage test can be found in Table S1 (see *Supplementary material*). Test results from repeated measures ANOVA and *posthoc* Scheffé for strain differences are also summarized in this table.

Correlations between day 1 and day 6 were calculated to detect whether there was a change in parameter values over the course of the experiment. For most parameters, day 1 was not significantly correlated to day 6, indicating indeed changes in behaviour over time (data not shown). Two parameters did show a significant correlation between day 1 and day 6; frequency of movement ($P = 0.012$) and duration on shelter ($P = 0.001$). To determine the reliability of the changes of the parameters over days, we calculated Cronbach's alpha values. Values ranged from 0.79 to 0.93 indicating a high internal consistency for all parameters (data not shown).

Circadian rhythmicity

Strains differed in circadian rhythmicity as reflected by the 24-h distribution of the parameter 'duration of movement' (Fig. 1). Circadian rhythmicity was calculated using the means of day 4–6. This was based on the course of 'duration of movement' over time (see Table S1, *Supplementary material*); the strongest decline in movement was seen on days 1–3, whereas from day 4 onwards, a more stable time–course was displayed. For all strains, the peak of activity was during the dark phase of each day, but patterns of activity during this phase differed between strains. A 'bimodal' curve was seen in C57BL/6, with two activity peaks, one at the onset of the dark phase and one just before the end of the dark phase. C57BL/6 mice showed more activity during the dark phase than any of the other strains. Duration of movement in C3H mice was higher than in DBA/2 mice during the first half of the dark phase. 129S2/Sv mice moved remarkably little compared with the other strains. During the light phase, it was noted that C57BL/6, C3H and DBA/2 mice showed a small increase in movement in the last hour before the dark onset. This increase was absent in 129S2/Sv mice. Notably, movement in 129S2/Sv mice was close to zero during the light phase.

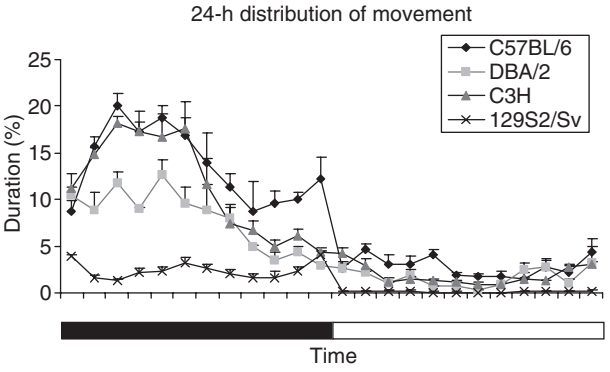

Figure 1: Circadian rhythmicity under baseline conditions as expressed by the duration of movement (% of total time). Means ± SEM of days 4–6 are used in 1-h bins. The black bar represents the 12-h dark period of the day, whereas the white bar represents the 12-h light period of the day.

PCA

The interrelation of parameters in the home cage test is represented by two factors, extracted by PCA (Table 1). These factors accounted for 76.89% of the variance. Factor 1 loads negatively on time spent inside the shelter and positively on, for example, distance moved and time spent on the shelter. These parameters reflect how active the animal is during the day and is therefore labelled as 'level of activity'. Factor 2 consists of the parameters 'velocity' and 'number of stops' which suggest a specific aspect of movement being independent of the amount of activity. This factor

Table 1: Principal component analysis across strains resulted in two factors with eigenvalues >1.0

Parameter	Factor 1	Factor 2
On shelter (duration)	0.790	
On shelter (frequency)	0.926	
In shelter (duration)	−0.897	
Movement (duration)	0.946	
Movement (frequency)	0.978	
Distance moved	0.944	
Variance explained (%)	60.02	
Velocity		0.753
Number of stops		−0.759
Variance explained (%)		16.84

Only behavioural parameters that loaded >0.6 are shown. Data from days 1–6 are included.

is labelled as 'velocity/stops'. To determine the robustness of the factors extracted, we performed a split half analysis resulting in a similar grouping of parameters (data not shown).

To investigate the effect of both strain and time on individual scores on each of the factors, we calculated factor scores for each individual mouse, and means per strain on consecutive days are shown as symbols in Fig. 2a,b. For both factors, only the active period of the day, i.e. the dark phase, is shown. Strains differences were found for both factor 1 [repeated measures ANOVA, between-subjects effect (strain) $F_{3,28} = 16.545$; $P < 0.001$] and factor 2 [repeated measures ANOVA, between-subjects effect (strain) $F_{3,28} = 28.193$; $P < 0.001$]. Notably, strain rankings were different between the two factors; for factor 1: C57BL/6 > DBA/2 = C3H > 129S2/Sv and for factor 2: C57BL/6 = DBA2 > C3H = 129S2/Sv (results from *posthoc* Scheffé-test, $P < 0.05$).

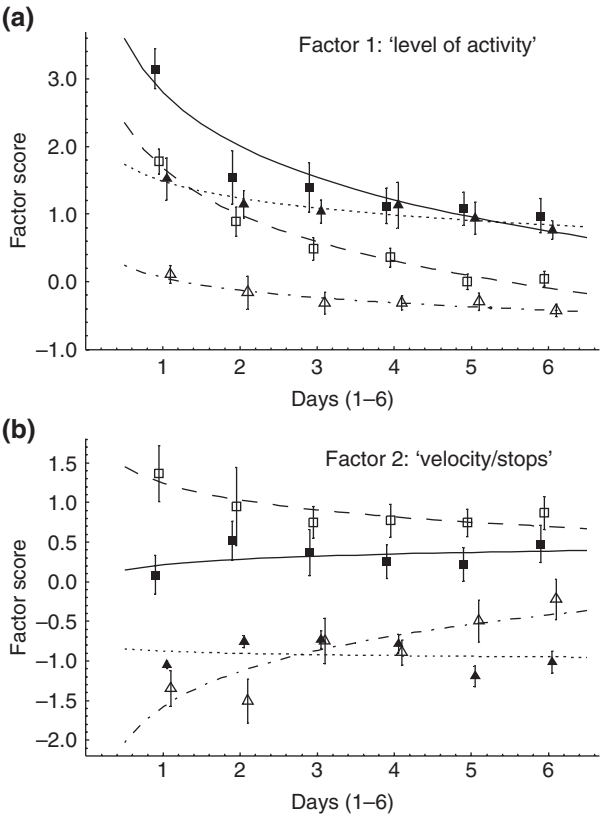

Figure 2: Observed (symbol with error bars) and predicted (lines) scores for (a) factor 1 and (b) factor 2 of C57BL/6 (■, ——), DBA/2 (□, – – –), C3H (▲, - - -) and129S2/SV (△, – . –) strains for day 1 through day 6. Observed factor scores are calculated for each individual animal using parameter loadings derived from Principal Component Analysis (see Table 3). Means ± SEM of 12-h bins are used. Predicted factor scores are calculated using mixed linear regression models (see *Materials and Methods* for details).

Development of locomotor activity over days

Development of locomotor activity over days was quantified using mixed linear regression models for factors 1 ('level of activity') and 2 ('velocity/stops'). Analysis was limited to the dark phase, as the minimal levels of activity during the light phase may confound the model. The graphical representation of these models for each factor is shown as lines in Fig. 2(a) (factor 1) and Fig. 2(b) (factor 2). Values of intercept and slopes are noted in Table 2. Changes in the interaction with the home cage environment are reflected by a decrease in 'level of activity' (factor 1, Fig. 2a) for all strains. However, there were significant strain differences in the rate of decrease, as reflected by the slope. C57BL/6 and DBA/2 mice showed a steeper slope compared with C3H and 129S2/Sv mice, which indicates a higher rate of adaptation and a more pronounced differentiation between novelty-induced and baseline activity. 'Velocity/stops' (factor 2, Fig. 2b) did not change over days in C57BL/6 and C3H animals, and only slightly for DBA/2. However, 129S2/Sv mice showed a marked increase in factor 2 over time, which reflects a change in the structure of movement towards a higher velocity and lower number of stops (see Table 1 for explanation of factors).

Individual differences in locomotor activity

To further distinguish strains on the level of individual animals, we plotted factor 1 and 2 for all individual mice (Figure S1, see *Supplementary material*). Separate plots were made for day 1 (Figure S1a) and day 6 (Figure S1b) to differentiate between the novel and baseline situation. In general, a more pronounced shift on factor 1 compared with factor 2 was seen from day 1 to day 6 for all individuals. Both plots revealed clusters of mice belonging to a certain strain, but individuals are clearly more scattered along the x-axis on day 1 compared with day 6. On day 1, factor 1 clearly separates 129S2/Sv mice from the other strains, whereas factor 2 clearly separates DBA/2 mice from 129S2/Sv and C3H mice and to a lesser extent from C57BL/6 mice. On day 6, 129S2/Sv mice are distinguished from C57BL/6 and C3H mice by factor 1, whereas DBA/2 and C3H mice are distinguished mostly by factor 2.

Table 2: Values of intercept and slopes derived from mixed linear regression models for factor 1 ('level of activity') and factor 2 ('velocity/stops')

Strain	Factor 1		Factor 2	
	Intercept	Slope	Intercept	Slope
C57BL/6	2.80^{*}	-1.14^{*}	0.21^{*}	0.10^{*}
DBA/2	$1.68^{\dagger}$	-0.99^{*}	$1.24^{\dagger}$	-0.31^{*}
C3H	$1.48^{\dagger}$	$-0.36^{\dagger}$	$-0.88^{\ddagger}$	-0.04^{*}
129S2/Sv	$0.06^{\ddagger}$	$-0.27^{\dagger}$	$-1.58^{\ddagger}$	$0.65^{\dagger}$

Estimates with the different superscript symbols are significantly different ($P < 0.017$) using *t*-test after Bonferroni correction.

Open-field and elevated plus-maze data

Table 3 summarizes data from the open-field and elevated plus-maze test. There were significant strain differences on all open-field parameters (main effect of 'strain': duration of movement KW = 29.682; $P < 0.001$, distance moved $F_{3,31} = 26.644$; $P < 0.001$, velocity $F_{3,31} = 19.017$; $P < 0.001$ and number of stops KW = 27.595; $P < 0.001$). C57BL/6 showed high, DBA/2 and C3H intermediate and129S2/Sv low levels of locomotor activity in terms of duration of movement. C3H and 129S2/Sv scored high compared with C57BL/6 and DBA/2 on the parameter number of stops.

On the elevated plus maze, significant strain differences were found on the parameters: head dips ($F_{3,31} = 18.927$; $P < 0.001$), total closed-arm entries ($F_{3,31} = 32.006$; $P < 0.001$), time in open arm (KW = 15.606; $P < 0.001$), time in centre (KW = 16.561; $P < 0.001$) and time in closed arm (KW = 19.592; $P < 0.001$). No effect of 'strain' was found on percentage of open-arm entries ($F_{3,31} = 0.612$; $P = 0.613$). DBA/2 mice showed the highest levels of anxiety-related behaviour compared with the other strains. General activity levels, expressed by the total number of closed arm entries, were high in C57BL/6 mice, intermediate in 129S2/Sv mice and low in DBA/2 and C3H mice.

Association between open-field, elevated plus-maze and home cage tests

Partial correlations between parameters from open-field, elevated plus-maze and the home cage tests are summed in Table 4. Only significant pairs are shown. Distance moved, velocity and number of stops in the first hour of the home cage test correlated with these parameters in the open field. In general, most of these correlations disappeared as time progressed in the home cage test. On the 6th day, only a correlation between open field and home cage on the parameter velocity was found. Several correlations were found between the first hour of the home cage test (velocity, time spent on the shelter and time spent in centre) and the elevated plus maze (head dips, time spent in open and closed arms and on the centre platform). Animals that moved with a higher velocity and less stops tended to have less head dips, spent more time in the closed arms and less time on the centre platform of the maze. Again, these correlations disappeared over time.

Table 3: Open-field and elevated plus-maze data

Strain	Open-field parameters				Elevated plus-maze parameters				
	Movement (duration, %)	Distance moved (m)	Velocity (cm/second)	Number of stops (per 50 cm moved)	Head dips (frequency)	Closed-arm entries (frequency)	Open-arm entries (% of total entries)	Open-arm time (duration, %)	Centre time (duration, %)	Closed-arm time (duration, %)
C57BL/6	$89.6 \pm 0.7^{*}$	$112.7 \pm 5.2^{*}$	$14.0 \pm 0.6^{*}$	$0.5 \pm 0.1^{*}$	$18.8 \pm 1.1^{*}$	$18.2 \pm 1.1^{*}$	27.7 ± 1.5	$25.6 \pm 2.9^{*}$	$21.4 \pm 2.8^{*}$	$53.1 \pm 3.0^{*}$
DBA/2	$80.7 \pm 1.6^{\dagger}$	$108.6 \pm 9.8^{*}$	$14.8 \pm 1.1^{*}$	$0.7 \pm 0.1^{*}$	$7.4 \pm 1.5^{\dagger}$	$5.7 \pm 0.8^{\dagger}$	27.5 ± 3.7	$8.3 \pm 2.4^{\dagger}$	$8.9 \pm 1.8^{\dagger}$	$82.8 \pm 3.8^{\dagger}$
C3H	$68.0 \pm 5.5^{\dagger,\ddagger}$	$51.0 \pm 6.8^{\dagger}$	$8.2 \pm 0.6^{\dagger}$	$3.0 \pm 0.7^{\dagger}$	$25.2 \pm 3.0^{*}$	$5.0 \pm 1.0^{\dagger}$	31.7 ± 2.9	$24.7 \pm 5.8^{*}$	$27.5 \pm 3.5^{*}$	$47.8 \pm 5.7^{*}$
129S2/Sv	$47.6 \pm 5.1^{\ddagger}$	$35.1 \pm 6.1^{\dagger}$	$7.7 \pm 0.7^{\dagger}$	$4.7 \pm 1.3^{\dagger}$	$27.5 \pm 2.7^{*}$	$11.7 \pm 1.2^{\ddagger}$	26.3 ± 2.3	$22.1 \pm 5.9^{*}$	$27.1 \pm 4.3^{*}$	$50.8 \pm 3.1^{*}$

Estimates with different superscript symbols are significantly different ($P < 0.05$).

Discussion

This study demonstrates the strength of automated home cage observations as a tool for the characterisation of mice. Known differences in locomotor activity between the four inbred strains used in this study could be reproduced, such as circadian rhythmicity and activity in a novel environment (Crawley *et al.* 1997; Kopp 2001; Tang *et al.* 2002). At the same time, important new information was obtained, such as different time–courses of factors of locomotor activity. Furthermore, associations between behaviour displayed

Table 4: Correlations between open-field, elevated plus-maze and home cage test

	Open field	*r*	Elevated plus maze	*r*
Home cage test (1 h)				
Distance moved	Distance moved	0.5470*		
	Velocity	0.5155*		
Velocity			Head dips	−0.5828*
			Closed-arm time	0.6136†
			Centre time	−0.5475*
			Head dips	0.5910†
Number of stops	Velocity	−0.5572*	Closed-arm time	−0.5802*
			Centre time	0.5048*
Time on shelter			Head dips	0.5579*
			Closed arm time	−0.6011†
			Centre time	0.5112*
Home cage test (day 1)				
Velocity	Distance moved	0.4821*	Head dips	−0.4787*
	Velocity	0.5670†	Closed-arm time	0.4874*
Number of stops	Distance moved	−0.4788*		
	Velocity	−0.4940*		
Home cage test (day 6)				
Velocity	Velocity	0.5115*		

Partial correlations were calculated for means of three different time intervals in the home cage test; the first hour after mice were introduced to the home cage (1 h), the dark phase of day 1 (day 1) and the dark phase of day 6 (day 6).

*$0.01 > P > 0.005$.

†$P < 0.005$, after Bonferroni correction of 10 for number of parameters compared.

during initial phase of the home cage test and the open field and elevated plus maze were found. For example, velocity and number of stops correlated positively with anxiety-related behaviour on the elevated plus maze. This method allows for continuous recordings of both novelty-induced activity and baseline locomotor activity in a home cage environment with minimal need for human intervention.

Characterization of inbred strains on home cage locomotor activity

Circadian activity under baseline conditions showed marked differences between strains, which are largely in line with what has previously been reported in literature (Kopp 2001; Tang & Sanford 2005; Tang *et al.* 2002). All strains show predominantly activity during the dark phase as compared with the light phase, and differences between strains were most conspicuous during the active dark phase. For example, a second peak in movement levels characterizes C57BL/6 mice at the end of the dark phase, which was also found by Tang *et al.* (2002) in this strain. In the present study, 129S2/Sv mice are characterized by overall low levels of activity during the complete period of the dark phase and an almost complete absence of activity during the light phase.

PCA cross strains revealed two main factors of home cage locomotor activity that together accounted for 79% of the variance. 'Duration of movement', 'distance moved' and 'time spent inside the shelter' most strongly loaded onto the first factor, which can be interpreted as 'level of activity', or 'how active is the animal?'. The second factor found is defined as 'velocity/stops', or 'when the animal is active, in what specific way is it moving through the cage?'.

In line with our findings, a study was found that used human observer recordings to observe home cage activity in heterogeneous stock mice (Mill *et al.* 2002). Here, PCA revealed a single factor, labelled 'general activity'. This factor is comparable with the factor 1 ('level of activity') found in the present study. Floor activity loaded positively on their factor 'general activity', whereas the time in nest area loaded negatively. In the study by Mill and colleagues, no parameters were included like velocity or number of stops. This might explain why the authors did not find a factor similar to factor 2 ('velocity/stops') found in the present study. Thus, the present findings reveal new insights in those aspects of locomotor activity that are informative on how mice interact with their surroundings. In addition, it can be concluded that strains show a high degree of variation in the development of the factors 'level of activity' and 'velocity/stops' over time. The results show a pronounced differential shift in behaviour that distinguishes strains under both novelty-induced and baseline conditions. For example, C57BL/6 mice respond to a new environment with enhanced levels of activity, but velocity and the number of stops remained largely constant over days. In contrast, the response of 129S2/Sv mice to the new environment is reflected by a low velocity and high number of stops, which increases over days.

The observation that in all strains the 'velocity/stops' factor is independent of 'level of activity' under both novelty and baseline conditions but not independent of time, suggests that different motivational systems may underlie these factors. The factor 'velocity/stops' might be interpreted as reflecting some degree of anxiety-related behaviour. For instance, Kafkafi *et al.* (2003) developed a comprehensive software package (SEE, for further information see Drai & Golani 2001) to extract a variety of behavioural parameters for open-field behaviour. One of these parameters is 'darting' behaviour, which is defined as having high acceleration during moving, while moving less during lingering episodes. This parameter is independent from other parameters of activity and discriminates between C57BL/6 and DBA/2 mice in an open field. The authors suggest that 'darting' behaviour 'may reflect the animal's tendency toward stress, jitteriness or anxiety' (Kafkafi *et al.* 2003, p. 203). Notably, the acceleration part of 'darting' behaviour is corresponding with the velocity parameter that is part of the 'velocity/stops' factor found in the present study. Furthermore, the parameters 'velocity' and 'number of stops' correlated with anxiety-related behaviour on the elevated plus maze. This supports the suggestion that the factor 'velocity/stops' might be related to anxiety.

However, in 129S2/Sv mice, velocity and number of stops do not seem indicative of anxiety in the initial phase when the animal is also becoming acquainted with its environment. They show a marked increase over time on this factor, which means that over time they move with higher velocity and lower number of stops. It does not seem likely that this increase is representative of an increase in anxiety-related behaviour over days as habituation to the environment normally leads to a decrease in anxiety-related behaviour in mice (e.g. Ohl *et al.* 2003). Thus, in a novelty situation, the factor 'velocity/stops' seems more indicative of thoroughness of exploration. This is reflected by the more careful way of exploration of 129S2/Sv mice when placed in a new environment as compared with C57BL/6 and DBA/2 mice, which make long and fast excursions. Under baseline conditions, when velocity and number of stops are in a minor degree affected by exploration, it may very well reflect a trait characteristic that is related to anxiety. Pharmacological experiments with standard anxiolytics, such as diazepam, are indispensable to further assess the influence of anxiety on the 'velocity/stops' factor.

The longitudinal nature of the method presented may have the potential to differentiate between 'state' and 'trait' characteristics. The concept of differentiating between 'state' and 'trait' is often used in relation to anxiety. 'Trait' anxiety refers to a persistent and endurable feature of an individual that is independent of context and reflects the way the individual interacts with their environment (Belzung & Griebel 2001; Sandford *et al.* 2000). 'State' anxiety, by contrast, is highly dependent on context and changes in response to the level of stress and the way stress is perceived (Belzung & Griebel 2001; Sandford *et al.* 2000). In the present study, the animals' scores on the 'level of activity' and 'velocity/stops' factors during the first day after introduction to the cage may reflect a 'state' characteristic that is strain specific and a response to the novel environment. After a few days, when the animals are adapted to their new home cage, the scores on 'level of activity' and 'velocity/stops' may reflect a 'trait characteristic', which is also strain specific but typical for the way animals interact with a familiar environment.

Association between open-field, elevated plus-maze and home cage tests

Several elements of home cage locomotor activity during the first hour after introduction to the cage were related to the behavioural elements displayed in an open-field test and elevated plus-maze test. Distance travelled and velocity in the home cage were correlated with these parameters in the open field. Velocity, number of stops and time spent on shelter were correlated with anxiety-related behaviour on the elevated plus maze. This suggests that behavioural reactions to novelty can be studied in the new home cage environment and is compatible with results obtained in an open field and elevated plus maze.

With respect to the comparison between home cage and open field, it must be noted that the home cage was provided with a shelter, where the animals can hide. This situation is substantially different from the open-field test and enabled the animals to display a broader array of responses to the new environment. Despite this different context, strain rankings on the open-field parameters are similar to strain rankings on home cage parameters during the first hour. Correlations between the three tests disappeared as time progressed in the home cage test. This confirms the hypothesis that this method is able to differentiate between behaviour under novel and baseline conditions.

Although both open-field and the home cage tests allow for the characterization of mice on novelty-induced behaviour, one cannot substitute for the other. For specific research questions involving the responses of mice in a new environment, for example after pharmacological treatment, open field and elevated plus maze are appropriate assays. Not unimportant, they are low cost and easy to perform. However, automated home cage observations offer the unique possibility to study long-term behaviour with a high temporal and spatial resolution in undisturbed mice under both novelty-induced and baseline conditions. The integration of programmable aversive, rewarding and neutral stimuli (e.g. running wheel, novel objects, aversive light gradients, sound and programmable feeder) in the home cage environment will extend the range of interacting motivational systems and cognitive functions that can be addressed.

References

Belzung, C. & Griebel, G. (2001) Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res* **125**, 141–149.

Cabib, S., Puglisi-Allegra, S. & Ventura, R. (2002) The contribution of comparative studies in inbred strains of mice to the understanding of the hyperactive phenotype. *Behav Brain Res* **130**, 103–109.

Casadesus, G., Shukitt-Hale, B. & Joseph, J.A. (2001) Automated measurement of age-related changes in the locomotor response to environmental novelty and home-cage activity. *Mech Ageing Dev* **122**, 1887–1897.

Crawley, J.N. (2000) *What's Wrong with My Mouse?: Behavioural Phenotyping of Transgenic and Knockout Mice.* Wiley-Liss, New York.

Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A. & Paylor, R. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology* **132**, 107–124.

Drai, D. & Golani, I. (2001) See: a tool for the visualization and analysis of rodent exploratory behavior. *Neurosci Biobehav Rev* **25**, 409–426.

Ferguson, G.A. (1981) *Statistical Analysis in Psychology and Education,* 5th edn. McGraw-Hill, Singapore.

Gerlai, R. (2002) Phenomics: fiction or the future? *Trends Neurosci* **25**, 506–509.

Griebel, G., Belzung, C., Perrault, G. & Sanger, D.J. (2000) Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology* **148**, 164–170.

Kafkafi, N., Pagis, M., Lipkind, D., Mayo, C.L., Bemjamini, Y., Golani, I. & Elmer, G.I. (2003) Darting behavior: a quantitative movement pattern designed for discrimination and replicability in mouse locomotor behavior. *Behav Brain Res* **142**, 193–205.

Kas, M.J.H. & Van Ree, J.M. (2004) Dissecting complex behaviours in the post-genomic era. *Trends Neurosci* **27**, 366–369.

Kopp, C. (2001) Locomotor activity rhythm in inbred strains of mice: implications for behavioural studies. *Behav Brain Res* **125**, 93–96.

Kopp, C., Vogel, E. & Misslin, R. (1999) Comparative study of emotional behaviour in three inbred strains of mice. *Behav Processes* **47**, 161–174.

Lister, R.G. (1987) The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* **92**, 180–185.

Mill, J., Galsworthy, M.J., Paya-Cano, J.L., Sluyter, F., Schalkwyk, L.C., Plomin, R. & Asherson, P. (2002) Home-cage activity in heterogeneous stock (hs) mice as a model of baseline activity. *Genes Brain Behav* **1**, 166–173.

Ohl, F., Sillaber, I., Binder, E., Keck, M.E. & Holsboer, F. (2001) Differential analysis of behavior and diazepam-induced alterations in C57BL/6n and Balb/c mice using the modified hole board test. *J Psychiatr Res* **35**, 147–154.

Ohl, F., Roedel, A., Binder, E. & Holsboer, F. (2003) Impact of high and low anxiety on cognitive performance in a modified hole board test in C57BL/6 and DBA/2 mice. *Eur J Neurosci* **17**, 128–136.

Rodgers, R.J., Boullier, E., Chatzimichalaki, P., Cooper, G.D. & Shorten, A. (2002) Contrasting phenotypes of C57BL/6JOlaHsd, 129S2/SvHsd and 129/SvEv mice in two exploration-based tests of anxiety-related behaviour. *Physiol Behav* **77**, 301–310.

Rogers, D.C., Jones, D.N.C., Nelson, P.R., Jones, C.M., Quilter, C.A., Robinson, T.L. & Hagan, J.J. (1999) Use of shirpa and discriminant analysis to characterise marked differences in the behavioural phenotype of six inbred mouse strains. *Behav Brain Res* **105**, 207–217.

Sandford, J.J., Argyropoulos, S.V. & Nutt, D.J. (2000) The psychobiology of anxiolytic drugs: Part 1: basic neurobiology. *Pharmacol Ther* **88**, 197–212.

Sherwin, C.M. (1998) Voluntary wheel running: a review and novel interpretation. *Animal Behav* **56**, 11–27.

Spink, A.J., Tegelenbosch, R.A.J., Buma, M.O.S. & Noldus, L.P.J.J. (2001) The EthoVision video tracking system – a tool for behavioral phenotyping of transgenic mice. *Physiol Behav* **73**, 731–744.

Spruijt, B.M. (1992) Progressive decline in social attention in aging rats: an informational-statistical method. *Neurobiol Aging* **13**, 145–151.

Spruijt, B.M. & Gispen, W.H. (1983) Prolonged animal observation by use of digitized video displays. *Pharmacol Biochem Behav* **19**, 765–769.

Tang, X. & Sanford, L.D. (2005) Home cage activity and activity-based measures of anxiety in 129P3/J, 129x1/SvJ and C57BL/6J mice. *Physiol Behav* **84**, 105–115.

Tang, X., Orchard, S.M. & Sanford, L.D. (2002) Home cage activity and behavioral performance in inbred and hybrid mice. *Behav Brain Res* **136**, 555–569.

Tecott, L.H. & Nestler, E.J. (2004) Neurobehavioral assessment in the information age. *Nat Neurosci* **7**, 462–466.

Van Gaalen, M.M. & Steckler, T. (2000) Behavioural analysis of four mouse strains in an anxiety test battery. *Behav Brain Res* **115**, 95–106.

de Visser, L., van den Bos, R. & Spruijt, B.M. (2005) Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. *Behav Brain Res* **160**, 382–388.

Wahlsten, D., Rustay, N.R., Metten, P. & Crabbe, J.C. (2003) In search of a better mouse test. *Trends Neurosci* **26**, 132–136.

Supplementary material

Table S1. Summary of observed values and statistics for all home cage parameters. Means and SEM are calculated in 12-h bins for both the dark/light period of each day (1–6). Repeated measures ANOVA performed separately for dark/light period, with factors 'day' and 'strain' and their interaction. Different superscripts following strain name represent differences between strains ($P < 0.05$) as tested *posthoc* with Scheffé.

Figure S1. Scatter plot presenting the distribution of factor scores of individual animals on day 1 and day 6. After Principal Component Analysis, scores on the extracted factor 1 and factor 2 were calculated for each individual animal. Means of 12-h bins are used.

These materials are available as part of the online article from http://www.blackwell-synergy.com

Acknowledgments

The authors thank M. Schenke and J.M. Baars for their practical assistance and Noldus Information Technology for their technical support. This study was part of the Utrecht University ABC Neurogenomics programme and supported by the NeuroBsik Consortium.