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Short communication

Temporal stability of novelty exploration in mice exposed to different open field tests

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Abstract

We investigated behavioural activity and temporal distribution (patterning) of mouse exploration in different open field (OF) arenas. Mice of 129S1 (S1) strain were subjected in parallel to three different OF arenas (Experiment 1), two different OF arenas in two trials (Experiment 2) or two trials of the same OF test (Experiment 3). Overall, mice demonstrated a high degree of similarity in the temporal profile of novelty-induced horizontal and vertical exploration (regardless of the size, colour and shape of the OF), which remained stable in subsequent OF exposures. In Experiments 4 and 5, we tested F1 hybrid mice (BALB/c-S1; NMRI-S1), and Vitamin D receptor knockout mice (generated on S1 genetic background), again showing strikingly similar temporal patterns of their OF exploration, despite marked behavioural strain differences in anxiety and activity. These results suggest that mice are characterised by stability of temporal organization of their exploration in different OF novelty situations.

Keywords: Novelty; Open field; Mice; Temporal patterning; Exploration stability

1. Introduction

The open field (OF) is one of the most popular tests in behavioural neuroscience (Belzing, 1999; Crawley, 1999; Drai et al., 2001; Crabbe et al., 1999), widely used for behavioural phenotyping of various mouse strains (Belzung and Griebel, 2001; Tang et al., 2002; Augustsson and Meyerson, 2004; Crabbe et al., 1999; Kafkafi and Elmer, 2005). Several factors determine rodent of behaviour, including anxiety, arousal, risk assessment, escape, locomotory activity and exploration (Paulus et al., 1999; Ohl et al., 2001). The mouse horizontal and vertical exploration, defecation/urination scores and grooming represent traditional OF measures (Flint et al., 1995; Choleris et al., 2001; Flint, 2002) sensitive to different stressors and psychotropic drugs (Homanics et al., 1999; Prut and Belzung, 2003), underlying wide application of this test in neurobehavioural research.

While one can view animal OF novelty exploration as a stochastic process, recent studies have shown well-organized OF behaviours in rodents (Eilam and Golani, 1988, 1989, 1990; Golani et al., 1993; Eilam et al., 1999, 2003; Tchernichovski and Golani, 1995), including establishing key places, such as a safe location (home base), from which they perform round-trip excursions with different speed and velocity (Drai et al., 2000, 2001; Kafkafi et al., 2001, 2003, 2005). Several other sophisticated kinematical, angular, dimensional, spatial and entropy-based indices have been recently suggested to assess the rodent OF activity in detail (Tchernichovski and Golani, 1995; Brudzynski and Krol, 1997; Tchernichovski et al., 1998; Paulus et al., 1999; Drai et al., 2000; Kafkafi et al., 2003; Lipkind et al., 2004). However, despite the extensive use in neuroscience research, the exact nature of the OF behaviours and their patterning is not yet fully understood (Calatayud et al., 2004), underlying the importance of further in-depth ethological analyses.

The key problem in animal exploration research is the relation between novelty and exploration. Although exploration largely depends on environment (Belzing, 1999; Crabbe et al., 1999; Wahlsten et al., 2003a,b), several recent studies have shown that rodent OF exploration withstands changes in basic novelty properties such as size, shape or colour (Golani et al., 1993; Eilam, 2003; Eilam et al., 2003), suggesting a highly conservative behavioural organization of novelty exploration (Drai et al.,

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2001; Eilam et al., 2003). In the present study, we analysed the mouse exploration in different OF arenas, varying their properties (e.g. colour, size and shape), assessing a wide spectrum of behaviours (including both exploratory and non-exploratory measures) and focusing on temporal patterning of their activity in these tests.

The 129S1 (S1) mouse strain was chosen for our study for its common use in behavioural research (MPD, 2001; Wahlsten et al., 2003b). F1 hybrid strains (NMRIS1, BCS1) were used as reference mouse strains, markedly differing from S1 mice and sharing many behavioural features (see further) of their other parental strains (active anxious BC, active non-anxious NMRI; see: MPD, 2001; Kalueff and Tuohimaa, 2005, for details). Knockout mice lacking functional Vitamin D receptors (VDR KO, Yoshizawa et al., 1997) were used here as an animal model of mutation-induced alteration in anxiety and activity (Kalueff et al., 2004, 2005; Burne et al., 2005). Expressing non-functional "truncated" VDR, these mice are insensitive to genomic effects of important neurosteroid hormone Vitamin D, and display high anxiety low activity phenotype, compared to the wild type S1 strain (Kalueff et al., 2004; Kalueff, 2005).

Here, we report that mice of several strains subjected to different OF arenas may vary the levels (quantity) of their horizontal activity but demonstrate a striking stability of temporal patterning (quality) of horizontal and vertical exploration.

2. Materials and methods

2.1. Animals

Subjects were adult male and female mice of different strains maintained in a virus/parasite-free facility under conditions of controlled temperature (22 ± 2 °C), humidity (60%) and a 12-h light:12-h dark cycle (lights on at 07:00 h) in the Animal House of the University of Tampere (Finland). The following animals were used in this study: S1 strain (25–30 g, 27 males and 10 females, Experiments 1–3); 15 males of F1 hybrid strains (7 NMRI × S1; 35–40 g and 8 BALB/c (BC) × S1; 30–35 g; Experiment 4) and VDR KO mice (20–25 g, 10 females, Experiment 5; generated on S1 genetic background and fed with special rescue Ca/P-rich diet (Lactamin AB, Sweden), to normalize mineral homeostasis). All animals used here were experimentally naïve and housed in groups of three to four animals per cage, with food and water freely available.

2.2. Apparatus and procedures

Several different OF were used here, including a circular (COF), square (SOF), big square (BOF) and small actimeter (AOF) OF tests. COF was an open plastic brown arena (90 cm in diameter), surrounded by a 50-cm wall, with a floor marked out by eight radial lines and two concentric circles 15 and 45 cm in diameter. The outer rings were divided by lines into 32 sectors each of the length of 15 cm. SOF was a grey plastic box (45 cm × 45 cm × 45 cm) with the floor divided into nine sectors (15 cm × 15 cm) by line drawing. AOF was a small transparent Plexiglas box (30 cm × 30 cm) with the floor divided into five squares (15 cm × 15 cm) by line drawing. BOF was a dimly lit isolated square room (5.5 m × 5.5 m) with white linoleum floor (divided into 484 squares 25 cm × 25 cm each) and white walls.

All testing was conducted between 14:00 and 19:00 h. On the days of experiments, the mice were transported to the dimly lit experimental room, and left undisturbed for 1 h for acclimation. In Experiment 1, we assessed the OF behaviours in three parallel groups of S1 male mice ($n = 10$ each) subjected to 10-min AOF, SOF or COF tests. In Experiment 2, we wanted to know if the same OF exploration strategy will be used by mice exposed to COF and BOF novelty. For this, 10 S1 mice were first tested in the COF (trial 1, 10 min), and then, 1-week later, in the BOF (trial 2, 10 min). In Experiment 3, we tested female ($n = 10$) S1 mice in the SOF (trial 1), re-exposing them to the same arena 1-week later (trial 2, 10 min each).

In Experiment 4, we wanted to extend our studies to several other mouse strains, markedly differing in activity and emotionality. For this, we tested S1 ($n = 7$) and F1 hybrid NMRIS1 ($n = 7$) and BCS1 ($n = 8$) males for 5 min in the SOF and COF (30 days after SOF). In addition, we assessed their anxiety and activity using the elevated plus maze (EPM), a test widely used tests in behavioural phenotyping of mice (Crawley, 1999). The EPM was made from Plexiglas and consisted of two open arms (30 cm × 10 cm) and two enclosed arms (30 cm × 10 cm × 10 cm) extending from a common central region (10 cm × 10 cm) elevated to a height of 60 cm. The EPM testing was performed 2 weeks after SOF.

In Experiment 5, we tested mutant animals with known aberrant activity and anxiety phenotypes, such as anxious hypoactive VDR KO mice (Kalueff et al., 2004, 2005; Burne et al., 2005). Female VDR KO mice were first compared to their WT controls ($n = 10$ in each group) in the 10-min SOF. One-week later, we re-exposed these VDR KO to the same test for 10 min, analysing the difference between these two trials. Three-week later, we exposed these mice for 5 min to the COF arena, comparing their performance with the first 5-min interval of the initial SOF trial, as described previously.

In all these tests, mice were exposed to the OF by placing them individually in the centre of the arena. After this, the experimenter quietly withdrew from the immediate vicinity of the test. The behaviour (frequency data) was recorded by a highly trained observer (intra-rater reliability > 0.90) for every minute of the test, using a custom-made register. Exploratory measures included horizontal locomotion (the number of sectors visited with four paws) and vertical activity—the number of times an animal stood erect on its hind-legs with its forelegs in the air (vertical rears) or against the wall (wall-leaning), as well as total vertical activity (rears + wall-leanings). In Experiment 2, we also assessed stopping behaviour (the number of stops; recorded whenever there was a cessation of progression > 3 s), and measured only cumulative (total) vertical activity. Non-exploratory behaviours in all experiments included the number of grooming bouts (licking, scratching and washing of the paws, head and body) and vegetative behaviours (defecation boli and urination episodes). For all indices (except vegetative behaviours), we calculated their temporal (per minute) distribution as the percentage of total scores (taken as 100%). In the EPM (Experiment 4), the

mouse was placed in the center of the apparatus facing the open arm, and observed for 5 min. Conventional measures were the numbers of open-, closed-arm and total arm entries (four-paw criterion), central platform crossing (two-paw criterion), vertical rears (wall leaning + unsupported rears), head dips, grooming bouts, urination episodes and defecation boli. Between sessions, all tests were cleaned with 70% ethanol and swept by paper towels (SOF, COF, AOF, EPM) or wet and dry cloths (BOF). All animal experiments reported here were performed in full compliance with the European legislation on animal experimentation (86/609/EEC) and approved by the Ethical Committee of the University of Tampere.

2.3. Statistical analysis

All data are presented as mean ± S.E.M. Differences between cumulative behavioural scores were analysed by Mann–Whitney *U*-test (Experiments 2, 3, 5), or one-way ANOVA (factors: type of the OF, Experiment 1; strain, Experiment 4), followed by a post hoc *U*-test. For horizontal activity in Experiment 1, the SOF and AOF data (squares visited) were analysed by *U*-test. Temporal distribution of activity (% of total measures scored) was analysed in all these experiments, using a one-way ANOVA with repeated measures (factor: OF type, Experiments 2 and 5; genotype, Experiment 5; strain, Experiment 4) or two-way ANOVA (factors: trial and genotype, Experiment 3; OF type and strain, Experiment 4), followed by a post hoc *U*-test. In all tests, a probability of less than 0.05 was considered statistically significant. Statistical analyses of data were performed using Microsoft Excel and Interactive Online Statistical Calculator (www.statpages.net).

3. Results

As can be seen in Fig. 1 (Experiment 1), the S1 male mice produced significantly less horizontal activity in AOF versus SOF (79 ± 7 versus 161 ± 12, $P < 0.05$, *U*-test), but showed similar total vertical ($F(2,29) = 1.84$, NS), unsupported vertical ($F(2,29) = 1.63$, NS) and wall-leaning ($F(2,29) = 0.39$, NS) activity as well as unaltered grooming ($F(2,29) = 3.13$, NS), defecation ($F(2,29) = 2.44$, NS) and urination ($F(2,29) = 1.50$, NS) scores in all three OF arenas. Analysing temporal patterning of horizontal and vertical exploration in Experiment 1, we found striking similarities in the mouse performance in all three parallel OF arenas (Fig. 1, $F(2,29) < 1$ for all measures, one-way ANOVA with repeated measures, factor: test).

Analysing the results of Experiment 2, we found similar temporal distribution of horizontal and vertical activity in COF and BOF arenas (Fig. 1, $F(1,19) < 1$, one-way ANOVA with repeated measures, factor: test). In addition, analysis of stopping behaviour in S1 mice shows that in both tests the animals displayed similar number of stops (26 ± 7 COF, 25 ± 5 BOF, NS, *U*-test), almost equally distributed over a 10-min observation period (Fig. 1, $F(1,19) < 1$, one-way ANOVA with repeated measures, factor: test). Although distance travelled was not directly assessed in this study, given more squares crossed in BOF, S1 mice also seem to display more horizontal activity in a bigger OF arena, but showed similar vertical, grooming, defecation and urination scores ($P > 0.05$, *U*-test) in both tests (Fig. 1).

Fig. 2 shows the performance of female S1 mice tested in two consecutive SOF trials (Experiment 3). Overall, we found similar cumulative scores and temporal distribution of horizontal and vertical (wall leaning, unsupported and total vertical rears) exploration in both trials ($F(1,38) < 1$, two-way ANOVA with repeated measures for trial, genotype and trial × genotype).

Fig. 3 shows the performance of male of S1 and two F1 hybrid strains tested in the EPM, SOF and COF. Although no difference was found in the EPM between the strains for the number of open entries ($F(2,21) = 0.46$; $P = 0.64$), open/closed ratio ($F(2,21) = 0.54$; $P = 0.59$), open/total ratio ($F(2,21) = 0.42$; $P = 0.66$), total entries ($F(2,21) = 1.99$; $P = 0.16$), urination ($F(2,21) = 2.11$; $P = 0.15$) and defecation scores ($F(2,21) = 2.05$; $P = 0.16$), there were significant genotype differences in the number of closed entries ($F(2,21) = 4.52$; $P = 0.03$), central platform crossing ($F(2,21) = 3.91$; $P = 0.04$), head dips ($F(2,21) = 3.75$; $P = 0.04$) and vertical rears ($F(2,21) = 3.79$; $P = 0.04$). Overall, these results indicate strain differences in anxiety and activity EPM measures, with NMRIS1 hybrids being the most active and S1 mice—the most anxious and hypoactive groups (Fig. 3). In the SOF, all three genotypes showed no significant strain differences in their horizontal ($F(2,21) = 0.29$; $P = 0.75$), total vertical ($F(2,21) = 0.6$; $P = 0.56$) and wall-leaning ($F(2,21) = 1.92$, $P = 0.17$) activity, grooming bouts ($F(2,21) = 0.84$; $P = 0.44$) and urination scores ($F(2,21) = 1.28$; $P = 0.30$), although showing a tendency to altered defecation scores ($F(2,21) = 3.10$; $P = 0.068$: BCS1 > S1, NMRI) and significant difference in vertical rears ($F(2,21) = 6.29$; $P = 0.008$: BCS1>NMRI; $P < 0.05$, *U*-test). One month later, these mice showed similar COF horizontal activity ($F(2,21) = 1.60$; $P = 0.23$), wall-leaning ($F(2,21) = 0.84$; $P = 0.44$), unsupported rears ($F(2,21) = 0.51$; $P = 0.61$), total vertical activity ($F(2,21) = 0.73$; $P = 0.49$), grooming bouts ($F(2,21) = 0.64$; $P = 0.54$), defecation ($F(2,21) = 0.74$; $P = 0.48$) and urination ($F(2,21) = 1.43$; $P = 0.26$) scores. Moreover, assessing temporal distribution of activity in these hybrid strains, we found strikingly similar patterns of their SOF and COF horizontal and vertical exploration (Fig. 3; $F(1,33) < 2$, two-way ANOVA with repeated measures for test, strain and test × strain).

Fig. 4 shows the performance of female VDR KO in different OF tests (Experiment 5). Compared to their WT counterparts, the VDR KO mice showed significantly lower horizontal and vertical SOF activity (Fig. 4), confirming our earlier data in male KO mice in this test (Kalueff et al., 2004). However, despite different baseline anxiety and activity levels, both genotypes showed a strikingly similar temporal organization of their horizontal and vertical activity in this test (Fig. 4, $F(1,19) < 1$, one-way ANOVA with repeated measures, factor: genotype). Furthermore, in two consecutive SOF trials, there was significant genotype effect ($F(1,36) = 13.71$; $P = 0.0007$) but no trial ($F(1,36) = 0.53$; $P = 0.47$) or trial × genotype interaction ($F(1,36) = 0.005$; $P = 0.94$) for horizontal activity and vertical activity scores (total rears: genotype ($F(1,36) = 15.8$; $P = 0.0033$); trial ($F(1,36) = 0.02$; $P = 0.89$), trial × genotype ($F(1,36) = 0.02$;

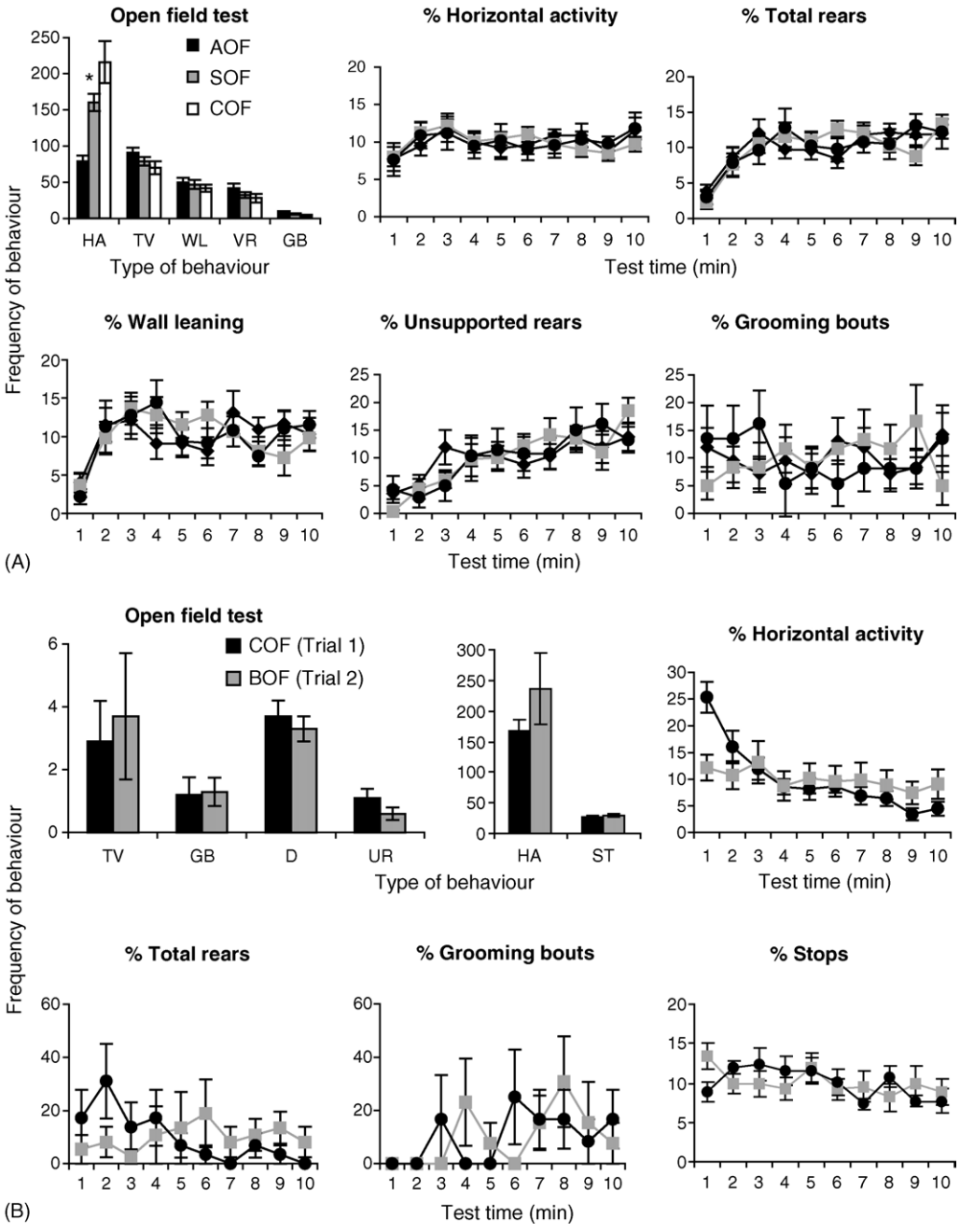

Fig. 1. Behavioural performance and temporal distribution of activity in 129S1 male mice tested in different open field (OF) tests. HA, horizontal activity; TV, total vertical activity; WL, wall leaning; VR, unsupported vertical rears; GB, grooming bouts (frequency). Data are presented as mean ± S.E.M. (A) Experiment 1. Three groups of mice ($n = 10$ each) tested in parallel in three different OF tests: actimeter box (AOF) and square (SOF) and circular (COF) OF arenas. Note predictably different horizontal activity levels (higher in the bigger arena), similar vertical exploration and other measures, and essentially the same temporal organization of exploratory activity in both trials. $^{*}P < 0.05$ (U-test) vs. actimeter box. (B) Experiment 2. One group of mice ($n = 10$) tested in the circular (COF, trial 1) and big square (BOF, trial 2) OF arenas with a 1-week interval. Note predictably different horizontal activity (higher in the bigger arena), similar vertical exploration and other measures, and essentially the same temporal organization of horizontal and vertical exploratory activity in both trials.

$P = 0.89$); wall-leaning: genotype ($F(1,36) = 9.15$; $P = 0.0045$); trial ($F(1,36) = 0.11$; $P = 0.73$), trial × genotype ($F(1,36) = 0$; $P = 1$); unsupported vertical rears: genotype ($F(1,36) = 4.13$; $P = 0.049$); trial ($F(1,36) = 0.63$; $P = 0.43$), trial × genotype ($F(1,36) = 0.36$; $P = 0.55$)). No significant strain effect was found for grooming bouts (genotype ($F(1,36) = 0.40$; $P = 0.53$); with significant trial ($F(1,36) = 6.45$; $P = 0.01$) but no trial × genotype ($F(1,36) = 0.07$; $P = 0.78$) effects. There was significant genotype effect for the number of defecation boli ($F(1,36) = 4.98$; $P = 0.031$) but no trial ($F(1,36) = 0.19$; $P = 0.66$) and trial × genotype ($F(1,36) = 0.09$; $P = 0.77$). Likewise, no significant difference was found for the number of urination episodes (genotype ($F(1,36) = 0.30$; $P = 0.59$; trial $F(1,36) = 1.63$; $P = 0.21$, trial × genotype $F(1,36) = 1.63$; $P = 0.21$). Finally, assessing temporal distribution of activity in these mice, we again observed strikingly similar patterns of

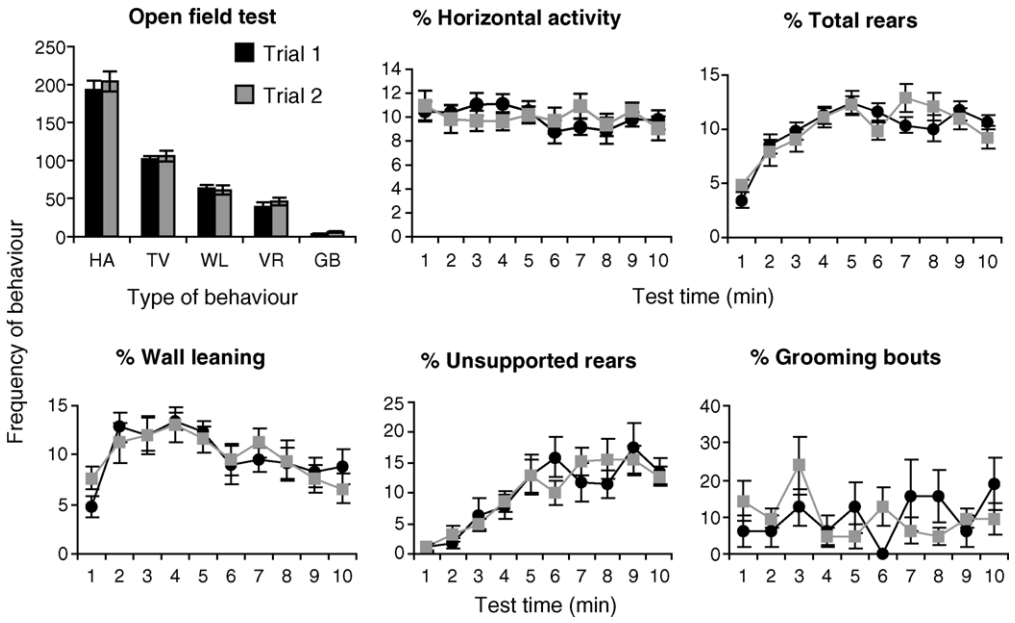

Fig. 2. Behavioural performance of female 129S1 mice ($n = 10$) tested in the square open field test with a 1-week interval (Experiment 3). Legend as in Fig. 1. Note similar behavioural scores and essentially the same temporal organization of horizontal and vertical exploratory activity in both trials. Data are presented as mean ± S.E.M.

their OF horizontal and vertical exploration (Fig. 4) activity in both SOF trials as well as in the COF test ($F(1,9) < 1$, one-way ANOVA, factor: test).

4. Discussion

Horizontal and vertical exploration is used by animals to assess novel environments and represents an important part of rodent natural behaviour (Crawley, 1999; Augustsson and Meyerson, 2004). Several interesting behavioural observations can be made based on the results of this study. Experiment 1 clearly shows that S1 mice display similar temporal patterning of their exploration if tested in parallel in three different OF tests (AOF, SOF, COF). This suggests that in any novel OF arena the mice seem to use the same temporal organization of their horizontal and vertical exploration (Fig. 1).

Experiment 2 shows that S1 mice use essentially the same exploration patterning if first tested in one arena (COF), and then exposed to a markedly different OF test (BOF). Thus, a dramatic alteration in the size, colour and shape of the OF (e.g. COF versus BOF) does not influence the mouse exploration temporal patterning, although (in line with previous rodent studies; e.g. Golani et al., 1993; Eilam, 2003) it does affect horizontal activity levels; also see similar results in Experiment 1 (Fig. 1). Moreover, as can be seen in Fig. 3, S1 mice do not change temporal patterning of their activity exploring the same OF 1-week after the initial exposure (Experiment 3). Collectively, this suggests that mice exploring novel OF arenas, or re-exploring previously exposed OF arenas, use essentially the same conservative temporal strategies as during the initial exploration.

The results of Experiment 4 suggest that these findings may be generalized to other mouse strains, since both hybrid F1 strains (despite behavioural strain in activity and emotionality) demonstrate essentially the same temporal patterning of their OF behaviours, as assessed in two different tests (SOF, COF); Fig. 3. This suggests that behavioural differences between strains do not alter their conservative temporal strategies in the OF arenas. In line with this, Experiment 5 (Fig. 4) shows that genetic mutations seriously affecting the mouse anxiety and activity (e.g. VDR KO) do not alter the temporal patterning of their behaviours in different OF situations. Interestingly, VDR KO mice of both sexes display similar behavioural scores and essentially the same temporal patterning of their activity in the OF test (Kalueff, 2005), further confirming our present observations.

Notably, although ANOVA test did not reveal significant differences between the groups in temporal distribution of their horizontal activity, there was a clear tendency to altered activity during the first minute of the test in some of our experiments. For example, in Experiment 2, reduced % horizontal and reciprocally increased % stopping activity were seen immediately after the exposure to the BOF (Fig. 1B), most likely reflecting a fear-like freezing response (to a more stressful BOF compared to the COF) placement procedure, generally in line with high anxiety/high freezing phenotype of S1 mice (MPD, 2001). Likewise, slightly higher % horizontal activity in VDR KO mice during the first minute of trial 2 (versus trial 1) in Experiment 5 (Fig. 4B) may reflect lower "initial" anxiety of these mice due to habituation to a placement procedure. Collectively, this indicates that various procedural factors, bi-directionally affecting animal activity immediately after the placement to the OF arena, have to be considered when interpreting behavioural data, especially using several strains displaying robust panic-like responses (e.g. S1, BC mice; MPD, 2001). Nevertheless, as can be seen in Figs. 1–4, these differences did not seem to affect generally stable temporal patterning of mouse exploration of the OF novelty reported here.

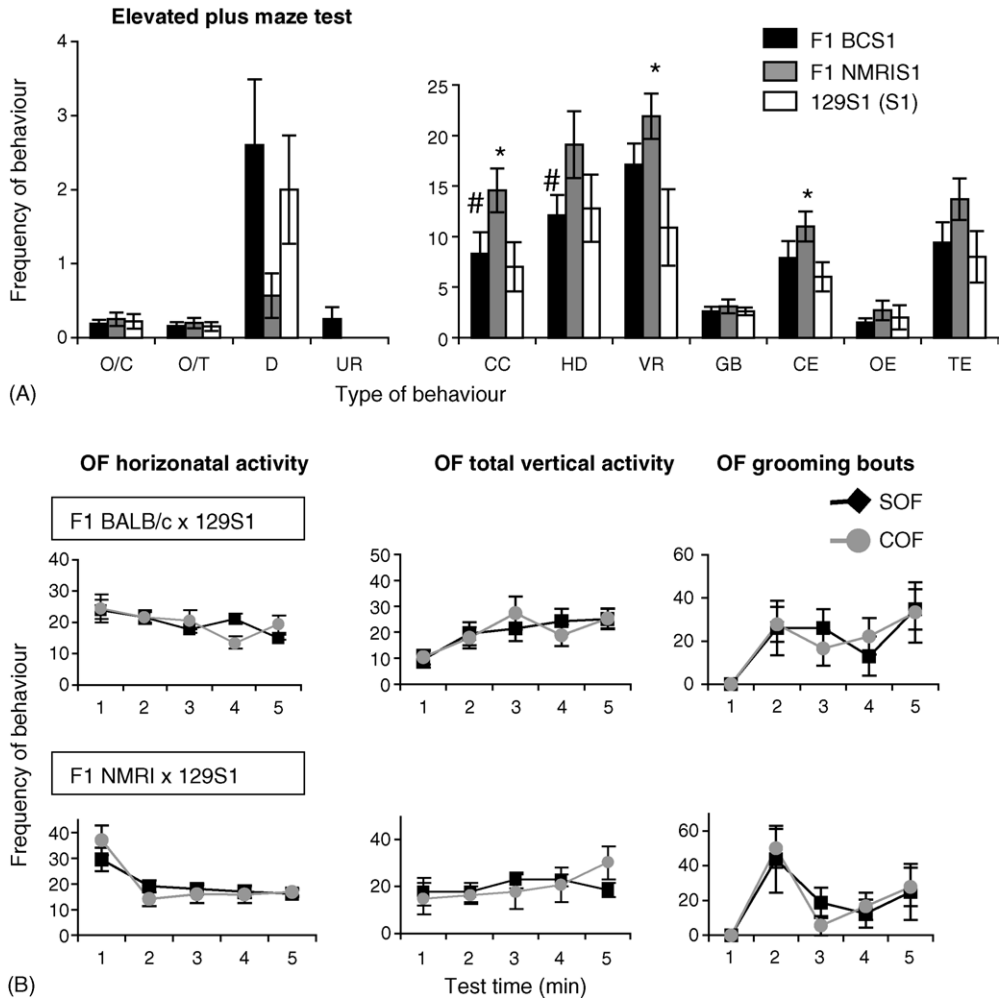

Fig. 3. Behavioural performance of 129S1 (S1, $n = 7$) and two F1 hybrid strains: BCS1 ($n = 8$) and NMRIS1 ($n = 7$); Experiment 4. Data are presented as mean ± S.E.M. (A) Gross behavioural measures in the elevated plus maze (EPM, 5 min) $^{*}P < 0.05$ vs. S1, $^{\#}P$ vs. NMRIS1 (U-test). O/C, open:closed entries ratio; O/T, open:total entries ratio; D, defecation boli; UR, urination episodes; CC, central platform crossing; CE, closed entries; OE, open entries; TE, total entries. Note different activity and anxiety levels in these strains. (B) Temporal distribution of activity of BCS1 and NMRIS1 hybrids tested in two different open field arenas (square, SOF and circular, COF; 5 min each) with a 4-week interval. Legend as in Fig. 1. Note a strikingly similar temporal organization of horizontal and vertical exploration in two different open field situations.

In general, the results of our study show that temporal organization of mouse OF exploration is stable and independent of the extrinsic properties of the novelty (such as size, shape and colour), unconfounded by alterations in non-exploratory behaviours (such as grooming) or different baseline anxiety and activity levels. Collectively, this suggests that the behavioural organization of animal OF exploration may be even more intrinsic and stable in nature than it was previously recognized (Golani et al., 1993; Eilam, 2003).

Importantly, such temporal stability was only observed for horizontal and vertical exploration (Figs. 1–4), and not for non-exploratory grooming (whose temporal distribution did not follow any consistent rule), suggesting that mice do not employ conservative patterning of their grooming activity in the OF novelty. Also interestingly, cumulative scores of vertical activity did not alter in our experiments (Figs. 1–4), suggesting that, unlike altering their horizontal activity, rodents do not scale their vertical activity to the size of the OF (see Eilam et al., 2003 for details). Furthermore, although our study was limited to mice with S1 genetic background or their F1 progeny, based on the results of Experiments 4 and 5, it is possible to assume that similar temporal stability of exploration may also be seen in other mouse strains. Clearly, further studies are needed to examine the phenomenon reported here in other strains widely used in behavioural neuroscience. In addition, given the similarity of exploration in mice and rats (Drai et al., 2001), it is of interest to assess temporal stability of exploration in rats and other rodent species. Finally, spatial and spatio-temporal aspects of exploration (Edut and Eilam, 2003; Eilam, 2003) are crucial for behavioural neuroscience, also meriting further in-dept investigation in different OF situations.

What can be potential applications of this study? First, we re-confirmed the OF test as a useful tool dissociating between "activity" (sensitive to OF novelty properties) and "exploration" (which appears to be stable) (Paulus et al., 1999; Kafkafi et al., 2001). This notion may not only minimize the risk of false positive and negative findings, but also find potential applications in psychopharmacology, behavioural genetics and neuroethol-

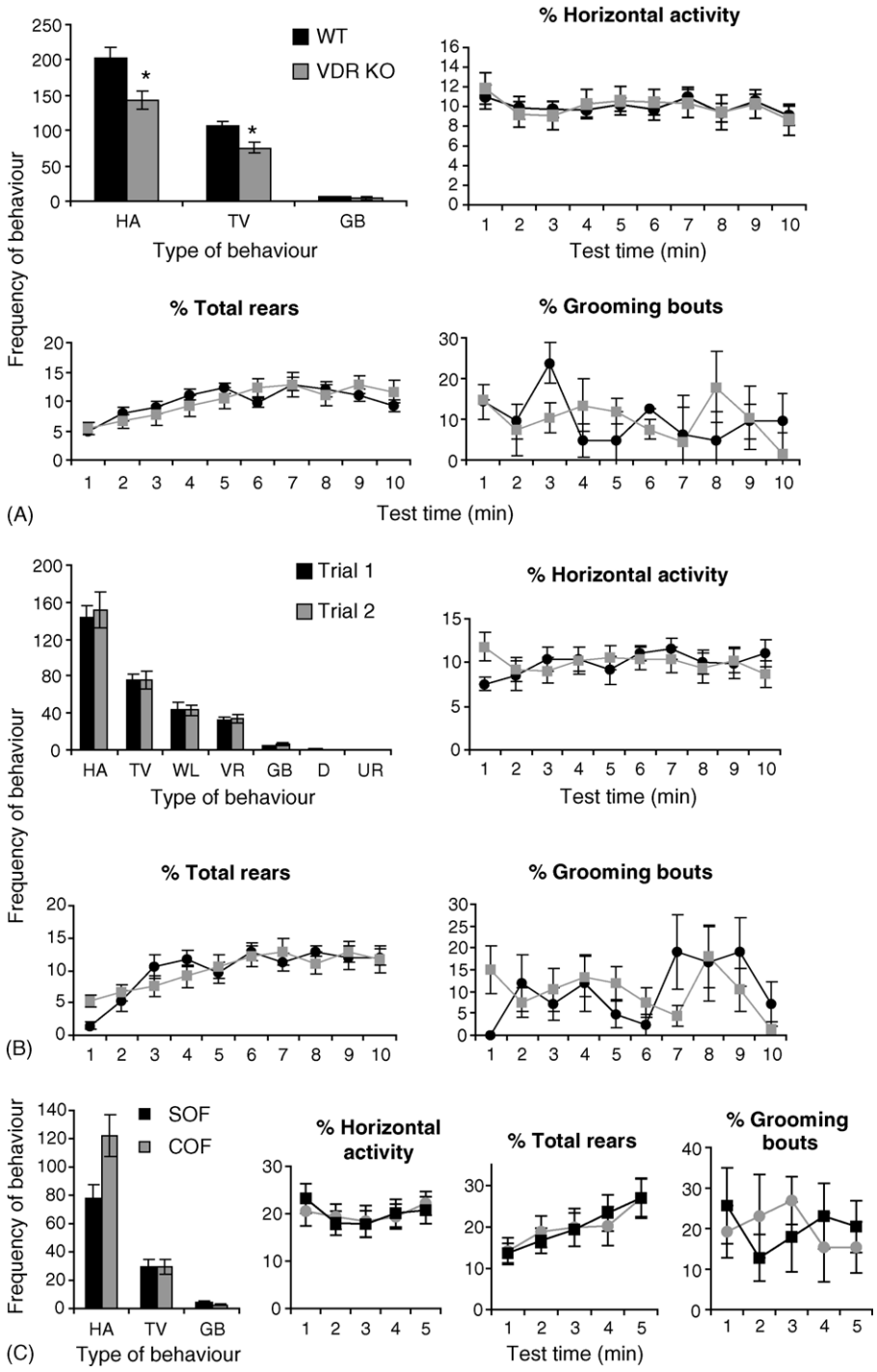

Fig. 4. Behavioural performance of Vitamin D receptor knockout mice (VDR KO) in the open field (OF) tests (Experiment 5). Data are presented as mean ± S.E.M. (A) Comparison of VDR KO female mice and their wild type controls (WT; $n = 10$ each) in the 10-min square OF (SOF). Legend as in Fig. 1. Note robust strain differences in activity levels (WT > KO), but a strikingly similar temporal distribution of their horizontal and vertical exploration in this test. $^{*}P < 0.05$ (U-test) vs. WT controls. (B) Behaviours of female VDR KO mice ($n = 10$) re-exposed to the SOF test 1-week later (10 min each trial). Legend as in Figs. 1 and 3. Note similar activity levels and essentially the same temporal distribution of mouse horizontal and vertical exploration in both trials. (C) Behaviours of female VDR KO mice ($n = 10$) tested for 5 min in the SOF (trial 1) and circular (COF, trial 2, 4-week later) OF tests.

ogy, where the drug-, mutation- or lesion-induced alterations in OF exploration patterning may reflect dramatic brain anomalies (Janus et al., 1995; Gross et al., 2000; Spreng et al., 2001; Kafkafi et al., 2003), thus using the OF exploratory stability as a "natural marker" of behavioural integrity.

Moreover, our results are relevant to the problem of replicability and reliability of results obtained in traditional behavioural tests, such as OF. This problem is presently central in the field of behavioural neuroscience, and widely debated in the literature (Crabbe et al., 1999; Crawley, 1999; Kafkafi et al.,

2003), with some authors advocating stringent standardization of experimental conditions (Crabbe et al., 1999; Wahlsten, 2001; Wahlsten et al., 2003a,b), and others insisting that standardization decreases the external validity of models (Wurbel, 2000, 2002). Our data, in line with findings of other groups (Golani et al., 1993; Eilam et al., 2003), show that OF exploration withstands marked changes in test properties and experimental design, thus, suggesting that standardization of the OF boxes per se may not be as crucial as it would seem. However, this notion allows the researchers to focus on more important standardization of other factors (affecting the mouse OF performance, e.g.: enrichment, handling, rearing conditions, circadian rhythms, early life events; Hall et al., 2000; Zimmermann et al., 2001; Valentinuzzi et al., 2000; Tang et al., 2003), thus outlining rational ways for further refinement and sophistication of the existing OF-based paradigms.

In conclusion, the main finding here is that temporal patterns of the OF exploration in mice are highly conservative and unaffected by the external (e.g. the novelty properties: size, colour, shape) or internal factors (e.g. genetically determined levels of activity or anxiety). This may contribute to our further understanding of the fundamental principles guiding the rodent novelty exploration.

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References

Augustsson, H., Meyerson, B.J., 2004. Exploration and risk assessment: a comparative study of male house mice (*Mus musculus musculus*) and two laboratory strains. Physiol. Behav. 81, 685–698.

Belzing, C., 1999. Measuring rodent exploratory behavior. In: Crisio, W.E., Gerlai, R.T. (Eds.), Handbook of Molecular-Genetic Techniques for Brain and Behavior Research. Elsevier Science, Amsterdam, pp. 738–749.

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

Brudzynski, S.M., Krol, S., 1997. Analysis of locomotor activity in the rat: parallelism index, a new measure of locomotor exploratory pattern. Physiol. Behav. 62, 635–642.

Burne, T.H., McGrath, J.J., Eyles, D.W., Mackay-Sim, A., 2005. Behavioural characterization of vitamin D receptor knockout mice. Behav. Brain Res. 157, 299–308.

Calatayud, F., Belzung, C., Aubert, A., 2004. Ethological validation and the assessment of anxiety-like behaviours: methodological comparison of classical analyses and structural approaches. Behav. Process. 67, 195–206.

Choleris, E., Thomas, A.W., Kavaliers, M., Prato, F.S., 2001. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequently pulsed magnetic field. Neurosci. Biobehav. Rev. 25, 235–260.

Crabbe, J.C., Wahlstein, D., Dudek, B.C., 1999. Genetics of mouse behavior: interaction with laboratory environment. Science 284, 1670–1672.

Crawley, J.N., 1999. Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res. 835, 18–26.

Drai, D., Benjamini, Y., Golani, I., 2000. Statistical discrimination of natural modes of motion in rat exploratory behavior. J. Neurosci. Methods 96, 119–131.

Drai, D., Kafkafi, N., Benjamini, Y., Elmer, G., Golani, I., 2001. Rats and mice share common ethologically relevant parameters of exploratory behavior. Behav. Brain Res. 125, 133–140.

Edut, S., Eilam, D., 2003. Rodents in open space adjust their behavioral response to the different risk levels during barn–owl attack. BMC Ecol. 3, 10.

Eilam, D., Golani, I., 1988. The ontogeny of exploratory behavior in the house rat (*Rattus rattus*): the mobility gradient. Dev. Psychobiol. 21, 679–710.

Eilam, D., Golani, I., 1989. Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. Behav. Brain Res. 34, 199–211.

Eilam, D., Golani, I., 1990. Home base behavior in amphetamine-treated tame wild rats (*Rattus norvegicus*). Behav. Brain Res. 36, 161–170.

Eilam, D., Dayan, T., Ben-Eliyahu, S., Schulman, I., Shefer, G., Hendrie, C.A., 1999. Differential behavioural and hormonal responses of voles and spiny mice to owl calls. Anim. Behav. 58, 1085–1093.

Eilam, D., 2003. Open-field behavior withstands drastic changes in arena size. Behav. Brain Res. 142, 53–62.

Eilam, D., Dank, M., Maurer, R., 2003. Voles scale locomotion to the size of the open field by adjusting the distance between stops: a possible link to path integration. Behav. Brain Res. 141, 73–81.

Flint, J., Corley, R., DeFries, J.C., Fulker, D.W., Gray, J.A., Miller, S., Colins, A.C., 1995. A simple genetic basis for a complex psychological trait in laboratory mice. Science 269, 1432–1435.

Flint, J., 2002. Genetic effects on an animal model of anxiety. FEBS Lett. 529, 131–134.

Golani, I., Benjamini, Y., Eilam, D., 1993. Stopping behavior: constraints on exploration in rats (*Rattus norvegicus*). Behav. Brain Res. 53, 21–33.

Gross, C., Santarelli, L., Brunner, D., Zhuang, X., Hen, R., 2000. Altered fear circuits in 5-HT(1A) receptor KO mice. Biol. Psychiatry 48, 1157–1163.

Hall, F.S., Huang, S., Fong, G.W., Sundstrom, J.M., Pert, A., 2000. Differential basis of strain and rearing effects on open-field behavior in Fawn Hooded and Wistar rats. Physiol. Behav. 71, 525–532.

Homanics, G.E., Quinlan, J.L., Firestone, L.L., 1999. Pharmacologic and behavioral responses of inbred C57BL/6J and strain 129/SvJ mouse lines. Pharmacol. Biochem. Behav. 63, 21–26.

Janus, C., Janus, M., Roder, J., 1995. Spatial exploration in transgenic mice expressing human beta-S100. Neurobiol. Learn. Mem. 64, 58–67.

Kalueff, A.V., Lou, Y.R., Laaksi, I., Tuohimaa, P., 2004. Increased anxiety in mice lacking vitamin D receptor gene. Neuroreport 15, 1271–1274.

Kalueff, A.V., Tuohimaa, P., 2005. Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). Behav. Brain Res. 160, 1–10.

Kalueff, A.V., Minasyan, A., Lou, Y.R., Laaksi, I., Tuohimaa, P., 2005. What can we learn from the VDR knockout mice about the link between vitamin D and anxiety? Psychopharm. Biol. Narcol. 2, 930–938.

Kalueff, A.V., 2005. Behavioural abnormalities in mice with partially deleted vitamin D receptor gene. Acta Univer. Tamperens. 1105, 1–105.

Kafkafi, N., Mayo, C., Drai, D., Golani, I., Elmer, G., 2001. Natural segmentation of the locomotor behavior of drug-induced rats in a photobeam cage. J. Neurosci. Methods 109, 111–121.

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

Kafkafi, N., Elmer, G.I., 2005. Activity density in the open field: a measure for differentiating the effects of psychostimulants. Pharmacol. Biochem. Behav. 80, 239–249.

Kafkafi, N., Benjamini, Y., Sakov, A., Elmer, G.I., Golani, I., 2005. Genotype-environment interactions in mouse behavior: a way out of the problem. Proc. Natl. Acad. Sci. U.S.A. 102, 4619–4624.

Lipkind, D., Sakov, A., Kafkafi, N., Elmer, G.I., Benjamini, Y., Golani, I., 2004. New replicable anxiety-related measures of wall vs. center behavior of mice in the open field. J. Appl. Physiol. 97, 347–359.

MPD, Mouse Phenome Database (http://aretha.jax.org), 2001. The Jackson Laboratory, Bar Arbor, Mainne, USA (May 2005).

Ohl, F., Toschi, N., Wigger, A., 2001. Dimensions of emotionality in a rat model of innate anxiety. Behav. Neurosci. 115, 429–436.

Paulus, M.P., Dulawa, S.C., Ralph, R.J., Geyer, M., 1999. Behavioural organization is independent of locomotor activity in 129 and C57 mouse strains. Brain Res. 835, 27–36.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463, 3–33.

Spreng, M., Cotecchia, S., Schenk, F., 2001. A behavioral study of alpha-1b adrenergic receptor knockout mice: increased reaction to novelty and selectively reduced learning capacities. Neurobiol. Learn. Mem. 75, 214–229.

Tang, A.C., Nakazawa, M., Reeb, B.C., 2003. Neonatal novelty exposure affects sex difference in open field disinhibition. Neuroreport 14, 1553–1556.

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

Tchernichovski, O., Golani, I., 1995. A phase plane representation of rat exploratory behavior. J. Neurosci. Methods 62, 21–27.

Tchernichovski, O., Benjamini, Y., Golani, I., 1998. The dynamics of long-term exploration in the rat. Part I. A phase-plane analysis of the relationship between location and velocity. Biol. Cybern. 78, 423–432.

Valentinuzzi, V.S., Buxton, O.M., Chang, A.M., Scarbrough, K., Ferrari, E.A., Takahashi, J.S., Turek, F.W., 2000. Locomotor response to an open field during C57BL/6J active and inactive phases: differences dependent on conditions of illumination. Physiol. Behav. 69, 269–275.

Wahlsten, D., 2001. Standardizing tests of mouse behavior: reasons, recommendations, and reality. Physiol. Behav. 73, 695–704.

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

Wahlsten, D., Metten, P., Phillips, T.J., Boehm, S.L., Burkhart-Kasch, S., Dorow, J., Doerksen, S., Downing, C., Fogarty, J., Rodd-Henricks, K., Hen, R., McKinnon, C.S., Merrill, C.M., Nolte, C., Schalomon, M., Schlumbohm, J.P., Sibert, J.R., Wenger, C.D., Dudek, B.C., Crabbe, J.C. 2003b. Different data from different labs: lessons from studies of gene-environment interaction. J Neurobiol. 54, 283–311.

Wurbel, H., 2000. Behaviour and the standardization fallacy. Nat. Genet. 26, 263.

Wurbel, H., 2002. Behavioral phenotyping enhanced—beyond (environmental) standardization. Genes Brain Behav. 1, 3–8.

Yoshizawa, T., Handa, Y., Uematsu, Y., Takeda, S., Sekine, K., Yoshihara, Y., Kawakami, T., Arioka, K., Sato, H., Uchiyama, Y., Masushige, S., Fukamizu, A., Matsumoto, T., Kato, S., 1997. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat. Genet. 16, 391–396.

Zimmermann, A., Stauffacher, M., Langhans, W., Wurbel, H., 2001. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behav. Brain Res. 121, 11–20.