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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