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Behavioural characterization of C57BL/6N and BALB/c female mice in social home cage – Effect of mixed housing in complex environment

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ABSTRACT

Developing reliable mouse models for social behaviour is challenging. Different tests have been proposed, but most of them consist of rather artificial confrontations of unfamiliar mice in novel arenas or are relying on social stress induced by aggressive conspecifics. Natural social interaction in home cage in laboratory has not been investigated well. IntelliCage is a fully automated home-cage system, where activity of the group-housed mice can be monitored along with various cognitive tasks. Here we report the behavioural profile of C57BL/6N (B6) and BALB/c (BALB) female mice in IntelliCage when separated by strain, followed by monitoring of activity and formation of 'home-base' after mixing two strains. For that purpose, 3 cages were connected. Significant differences between the strains were established in baseline behaviour in conventional tests and in IntelliCage. The B6 mice showed reduced anxiety-like behaviour in open field and light-dark box, slightly enhanced exploratory activity in IntelliCage during initial adaptation and clearly distinct circadian activity. Mixing of two strains resulted in reduction of body weight and anhedonia in B6 mice. In addition, the B6 mice showed clear preference to previous home-cage, and formed a new home-base faster than BALB mice. In contrast, BALB mice showed enhanced activity and moving between the cages without showing any preference to previous home-cage. It could be argued that social challenge caused changes in both strains and different coping styles are responsible for behavioural manifestations. Altogether, this approach could be useful in modelling and validating mouse models for disorders with disturbed social behaviour.

1. Introduction

Home-cage can serve as a comfortable quarter for animals where spontaneous, undisturbed behaviour is monitored and recorded. However, it can contain additional features allowing some specific tests to be conducted without removal of the animals. Novel, automated approaches are needed for behavioural phenotyping of increasing number of mutant mouse models and for enhancing the translational value of biomedical research [1–3]. It has been argued that testing in home-cage will add potential benefits to translational research and it is also compatible with 3R principle of animal experiments [4,5]. Despite the increasing number of studies applying the home-cage technology there is a clear need for advancing the field regarding the basic knowledge of mouse behaviour, but also for development and validation of novel methods based on ethological perspective [6].

Most of the currently available home-cage systems for behavioural monitoring require single housing. However, social separation is known to affect the behaviour of mice in various aspects [7,8]. IntelliCage is a special platform as compared to many other systems designed for home-cage testing. Namely, it allows social housing along with implementation of wide range of behavioural and cognitive tasks [9,10]. Testing of mice in social home cage offers several advantages as compared to conventional testing of individual animals. Most importantly, handling by experimenter is reduced to minimum. The effects of the experimenter on mouse behaviour have been well documented [11–13]. Moreover, handling and placement of the animal in novel arenas and mazes causes acute stress and changes in behavioural and physiological parameters [14]. Therefore, monitoring the mice in home-cage environment provides ethologically valid profile of behaviour with high between-laboratory consistency [15].

The role of social factors, especially social stress, in modulating behaviour is well known [16,17]. Most of the methods applied concern

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social isolation or separation, social instability or social defeat, situations specifically designed for stressful social experience. However, group-housing of laboratory rodents is a mandatory requirement by legislation, whereas little is known about the effects of mixing the animals with different genotypes and phenotypes. The latter is standard for maintenance of mutant strains where knockout and wild-type littermates are kept together. It has been shown that housing of transgenic mice with impaired memory function together with wild type animals can improve their performance [18]. Moreover, social deficits in BTBR mouse strain are alleviated by rearing together with C57BL/6 mice [19]. On the other hand, co-housing of C57BL/6 mice with DBA/2 strain can be stressful and anxiogenic for C57BL/6 mice [20]. Therefore, mixing of strains with different or even opposite phenotypes can open novel ways for modelling social environment and its effects on behaviour and physiology. Importantly, such information can be valuable for characterizing the mouse models of disorders where social behaviour is affected (e.g. mood disorders, schizophrenia, autism).

C57BL/6 and Balb/c mice are well characterized inbred strains and widely used in biomedical research. These strains exhibit differences in anxiety-like behaviour, motor performance, learning and memory, sociability [21–25]. In general, BALB/c mice are suggested to be more anxious and less social as compared to C57BL/6. Also, BALB/c mice are more vulnerable to social defeat stress [26,27]. In the present study we aimed at measuring the behavioural outcome of mixing C57BL/6 and BALB/c female mice in automated home-cage, IntelliCage. Only female mice were used in order to avoid aggression and fighting that may occur in large group of unfamiliar male mice.

2. Material and methods

The animal experiments were performed according to the EU legislation harmonized with Finnish legislation and have been approved by the National Animal Experiment board of Finland (License: ESAVI/7548/04.10.07/2013).

Thirty female mice (15 C57BL/6NHsd and 15 BALB/cOlaHsd, abbreviated as B6 and BALB in the following sections) were purchased from the commercial breeder (Harlan, The Netherlands) and arrived in the laboratory at the age of 8 weeks. At arrival the mice were allocated to the individually ventilated cage (IVC) system (Tecniplast, Italy) in groups of five animals of the same strain per cage. Ambient room temperature was 22 ± 2 °C and relative humidity at 50 ± 15%. The bedding (aspen chips 5 × 5 × 1 mm, Tapvei Oy, Finland) was changed weekly. Nesting material (aspen strips, PM9 0 L/R, 3 mm × 20 cm, Tapvei Oy, Finland) and wooden block (100 × 20 × 20 mm, Tapvei Oy, Finland) were provided as environmental enrichment. Food and water was available ad libitum. The lights were on between 6:00 and 18:00. One week after arrival the RFID transponders (Planet ID GmbH, Essen, Germany) were injected subcutaneously in the dorso-cervical region under isoflurane inhalation anaesthesia. One week after implantation of the transponders behavioural testing began (schedule shown in Fig. 1A).

2.1. Open field

The mice were released in the corner of novel open field arena (30 × 30 cm, Med Associates) with white floor and transparent walls (light intensity ~150 lx). Horizontal and vertical activity was recorded for 30 min. Peripheral zone was defined as a 6 cm wide corridor along the wall, corner zones were defined as 6 cm squares.

2.2. Light-dark box

The test was carried out in the open field arena (30 × 30 cm, Med Associates, St. Albans, VT) equipped with infrared light sensors detecting horizontal and vertical activity. The dark insert (non-transparent for visible light) was used to divide the arena into two halves, an opening (a door with a width of 5.5 cm and height of 7 cm) in the wall of the insert allowed animal's free movement from one compartment to another. Illumination in the centre of the light compartment was ~550 lx. Animal was placed in the light compartment and allowed to explore the arena for 10 min. Distance travelled, number of rearings, and time spent in different compartments were recorded by the program. The number of faecal boli was counted by experimenter after the end of trial. Testing in light-dark box was repeated 15 days later (after first day of mixed housing, see below).

2.3. Tube test of social dominance

Tube test is commonly used to measure social dominance in mice. Two unfamiliar mice of the same sex but different genotypes were placed in the opposite ends of a 30 × 3.8 cm (inner diameter) transparent plastic tube and released simultaneously. The match ended when one mouse completely retreated from the tube. The mouse remaining in tube was designated as the winner, and the retreated mouse was the loser, respectively. Each animal was tested against six unfamiliar animals from the opposed group. The percent of retreated matches as well as aggressive postures were scored for each animal. Matches lasting > 2 min or in which animals crossed over each other were not scored.

2.4. IntelliCage

The IntelliCage apparatus (TSE Systems, Bad Homburg, Germany) is placed in a polycarbonate cage (20.5 cm high, 58 × 40 cm top, 55 × 37.5 cm bottom, Tecniplast, 2000P, Buguggiate, Italy) and accommodates up to 16 mice. Its aluminium top contains a freely accessible food rack filled with standard mouse chow (Teklad 2016, Harlan). The floor is covered with bedding (aspen chips 5x5x1 mm, Tapvei Oy, Finland) and provides 4 central red shelters (Tecniplast, Buguggiate, Italy). Four triangular conditioning chambers (15 × 15 × 21 cm) are fitted in the cage corners and provide room for one mouse at a time. Each chamber contains two drinking bottles, accessible via round openings (13 mm diameter) on the side walls and which can be closed by motorized doors. Three multicolour LEDs are mounted above each door and the chamber ceiling contains a motorized valve for delivery of air puffs. Mice entering a chamber are identified by a circular RIFD antenna at its entrance (30 mm inner diameter) and the duration of their visit is determined by both the antenna reading and a temperature sensor that detects the presence of the animal inside the corner. During a visit, number and duration of individual nosepokes at each door are recorded using IR-beam sensors. Licking episodes at each bottle are monitored using lickometers (duration of the episode, number of licks, total contact time). IntelliCages have individual controllers and are connected to a central PC running the software that permits to design and run experiments, as well as to analyse the recorded data (IntelliCage Plus, NewBehavior AG). The following experimental designs were applied in the IntelliCage (shown also on Fig. 1A). Switching of the protocols occurred around 10:00 in the morning, and initial period until beginning of the dark phase (at 18:00) was defined as a Day-0 for respective protocol (subsequent full days were counted as 24 h periods, 12 h dark + 12 h light).

- Novelty induced exploration and habituation (Free Adaptation – FA, 6 days): Mice were released in two separate IntelliCages (15 B6 in one, and 15 Balb/c in another); all corners in the IntelliCage had doors open for unrestricted access to water. Exploratory activity – visits to corners, nosepokes, lick number, circadian activity.
- Extended adaptation (EA, 5 days): The mice were removed from the cages for measuring the body weight, and then they were returned to the cleaned cages. The doors in the corners were closed, both doors opened for 7 s after start of the visit to given corner. For further drinking the animals had to re-enter any corner. The corners