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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

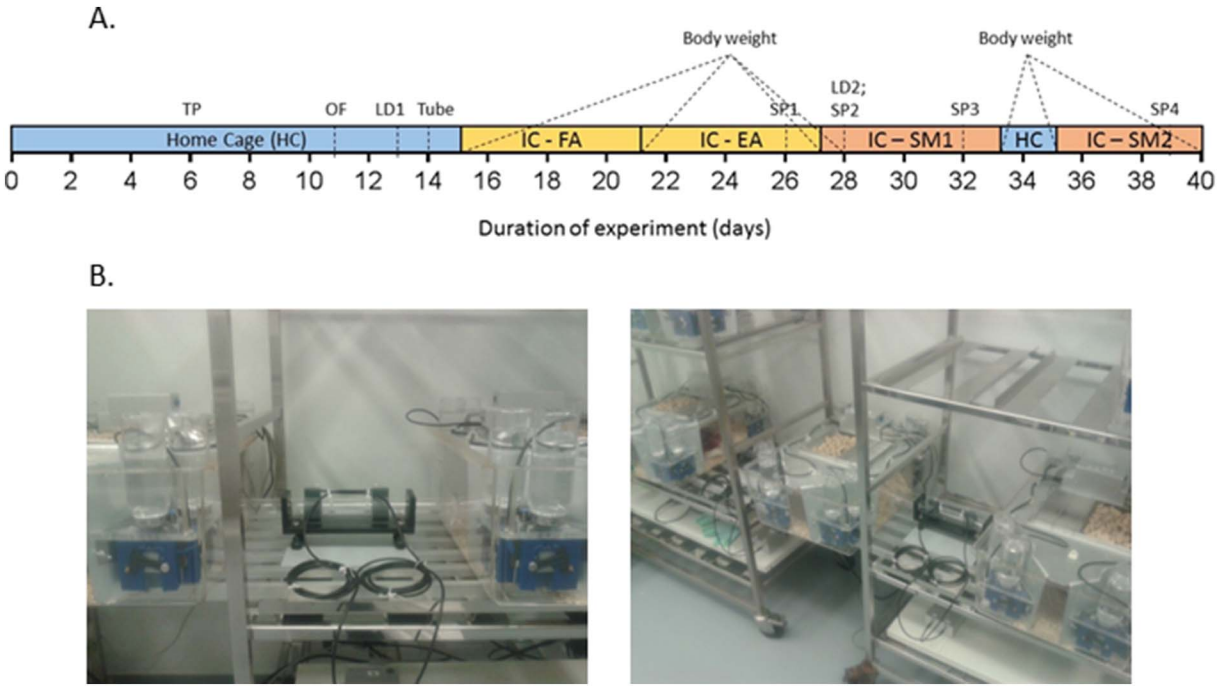

Fig. 1. A. Workflow of the experiment: total duration, procedures on specific days (TP – transpondering, OF – open field, LD – light-dark test, SP – saccharin preference), timing and duration of IntelliCage (IC) sessions – free adaptation (FA), extended adaptation (EA), social mixing (SM). B. Setup of three IntelliCages connected by tubes into one system.

and doors operated in a similar manner in all subsequent phases of the experiment.

- Saccharin preference (SP, 1 day) – each corner contained one bottle with plain water and one bottle with 0.5% saccharin (sides counterbalanced).
- Social competition and interaction (SM1, 6 days): The mice were removed from the cages. Three IntelliCages were connected by transparent tubes (diameter 3.8 cm, length 46 cm, equipped by RFID-antennas, see also in [28]) to each other and all mice were released in the central (clean, neutral) cage (setup shown on Fig. 1B), from where they had access through tunnels to the neighbouring cages (previous home cages, not cleaned). The entries through the tunnels into different cages were recorded in addition to activity (visits, nosepokes, licks) in three cages. On the second day, the mice were removed from the IntelliCages for second Light-Dark test. Thereafter, they were returned to the IntelliCages and saccharin preference was measured again (all corners in three cages contained bottles with water and saccharin).
- After 6 days the mice were removed from the IntelliCages and separated by strain in standard cages for 2 days. Thereafter, the mice were returned to the IntelliCage system with three cages interconnected by tubes. However, all cages and corners were thoroughly cleaned before start of this phase (SM2). The mice were released in the central cage and their activity was recorded as during previous phase (visits to the cages, visits, nosepokes and licks in the corners). Monitoring lasted for 5 days and during last day, the preference to saccharin was measured again.

2.5. Statistics

Analysis of variance (ANOVA) model with strain (B6 and BALB) as between-subjects factor, repeated measures ANOVA for analysis of the effect of time in open field, effect of repeated testing in light-dark box and effect of time in IntelliCage (initial exploration, circadian activity). Newman-Keuls post-hoc analysis was used after significant ANOVA results. Significance was set at $p < 0.05$. Programs STATISTICA v. 12 (StatSoft, Inc.) and Prism 7 for Windows (GraphPad Software, Inc.) were used to analyse and present the data.

3. Results

3.1. Open field

The B6 mice displayed increased locomotor activity, especially during first 5 min (Fig. 2A, effect of strain $F(1,28) = 7.5$, $p = 0.01$; time $F(5,140) = 33.0$, $p < 0.0001$; interaction $F(5,140) = 5.3$, $p < 0.001$). In addition, proportion of distance in centre (Fig. 2B, effect of strain $F(1,28) = 58.6$, $p < 0.0001$) and time in centre (Fig. 2C, effect of strain $F(1,28) = 40.0$, $p < 0.0001$) was significantly increased in B6 mice, whereas BALB mice spent more time in the corners. Number of rearings was reduced in BALB mice, especially during the first 10 min of the test (Fig. 2D, interaction of strain and time $F(5,140) = 3.0$, $p = 0.01$). Moreover, significantly less rearings were shown by BALB mice in the centre of open field (Fig. 2D, effect of strain $F(1,28) = 23.0$, $p < 0.0001$).

3.2. Light-dark box

The test was performed twice, before introducing the animals to the IntelliCage and after first day of social mixing in the IntelliCage (interval between two tests was 2 weeks). Therefore, repeated measures ANOVA was used for analysing the data. Time spent in the light compartment was not different between the strains and increased on the second exposure (Fig. 2E, effect of repetition $F(1,28) = 12.5$, $p < 0.01$). However, activity (total distance moved in 10 min) was higher in B6 mice (Fig. 2F, effect of strain $F(1,28) = 29.2$, $p < 0.0001$) on both days and did not change in either group on the second exposure. In contrast, proportion (%) of distance in light compartment (Fig. 2G) increased was in the second test (effect of repetition $F(1,28) = 68.7$, $p < 0.0001$) and when it was significantly lower for BALB mice in the first day, the difference disappeared in the second test (interaction of strain and repetition $F(1,28) = 7.7$, $p < 0.01$). Moreover, the B6 mice defecated significantly less (Fig. 2H, effect of strain $F(1,28) = 69.0$, $p < 0.0001$), but the number of defecations was reduced in BALB mice on the second exposure as compared to the first test (interaction of strain and repetition $F(1,28) = 10.9$, $p < 0.01$). The number of rearings was higher in B6 mice (Fig. 2I, effect of strain F

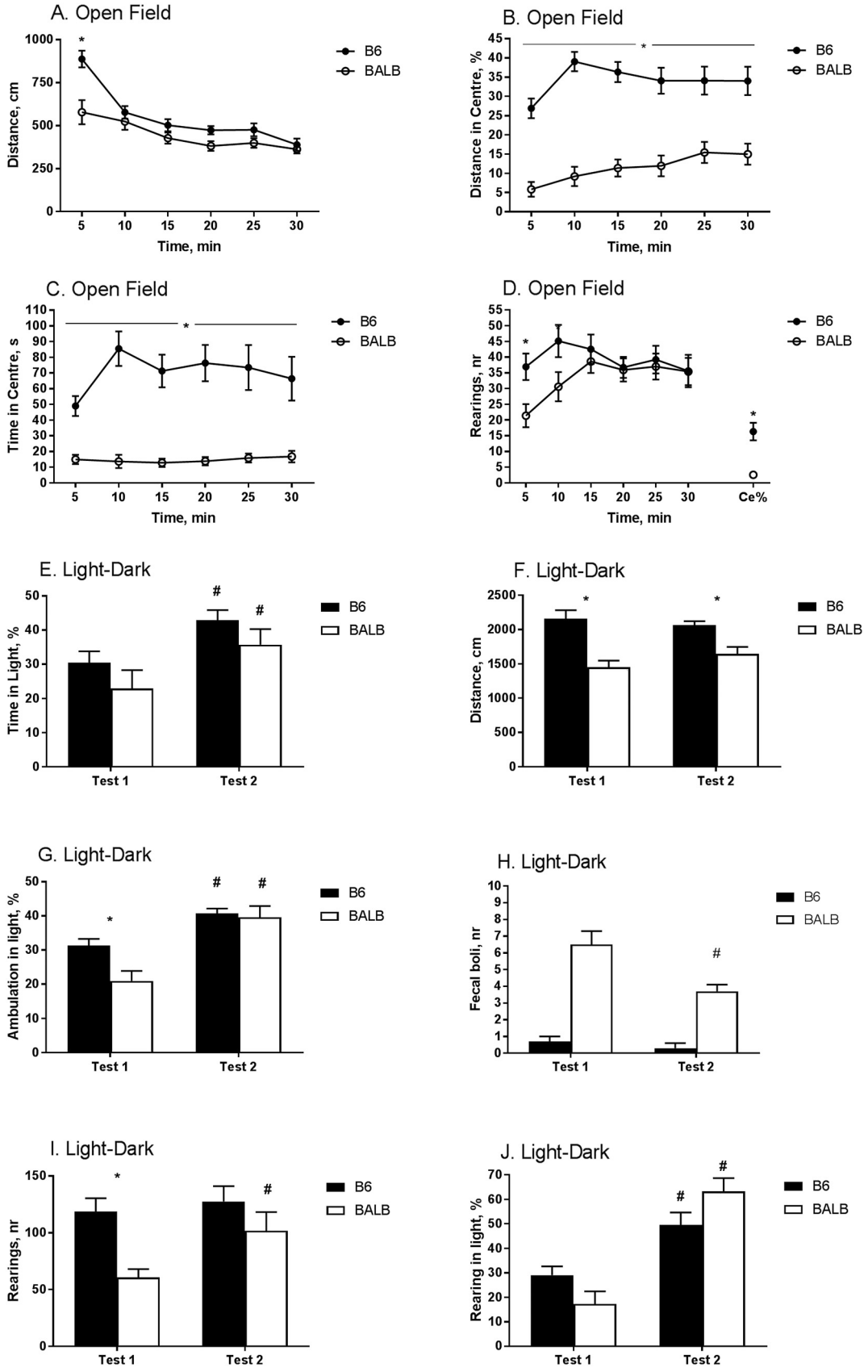

Fig. 2. Exploratory and anxiety-like behaviour in B6 and BALB mice assessed by open field and light-dark box. A. Distance travelled in the open field during 30 min session. B. Percent of distance in the centre of open field arena. C. time spent in the centre of the open field. D. Number of rearings during 30 min session and percentage of rearings in the centre of the arena. E. The percentage of time spent in the light compartment during 10 min test. F. Distance travelled in the light-dark box. G. Percentage of distance travelled in the light compartment. H. Number of faecal boli left in the light-dark box. I. Number of rearings in the light-dark box. J. Percentage of rearings in the light compartment. *$p < 0.05$ between B6 and Balb/c, #$p < 0.05$ between test 1 and test 2 (LD).

(1,28) = 7.6, p = 0.01) and although the interaction between strain and exposure was not significant, the post hoc analysis indicated that difference between groups was highly significant in first test, but not on the second exposure, and that rearings in BALB were significantly increased in the second test. Moreover, proportion of rearings made in light compartment was increased in second test and especially in BALB mice (Fig. 2J, effect of repetition F(1,28) = 48.9, p < 0.0001, interaction between strain and repetition F(1,28) = 7.2, p = 0.012). Overall, these results suggest that anxiety-like behaviour was substantially reduced in BALB mice after environmental and social enrichment in the IntelliCage.

3.3. Tube test

BALB mice displayed clearly more dominant behaviour when faced to unfamiliar B6 mice in tube and won > 90% of trials (data not shown). It has to be noted that the body weight of BALB mice was significantly higher and could have an effect in this test (Fig. 6B). However, our aim was to test animals at the same age and therefore, matching by body weight was not feasible.

3.4. IntelliCage

Free adaptation, strains separated. Initial exploration (number of corner visits) during the first 8 h in novel environment was not different between the groups with BALB mice showing faster adaptation and decrease in activity (Fig. 3A, interaction between strain and time F(7,196) = 4.0, p < 0.001). Profile of the circadian activity (number of corner visits) differed significantly between the strains (Fig. 3B). BALB mice were more active in the beginning of the dark phase, whereas B6 mice showed two peaks of activity – in the beginning and end of the dark phase (effect of strain not significant, effect of time F(23,644) = 56.1, p < 0.0001, interaction of strain and time F(23,644) = 19.2, p < 0.0001). The B6 mice were more active in the end of the dark phase and during the first 4–5 h of the light phase, whereas the BALB mice displayed more corner visits (and were more active than B6) in the beginning of the dark phase (Fig. 3B). Drinking (Fig. 3C, number of licks) was increased in B6 mice (effect of strain F(1,28) = 16.8, p < 0.001, effect of time F(23,644) = 23.8, p < 0.0001, interaction of strain and time F(23,644) = 8.3, p < 0.0001).

Extended adaptation, strains separated. The mice were removed from the Intellicage for changing the bedding, shelters and water bottles (~1 h). After returning to the IntelliCages, the mice showed enhanced activity during 1st hour and rapid decline of exploration. Interestingly, in contrast to start of the previous phase, BALB mice were more active in visiting the corners in cleaned, but otherwise already familiar environment (Fig. 2D, interaction between strain and time F(5,140) = 5.2, p < 0.001). Difference in circadian activity was still remarkable in the end of the dark phase and in the beginning of light phase when B6 mice showed significantly more corner visits (Fig. 3E). In contrast, difference between the strains in the beginning of dark phase had disappeared (Fig. 3E). Lick number was still higher in B6 mice (Fig. 3F, effect of strain F(1,28) = 10.5, p < 0.01).

3.5. Social mixing

The BALB mice showed significantly increased number of corner visits (F(1,28) = 18.8, p < 0.001) and tube visits (F(1,28) = 142.6, p < 0.0001) during initial 7 h after connecting of 3 cages (Fig. 4A-B). Thereafter, the total number of corner visits did not differ between the strains (Fig. 4C), and the pattern in circadian activity for corner visits remained similar to what was established during previous phases (Fig. 4C). In contrast, BALB mice displayed substantially higher activity in visiting the connecting tubes (F(1,28) = 95.0, p < 0.001), specifically during dark phase (Fig. 4D). Regarding preference to the IntelliCages, starting from the second day the B6 mice showed clear preference to corners in their previous home-cage, and > 90% of drinking took place there. In contrast, visits and drinking of BALB mice were rather equally distributed over all 3 cages, without clear preference (Fig. 4E–F).

After 6 days the mice were removed from the IntelliCage environment, separated by strain and kept for two days in standard cages. Thereafter, they were returned to thoroughly cleaned IntelliCages and monitored for another 5 days. The B6 mice showed significantly reduced activity in visiting the corners (F(1,28) = 6.8, p < 0.05) and connecting tubes (F(1,28) = 67.8, p < 0.001) during the first hours of the experiment (Fig. 5A–B). The activity patterns were essentially similar to the previous phase (Fig. 5C–D) with B6 mice visiting corners more in the early hours of light phase and BALB in the beginning of dark phase, and BALB being significantly more active throughout 24 h in the tubes (F(1,28) = 40.8, p < 0.001). During first 2 days neither strain showed clear preference to any of the IntelliCages (as measured by the proportion of corner visits and licks in 3 cages). Thereafter, B6 mice developed gradually strong preference to one cage, and BALB mice followed there, although at lower level of preference (Fig. 5E–F).

Testing for anhedonia at different stages of the experiment showed that initially both groups had high saccharin preference (B6 more than BALB, but not significantly). However, the preference for saccharin was significantly reduced in B6 mice after mixing of two strains, whereas no change was evident in BALB mice (Fig. 6A, effect of repeated measurement F(3,84) = 5.7, p < 0.01; interaction between strain and repetitions F(3,84) = 4.4, p < 0.01). The body weight of the B6 mice was lower throughout the experiment, but it is noteworthy that there was a significant drop immediately after mixing the two strains (Fig. 6B).

4. Discussion

The C57BL/6 and BALB/c mice have been shown to exhibit several differences in behaviour (including anxiety-related and social behaviour) and these differences were confirmed by the current study. However, we extended our analysis by observing the effect of mixing and confronting two strains of mice in the extended environment of IntelliCages where the animals had previously habituated. The predominant consequence was the aversive influence of BALB mice on the behavioural repertoire of B6 strain. The latter showed acute aggravation of anxiety and depression-like behaviours such as anhedonia, decrease of body weight and supressed activity during the social mixing.

Earlier studies have reported that C57BL/6 mice show less anxiety, they are more resilient to stress and emotionally more stable than BALB/c mice [29–32]. At first this seems rather contradictory to the results presented in the current study. What could be the main difference in the experimental setup that leads to such opposite results? In aforementioned studies the experiments have been performed on animals in standard conditions. Duration of the experiments has been short and social interaction is often possible only through perforated walls. The main aim of these studies is only to document the interest and exploration of test animals towards unfamiliar stimulus mouse, either spontaneous or after social defeat procedures. At the end of the experimental manipulation all mice were returned to their home cage to stay with their mates of the same genotype. In our experimental design animals from different genotypes were housed together in a complex environment which gave all of the animals from different genotypes access to the same visual, tactile and very importantly olfactory stimuli for over 6 days at a time aiming at observing changes in behavioural patterns.

Olfactory cues are perhaps the most important sensory information for the mice, particularly for social communication. Olfactory investigation is the first part in the process of social interaction between the animals. The relevant odours emit from the urine, faeces, scent glands and saliva [33]. However, it has been suggested that extreme

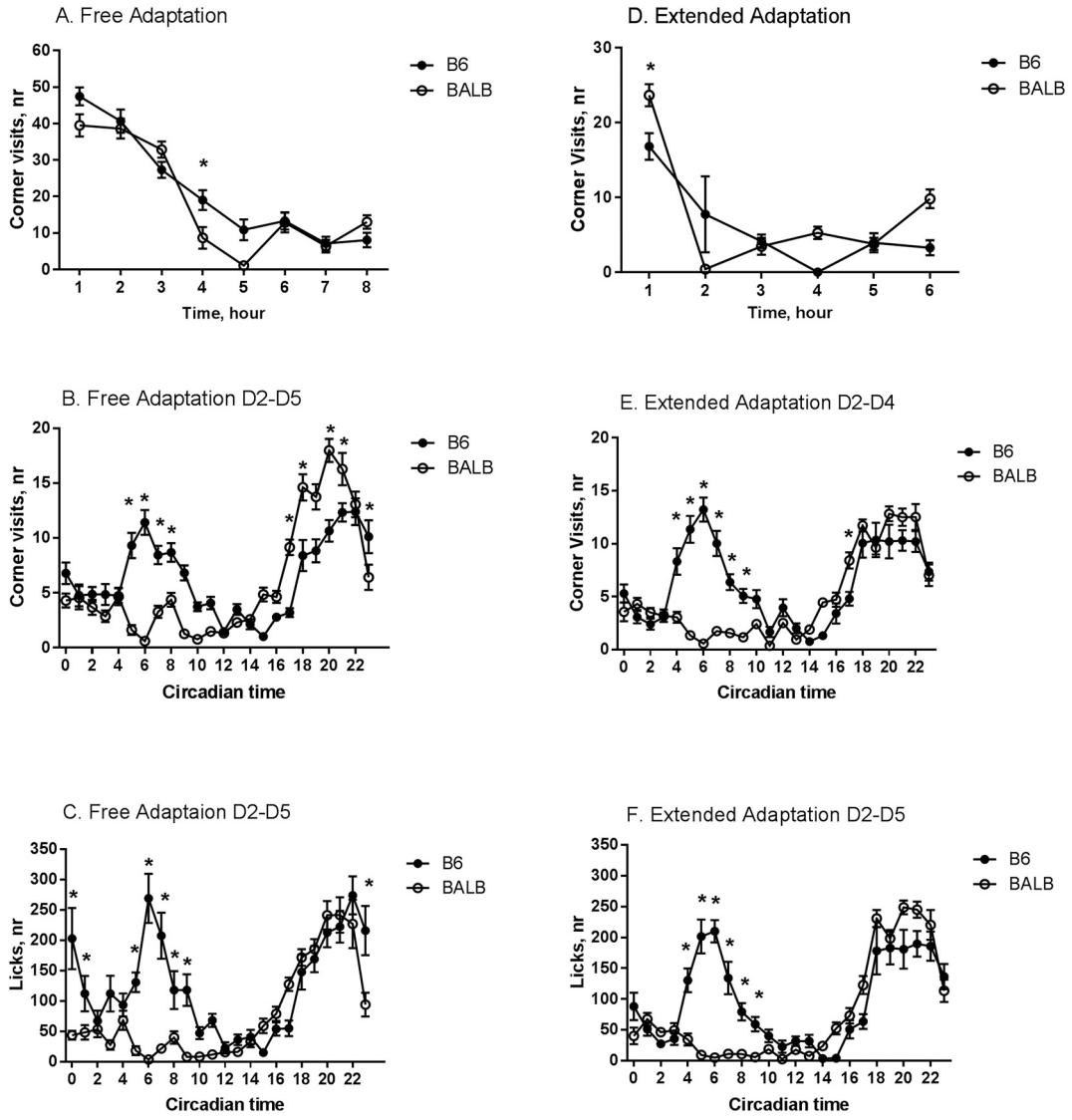

Fig. 3. Activity of B6 and BALB mice in the IntelliCage during adaptation sessions when strains were separated. A. Number of corner visits during initial 8 h after introducing the mice in the IntelliCage. B. Average number of corner visits per hour during circadian cycle (data averaged from days 2–5 of free adaptation). C. Average number of licks per hour during circadian cycle (data averaged from days 2–5 of free adaptation). D. Number of corner visits during initial 6 h after returning the mice to cleaned IntelliCages for extended adaptation. E. Average number of corner visits per hour during circadian cycle (data averaged from days 2–4 of extended adaptation). F. Average number of licks per hour during circadian cycle (data averaged from days 2–5 of extended adaptation). *$p < 0.05$ between the strains (Newman-Keuls post hoc test).

inbreeding is likely to severely compromise an animal's ability to discriminate between individuals that are genetically very similar or identical [34,35]. Therefore, social interaction followed by changes in the behaviour and physiology can be most effectively achieved by confronting the conspecifics from different genetic backgrounds.

In our experiment, anxious BALB mice excrete the anxiogenic signals into their shared environment. For the BALB mice, whose baseline anxiety is already high and who are used to being surrounded by these signals, there is no significant effect in their behavioural response. On the other hand, B6 mice, which are accustomed to much calmer environment, sense these signals and their behaviour changes thereafter drastically. Combined with the stress of being housed in the common area with BALB mice that dominated over them in the tube test earlier, this new situation causes B6 mice to express anxious and depressive-like symptoms.

In this study we have shown that in complex (and more ethologically valid) situations well studied inbred mice like C57BL/6 can behave in a rather unexpected way. As social enrichment in rodents is becoming increasingly popular method while studying autism spectrum disorders, it is important to notice the complex nature of social encounters and the possible consequences. The standard tests of social interaction (such as 3-chamber test) have suggested that BALB/c mice could be used as a strain modelling some aspects of autism spectrum disorders (reduced sociability) [21,36,37]. However, recent findings have challenged this view by showing that C57BL/6 mice are able to respond to and process social cues in a vicinity, not requiring physical contact with the stimulus, while BALB/c mice predominantly process social cues by direct contact with the source without any evidence of defects in sociability [38]. Moreover, social activities can be classified as active (sniffing, investigating other animals) and passive (e.g. huddling) behaviour, and it has been shown that development of these behaviours is different in BALB/c and C57BL/6 mice between 30 and 70 days of age [39]. It is important to emphasize that the passive social behaviours cannot be expressed in situations where the (unfamiliar) stimulus mice are placed in the perforated cylinders. Another method for testing social interaction of mice was recently proposed [40]. There, the mice could voluntarily enter a tube in an aversive open field and co-occupancy time was measured and no difference between the C57BL/6 and BALB/c mice was detected. Interestingly, inbred strains did not differ in preference for siblings or strangers whereas outbred mice were

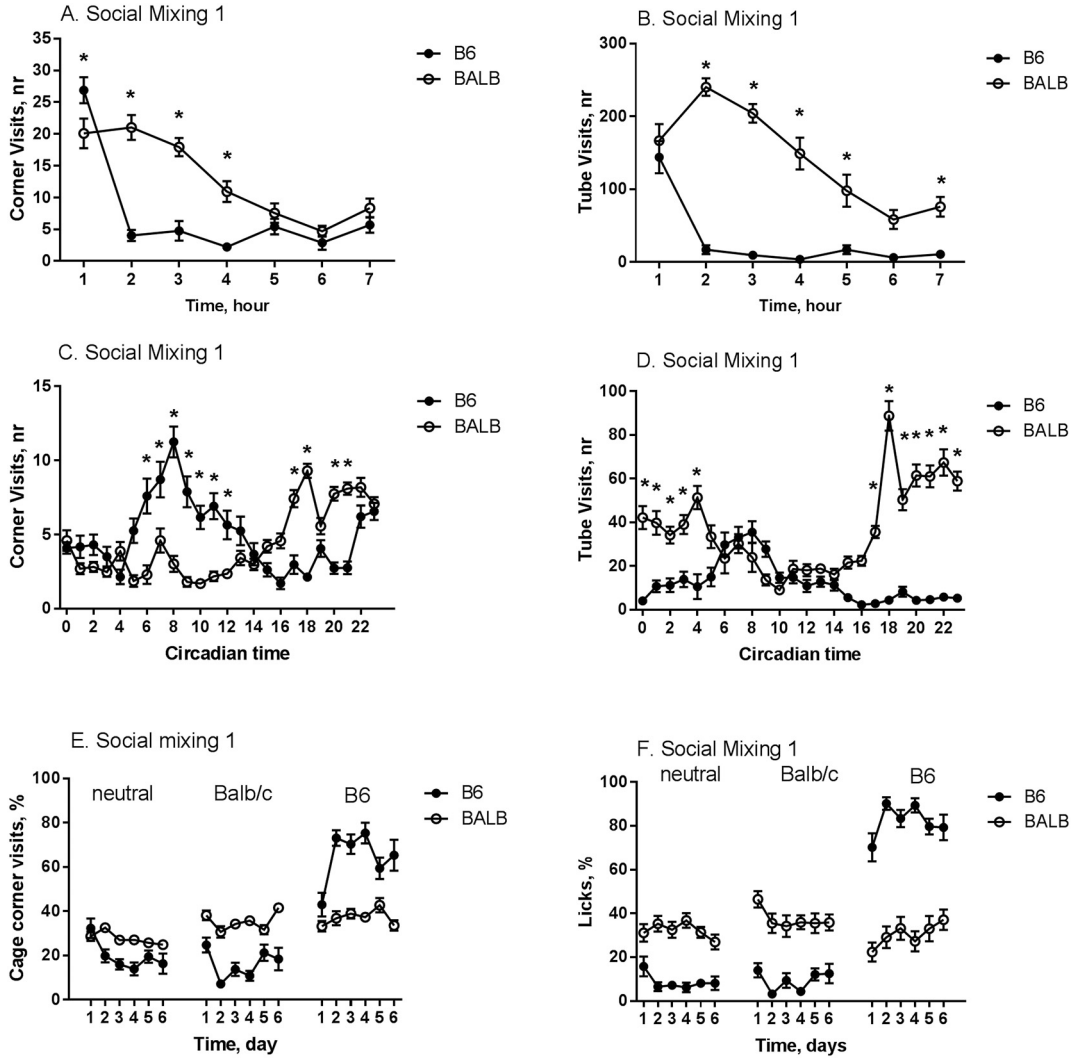

Fig. 4. Activity of mice in and between IntelliCages during first social mixing with neutral cage connected to previous home cages. A. Number of corner visits per hour during initial 7 h. B. Number of tube visits per hour during initial 7 h. C. Average number of corner visits per hour during circadian cycle (data averaged from days 2–5). D. Average number of tube visits per hour during circadian cycle (data averaged from days 2–5). E. Total number of corner visits over 5 days. F. Total number of tube visits over 5 days. G. Percentage of corner visits in three different IntelliCages (neutral middle cage connected to previous home cages for BALB and B6 mice, respectively). H. Percentage of licks in three different IntelliCages (neutral middle cage connected to previous home cages for BALB and B6 mice, respectively). *$p < 0.05$ between the strains (Newman-Keuls post hoc test).

clearly less likely to co-occupy the tube with strangers [40].

One confound related to the standard tests is brief duration of observation and recording (10–15 min in most of the protocols) combined with stress induced by handling and unfamiliar novel arenas. For instance, exploratory behaviour of BALB/c mice can change remarkably over extended period of testing rendering interpretation of anxiety-like behaviour in standard terms questionable [41,42]. Therefore, more reliable characterization of behavioural profile could be yielded by prolonged monitoring of unrestricted activity and importantly, involving also active (dark) period of the circadian cycle.

IntelliCage as a tool for behavioural profiling of mouse strains and disease models has been available for > 10 years. The major benefits and advantage of the system is elimination of human interference by handling during experiments and possibility to carry out complex cognitive tasks in group-housed animals. However, little is known about how such extensive social interaction affects and interacts with the performance of animals. Depending on the genotype and phenotype of interacting animals, the effects can be either positive (e.g. improvement in learning [18]) or negative (stress, anxiety-like behaviour [20]). Nevertheless, with special experimental designs it is also possible to investigate some specific aspects of social behaviour. For instance, social dominance can be measured with protocols where thirsty animals are competing for access to the water [43,44], social transmission of fear and avoidance can be studied by designating “demonstrator”- and “observer”-mice [45]. There have been also attempts to model endophenotypes related to autistic-like behaviour of mice in the IntelliCage, based on social status and interest towards conspecifics [46–48]. The major limitation of the IntelliCage is that activity of the animals is recorded only when they visit the corners, motivated either by curiosity or thirst, while exact nature and duration of social contacts between the animals remains unknown in the present setup. However, corner visits in the IntelliCage and locomotor activity measured continuously in single housed animals correlate well and reveal similar rankings [10,49]. Moreover, combination of different readouts (activity and preference patterns in corners and between cages, anhedonia as measured by saccharin preference, changes in body weight) allows to draw some conclusions about the effects of confronting animals with different behavioural phenotypes.

In addition to well-known profound phenotypic differences between inbred strains of mice, during last 15–20 years increasingly more evidence accumulates on subtle differences between substrains of inbred mice [50]. Indeed, substrains of C57BL/6 mice have been shown to exhibit several genetic and phenotypic differences which may have a major impact on the interpretation of data [51–56]. For our study, and

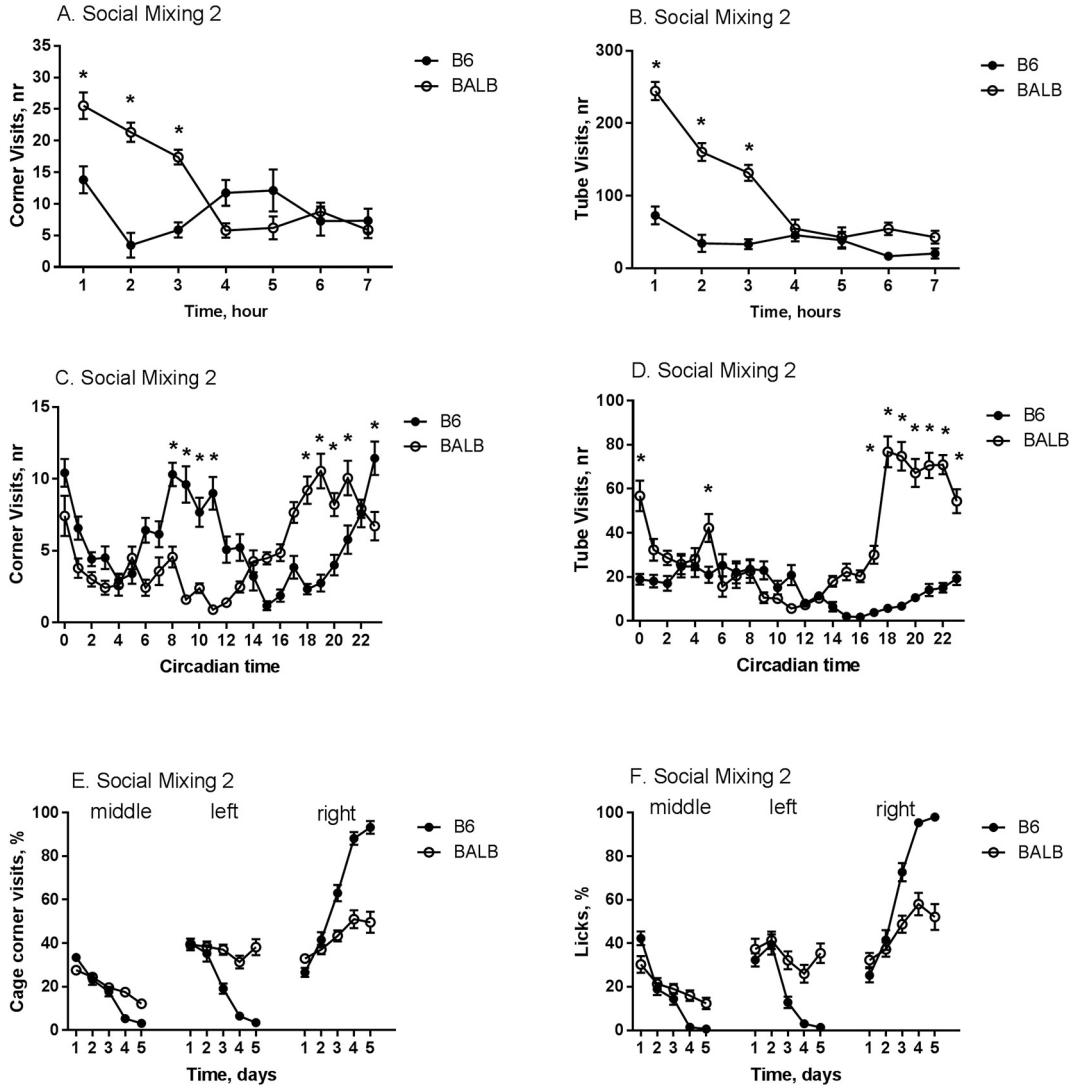

Fig. 5. Activity of mice in and between IntelliCages during second social mixing in three clean IntelliCages. A. Number of corner visits per hour during initial 7 h. B. Number of tube visits per hour during initial 7 h. C. Average number of corner visits per hour during circadian cycle (data averaged from days 2–4). D. Average number of tube visits per hour during circadian cycle (data averaged from days 2–4). E. Total number of corner visits over 5 days. F. Total number of tube visits over 5 days. G. Percentage of corner visits in three different IntelliCages (all cleaned before start of the session). H. Percentage of licks in three different IntelliCages (all cleaned before start of the session). *$p < 0.05$ between the strains (Newman-Keuls post hoc test).

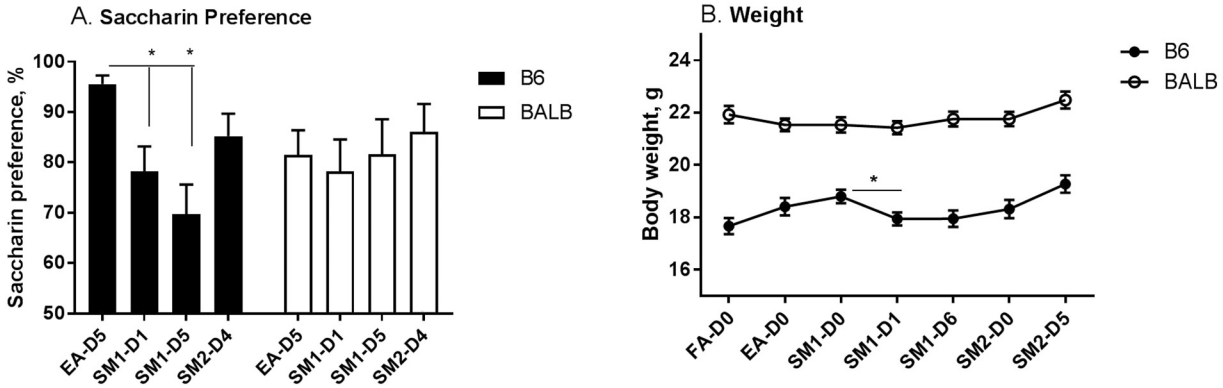

Fig. 6. Assessment of body weight and anhedonia-like behaviour during different phases of experiment. A. Saccharin preference (percentage of 0.5% saccharin consumed) on last day of extended adaptation (EA-D5), first and fifth days of first social mixing (SM1-D1, SM1-D5) and last day of second social mixing (SM2-D4). B. Body weight of the mice as measured before beginning of testing in the IntelliCage (FA-D0), before beginning of extended adaptation (EA-D0), and during social mixing (SM1-D0,D1,D6; SM2-D0,D5). *$p < 0.05$ between the indicated measurements (Newman-Keuls post hoc test).

for assessment of social behaviour in general, it is important and interesting to note that C57BL/6N mice show reduced place preference or even aversion in socially conditioned place preference paradigm as compared to C57BL/6 J mice [57] and therefore, C57BL/6N mice may be less “sociable” also in the other tests measuring social interest and interaction. Unfortunately, not all papers indicate explicitly the strains and substrains of the animals used.

Our findings suggest that environmental complexity and social enrichment have a strong effect on the behaviour of both C57BL/6N and BALB/c mice. In theory, as a result of mixing animals from different backgrounds, one could expect either spontaneous separation of the strains to different cages (social avoidance based on strain characteristics) or sharing and preferring one cage (high sociability), or indistinguishable preference. BALB/c in the present experiment tend to lean towards the latter (indistinguishable preference) and C57BL/6N clearly favour one cage. Regarding the social interaction between the two strains, it is unclear from the present data if the BALB/c were dominating over entire environment forcing the B6 mice to stay mostly in one IntelliCage out of three (expression of avoidance or social withdrawal), or another explanation could be that the C57BL/6N mice dominated in one cage, not allowing cohabitation of the BALB/c mice. Neophobia and novelty-induced anxiety-like behaviour was clearly reduced in BALB/c mice as a consequence of such experience. On the other hand, it may also be interpreted as an exaggerated activity and inability to adapt with the novel environment along with avoidance of C57BL/6N strain. Interestingly, BALB/c strain (compared to C57BL/6) has been shown to be more sensitive to the effects of phencyclidine and MK-801, thus suggesting “psychosis-prone” phenotype in this strain [58–60]. Environmental challenges may well contribute to manifestation of psychotic-like behaviour. Further studies with combinations of different inbred strains and also with established genetic models for e.g. autism-like phenotypes in mice are warranted for elucidation of proposed method. Moreover, other behavioural domains (e.g. social behaviour, learning and memory) could be investigated by conventional tests more thoroughly after this kind of challenge.

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