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Research report

The Visible Burrow System: A behavioral paradigm to assess sociability and social withdrawal in BTBR and C57BL/6J mice strains

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ABSTRACT

Disrupted sociability and consequent social withdrawal are (early) symptoms of a wide variety of neuropsychiatric diseases, such as schizophrenia, autism spectrum disorders, depressive disorders and Alzheimer's disease. The paucity of objective measures to translationally assess social withdrawal characteristics has been an important limitation to study this behavioral phenotype, both in human and rodents. The aim of the present study was to investigate sociability and social withdrawal in rodents using an ethologically valid behavioral paradigm, the Visible Burrow System (VBS). The VBS mimics a natural environment, with male and female rodents housed together in an enclosure where a large open arena is connected to a continuously dark burrow system that includes 4 nest boxes. In this study, mixed-sex colonies of C57BL/6J and of BTBR mice have been investigated (n = 8 mice per colony). Results showed marked differences between the two strains, in terms of sociability as well as social withdrawal behaviors. In particular, BTBR mice performed less social behaviors and have a preference for non-social behaviors compared to C57BL/6J mice. Neurobiologically, the decreased sociability of BTBR was accompanied by reduced GABA and increased glutamate concentrations in brain prefrontal cortex (PFC) and amygdala regions. In conclusion, our study validated the use of the VBS as an ethologically relevant behavioral paradigm in group-housed mice to investigate individual sociability and social withdrawal features and their underlying neurobiology. This paradigm may provide new insights to develop new therapeutic treatments for behavioral dysfunctions that may be relevant across neuropsychiatric diseases.

1. Introduction

Several neuropsychiatric diseases share the same behavioral dysfunctions, such as anxiety, delusion, apathy and impaired social functioning (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, DSM-5). Among these behavioral alterations, social withdrawal, defined as "disengagement from social activities that derives from indifference or lack of desire to have social contact", appears to be an early manifestation of a wide variety of neuropsychiatric diseases, such as schizophrenia, major depressive disorders (MDD), Alzheimer's disease and autism spectrum disorders (ASD) [1,2]. Indeed, a deep analysis of social withdrawal behaviors and their underlying neurobiology has become necessary in order to find new therapeutic strategies to curb this debilitating neuropsychiatric symptom. In this regard, mice can provide a good opportunity to study social behaviors, as they are highly social animals that live naturally in large groups with organized social structures and dominance hierarchies, and consequently show a wide variety of complex social interactions [3]. Yet, most laboratory studies of social behaviors primarily focus on short-term social encounters between familiar or unfamiliar dyads in relatively small cages under rather artificial conditions, thereby limiting the translational value of the obtained rodent data to humans. To enhance translational validity of rodent sociability and/or social withdrawal dynamics, colony housing systems that more closely approximate the natural habitat of rodents living together in groups have been developed [4–6]. One such a group-housing system is the Visible Burrow System (VBS) [7–9]. The VBS mimics a natural environment where male and female animals are housed together in an enclosure where an open arena, with an imposed diurnal photoperiod, is connected to a continuously dark burrow system, consisting of tunnels and small

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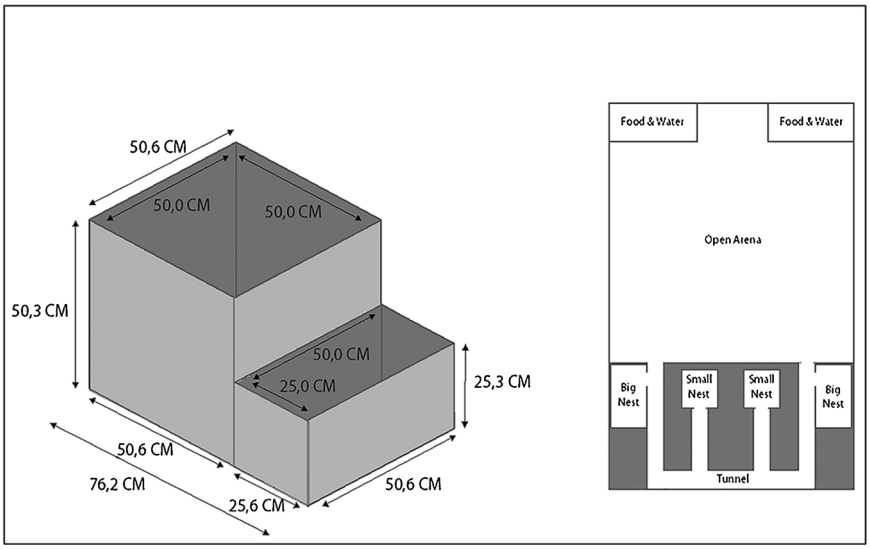

Fig. 1. The modified Visible Burrow System (VBS).

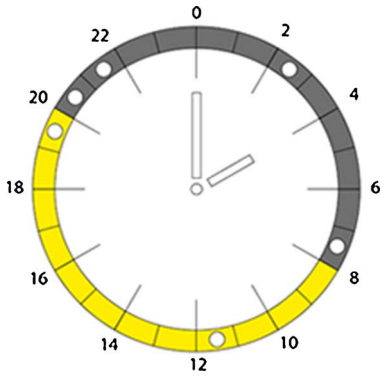

Fig. 2. Scoring schedule of the Visible Burrow System.
The system was kept under a light/dark cycle of 12L:12D with ZT0 at 8:00. Behavior was analyzed for the first 10 min of ZT3, 12–14, 18 and 23, denoted by small white circles.

chambers as the underground burrows and nests of colonies into the wild [10–13]. Although it has been used mainly to study social dominance hierarchy and consequent subordination stress, this social housing model appears to be a very useful setting to analyze social group behavior dynamics that naturally occur in a mixed-sex colony [14]. To validate the suitability of the VBS for studying sociability and social withdrawal behaviors, mouse models with behavioral phenotypes affecting the social sphere need to be used. In particular, the BTBR T + tf/J (BTBR) inbred mouse strain shows robust behavioral phenotypes with analogies to the core symptoms of ASD, such as deficits in social interaction, impaired communication, and repetitive behaviors [13,15,16]. BTBR mice show consistently low levels of sociability in the three-chamber social approach assays and they spend less time investigating a stranger mouse during direct social interaction [17,18].

At the neurobiological level, recent studies are focusing on the neural circuits and neuromolecular mechanisms underlying social behavioral alterations. A large body of evidence suggests a key role played by corticolimbic circuitry, including the medial prefrontal cortex (PFC) and basolateral amygdala. For example, it has been reported that activation of PFC and amygdala leads to a reduced social preference in the three chamber preference test and reduced social interaction in the social interaction test and in the resident-intruder paradigm [19,20], while NMDA and AMPA receptor blockade, with consequent glutamatergic neurotransmission suppression, ultimately leads to an increase in social interaction in the social interaction test [21]. Accordingly, in an elegant study, Paine and colleagues showed that a decrease in GABA functioning in either medial PFC or basolateral amygdala, due to a bilateral injection of a GABA A antagonist, decreased social preference in the three chamber preference test and social interaction in the social interaction test [22]. Thus, changes in GABA signaling might mediate sociability dysfunctions, such as social withdrawal, which is an important early symptom of several neuropsychiatric diseases. Accordingly, the GABAergic system has also been investigated in clinical research focused on schizophrenia, depression and bipolar disorders [23,24]. Patients suffering from these diseases appear to have lowered central and peripheral GABA levels when compared to healthy controls [23,24]. Moreover, this lowered functionality is visible during the prodromal stage of the diseases [25] and might ultimately represent a biomarker of symptomatic states in these patients [24].

Intriguingly, literature about GABAergic neurotransmission and colony housing systems is poor and need to be elucidated, especially considering that these semi-natural environments provide highly social and enriched conditions. In this regard, few studies reported a significant and beneficial role of social and environmental enrichments on different neurochemical parameters [26–28].

In the present study, the VBS has been validated as a behavioral paradigm to study sociability and social withdrawal behaviors in mice colonies. Hence, we studied BTBR and C57BL/6J mixed-sex colonies housed in the VBS continuously for 5 days, evaluating all the kinds of social and non-social behaviors. To further investigate the neurobiological mechanisms underlying sociability and social withdrawal, we quantified GABA and glutamate in PFC and amygdala of each mouse in our colonies.

Moreover, we also quantified GABA and glutamate in PFC and amygdala of mice housed in standard cages, comparing them with the mice housed in VBS, in order to evaluate whether this highly social and enriched environment might induce altered neurotransmission as a function of sociability.

2. Methods

2.1. Animals

Adult C57BL/6J and BTBR male and female mice aged 14–22 weeks were used in this study. C57BL/6J mice were offspring of breeding pairs obtained from Janvier Labs (Le Genest-Saint-Isle, France) and BTBR mice were offspring of breeding pairs obtained from Jackson Laboratory (Bar Harbor, Maine, U. S.). Animals were bred in the animal

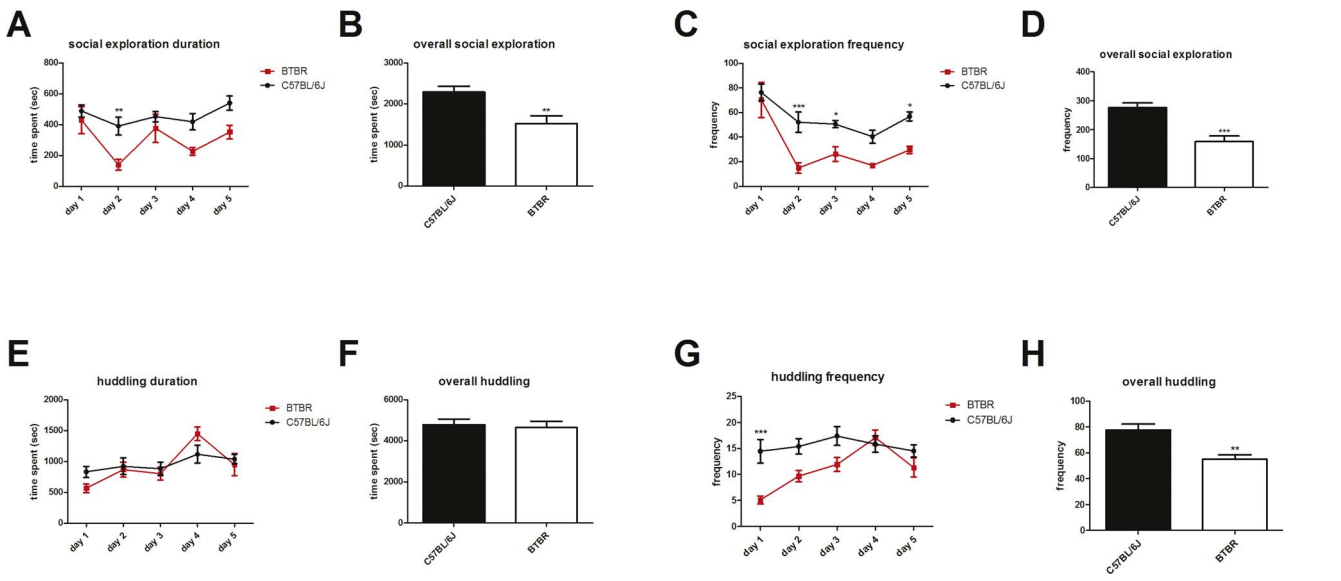

Fig. 3. Reduced social behaviors in BTBR colonies.
Duration and frequency of social behaviors in BTBR and C57BL/6J mice. Time spent performing social exploration per day (A) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, $^{**}p < 0.01$ vs. C57BL/6J. Overall time spent performing social exploration (B) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, $^{**}p < 0.01$ vs. C57BL/6J. Frequency of social exploration per day (C) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. C57BL/6J. Overall frequency of social exploration (D) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, $^{***}p < 0.001$ vs. C57BL/6J. Time spent performing huddling per day (E) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall time spent performing huddling (F) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, $^{**}p < 0.01$ vs. C57BL/6J. Frequency of huddling per day (G) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, $^{***}p < 0.001$ vs. C57BL/6J. Overall frequency of huddling (H) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, $^{**}p < 0.01$ vs. C57BL/6J. Data are expressed as mean ± SEM (n = 12 for BTBR group, n = 18 for C57BL/6J group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

facilities of the University of Groningen. Animals were housed in standard polypropylene cages, 34 cm × 18 cm × 14 cm, in a group of two mice in a temperature-controlled room (temperature 21 ± 2 °C). All subjects were maintained on a 12-h light/dark cycle, with access to water and standard chow *ad libitum* in their home cages. All procedures were conducted in accordance with protocols approved by the University of Groningen. Procedures involving animals and their care were conducted in conformity with the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and in accordance to ARRIVE guidelines. Animal welfare was daily monitored through the entire period of experimental procedures. No signs of distress were evidenced, anyway all efforts were made to minimize the number of animals used and their suffering.

2.2. Apparatus

The VBS' were built in-house at the University of Groningen, based on the design by Blanchard et al. (1995). Extra chambers (nests) were added to better study the social dynamics. The system consisted of two parts: an open arena (50cm × 50cm) with two stations where animals had access to food and water *ad libitum*, and a burrow (50cm × 25cm) with 4 chambers and a corridor. The open arena was subjected to a 12:12 L/D cycle (ZT0 at 08:00, see Fig. 3) and was open to the outside. Each VBS was illuminated with infrared LED lights (RM25-120 RAYMAX 25 IR Lamp- 120 °− 850 nm). The burrow of the VBS was closed using a black lid (Perspex 962 IR) that functioned as an infrared-pass filter. Thus the burrow was in complete darkness at all times, resembling the natural environment. Within the burrow 2 big chambers (7,5cm × 12,5cm) and 2 small chambers (7,5cm × 7,5cm) were placed with a tunnel connecting them to each other and to the open arena (see Fig. 1). Behavior in the VBS was recorded using a Bassler Cam GigE monochrome infrared sensitive camera (acA1300-60 gm). Thus, due to its infrared sensitivity, the camera not only recorded behavior in the open arena, but also could capture behavior in the burrow through the Perspex lid.

2.3. Experimental procedures

Animals were placed in the experimental room two weeks before the start of the experiments. Each colony consisted of 6 male mice and 2 female mice of the same strain. Every colony contained no more than 2 littermates. Many of the rodent studies on consequences of mixed-sex colony housing on brain, behavior and physiology are performed in male biased colonies [29–33]. The levels of perceived stress in males housed in a male-biased colony are reduced in comparison with males that are housed in a female-biased colony [34]. Since females are essential in facilitating natural hierarchy formation and behavioral expression [34] whereas high levels of perceived social stress could affect our behavioral outcomes and thus obscure our results, we chose to include a minimum amount of females without inducing a possible monopoly situation for the males. Thus, stimulating natural (and stable) hierarchy formation, while reducing the impact of social stress to the minimum. Females were previous sterilized by ligating the oviducts and leaving the ovaries intact in order to maintain the estrous cycle. Estrous cycle was monitored every day before the start of the experiments. Two days before the start of the experiment males were marked with a commercial crème-based hair dye (Garnier Olia B + + Super blonde) to facilitate individual recognition of the animals. Animals were housed in the system for 8 days. During the experiment, the animals were video-recorded continuously. The animals were weighed only at the beginning and at the end of the experiment in order to leave them undisturbed in the system.

2.4. Behavioral ethogram

Social and non-social behaviors scored are described in Table 1.

2.5. Behavioral analyses

Behavior was analyzed using The Observer 13 XT (Noldus Information Technology, Wageningen, The Netherlands). Each colony

was observed for 10 min of six different time points divided over the day (see Fig. 2). To provide a representative amount and distribution of all daily activities in our manual scoring, we chose six distinct time points during the 24 h, three at the beginning of dark period, one in the middle of dark period, one at the end of the dark period and one in the middle of the light period. We validated the chosen time points by scoring the first full day for two control colonies, showing no proportional behavioral differences between the six time points chosen and the full day (data not shown). Moreover, we scored the full hour of the six time points chosen and then compared the results with only 10 min of that hour and we found that the 10 min data sets accurately represented the full hour behavioral patterns (data not shown). A total of 3 C57BL/6J and 2 BTBR colonies were scored manually for 5 days. Frequency and duration of behaviors were scored for 10 min of each of the chosen six hours and data were shown as frequency per day and time spent per day. The data of the 5 days scored were summed and shown in the overall behavior in order to analyze strain differences. Two well-trained scorers, previous tested for inter-rater reliability, were involved in the scoring procedures and they were blind with respect the genotype of the colonies.

2.6. Post mortem analyses

After 5 days of VBS colony housing, male mice were immediately euthanized by cervical dislocation. Every male mouse within each colony was euthanized at the same time.

For the standard-housed measurements, 4 adult C57BL/6J and 4 adult BTBR male mice, housed in standard cages, two per cage, were euthanized by cervical dislocation.

The PFC and the amygdala were isolated from brains collected immediately after cervical dislocation using the Mouse Brain Matrix, making coronal sections of 1 mm of thickness. PFC and amygdala were then dissected from the slices according to the Mouse Brain in Stereotaxic Coordinates, 3rd Edition, Paxinos and Franklin, 2008. Tissues were frozen in isopentane and stored at −80 °C until analysis was performed.

2.7. HPLC quantifications

GABA and glutamate concentrations in PFC and amygdala were determined by HPLC using ODS-3 column (150 × 4.6 mm, 3 µm; INERTSIL) with fluorescence detection after derivatization with ophthalaldehyde/mercaptopropionic acid (emission length, 4.60 nm; excitation length, 3.40 nm). The mobile phase gradient consisted of 50 mM sodium acetate buffer, pH 6.95, with methanol increasing linearly from 2 to 30% (v/v) over 40 min. The flow rate was maintained by a pump (JASCO, Tokyo, Japan) at 0.5 ml/min. Results were analyzed by Borwin software (version 1.50; Jasco) and substrate concentration was expressed as µM.

2.8. Statistical analyses

Frequency and duration of each behavior were tested for normality and then analyzed per day using Two-way ANOVA for repeated measures followed by a Bonferroni's post-hoc test. Differences in the overall behavior were tested for normality and then analyzed using unpaired *t*-test. Neurochemical data were tested for normality and then analyzed using Two-way ANOVA followed by a Bonferroni's post-hoc test. Correlation between social behaviors and GABA tissue levels were analyzed by using Pearson correlation. Results were expressed as mean ± S.E.M. Statistical analyses were performed using Graph Pad 5.0 (GraphPad Software, San Diego, CA) for Windows. Differences were considered statistically significant when P value was less than 0.05.

3. Results

In order to validate the VBS as suitable tool to study social group behavior dynamics that naturally occur in a mixed-sex colony, we analyzed BTBR and C57BL/6J colonies.

3.1. BTBR mice showed reduced social behaviors in VBS colony housing

We scored both frequency and duration of social exploration and huddling as social behaviors. In particular, we found that the time spent on social explorative activities during day 2 was significantly decreased in BTBR compared to control (Fig. 3A, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 10.98$, $p < 0.01$ BTBR vs. C57BL/6J). In addition, the overall duration of social exploration was significantly decreased in BTBR compared to controls (Fig. 3B, Unpaired *t*-test, $p < 0.01$ BTBR vs. C57BL/6J). Moreover, the frequency of social exploration during day 2, 3 and 5 was significantly decreased in BTBR compared to control strain (Fig. 3C, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 20.72$, $p < 0.001$, $p < 0.05$ BTBR vs. C57BL/6J), and also the total frequency of social exploration was significantly decreased (Fig. 3D, Unpaired *t*-test, $p < 0.001$ BTBR vs. C57BL/6J). On the other hand, there were no differences in time spent performing huddling in BTBR compared to controls (Fig. 3E, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 1.753$, $p > 0.05$ BTBR vs. C57BL/6J, Fig. 3F Unpaired *t*-test, $p = 0.7175$ BTBR vs. C57BL/6J), while frequency during day 1 was significantly decreased in BTBR compared to controls (Fig. 3G, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 12.47$, $p < 0.001$ BTBR vs. C57BL/6J). Ultimately the total frequency of huddling was

Table 1
Behavioral ethogram describing all the different behavior scored.

Behaviors	Description
Social Exploration	Sniffing another animal, following another animal, playing with another animal
Approach	Moving towards another animal
Aggression	When the subject is biting, chasing, fighting other animals
Avoidance	Submissive and avoidance behavior. Submissive reactions to aggressive behavior (i.e. not fighting back/surrendering). Also moving/running away from aggressive encounters and social contact/approaches
Huddling	Resting/huddling while in contact with conspecifics. When the subject resumes activity for more than 5 seconds, behavior is not considered part of resting/huddling
Sexual activity	Mounting female
Passive/Receiving social contact	Receiving social contact is scored when an animal does not react to social exploration of another animal (i.e. passive social interaction)
Allogrooming	When an animal is grooming another animal
Autogrooming	When an animal is grooming itself
Feeding/Drinking	Feeding/drinking from the feeding station
Environmental Exploration	Animal explores the surrounding environment, behavior is not aimed towards another animal. (e.g. digging, locomotion, sniffing the walls)
Alone Inactivity	Resting while not being in bodily contact with another animal. When the subject resumes activity for more than 5 seconds, behavior is not considered part of resting

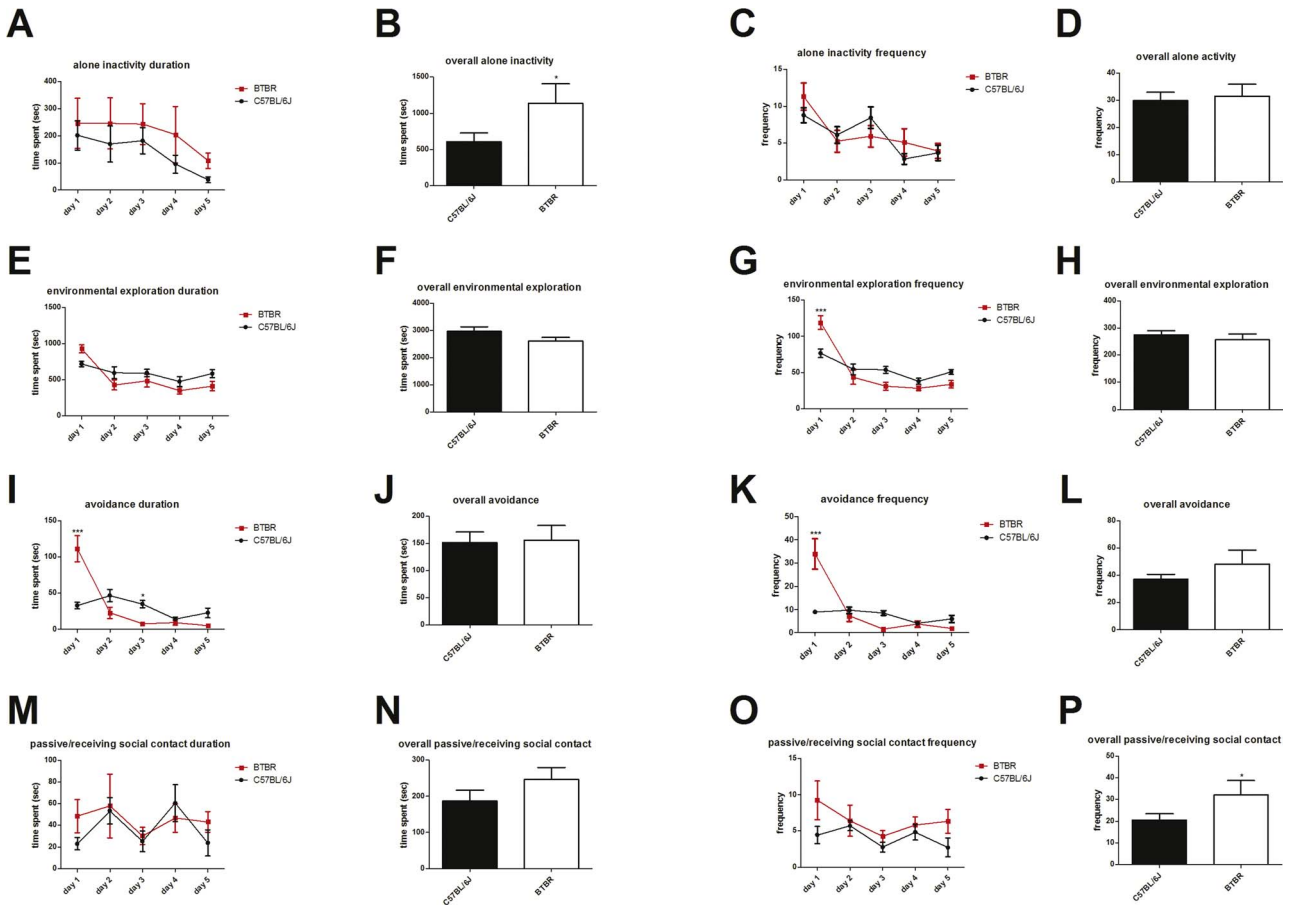

Fig. 4. Increased non-social behaviors in BTBR colonies.
Duration and frequency of non-social behaviors in BTBR and C57BL/6J mice. Time spent performing alone inactivity per day (A) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall time spent performing alone inactivity (B) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, *p < 0.05 vs. C57BL/6J. Frequency of alone inactivity per day (C) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall frequency of alone inactivity (D) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Time spent performing environmental exploration per day (E) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall time spent performing environmental exploration (F) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Frequency of environmental exploration per day (G) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, ***p < 0.001 vs. C57BL/6J. Overall frequency of environmental exploration (H) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Time spent performing avoidance per day (I) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, ***p < 0.001 vs. C57BL/6J. Overall time spent performing avoidance (J) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Frequency of avoidance per day (K) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, ***p < 0.001 vs. C57BL/6J. Overall frequency of avoidance (L) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Time spent performing passive/receiving social contact per day (M) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall time spent performing passive/receiving social contact (N) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Frequency of passive/receiving social contact per day (O) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall frequency of passive/receiving social contact (P) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, *p < 0.05 vs. C57BL/6J. Data are expressed as mean ± SEM (n = 12 for BTBR group, n = 18 for C57BL/6J group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

significantly reduced in BTBR compared to controls (Fig. 3H, Unpaired *t*-test, p < 0.01 BTBR vs. C57BL/6J).

3.2. BTBR mice showed increased non-social behaviors in VBS colony housing

We scored inactivity, environmental exploration, avoidance and passive/receiving social contact behaviors as non-social behaviors. Our results showed that there were no differences in time spent performing inactivity per day between the two strains (Fig. 4A, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 0.1020$, p > 0.05 BTBR vs. C57BL/6J), while the overall duration of alone inactivity was significantly increased in BTBR compared to control strain (Fig. 4B, Unpaired *t*-test, p < 0.05 BTBR vs. C57BL/6J). Regarding frequency, there were no differences in both daily and overall alone inactivity between the two strains (Fig. 4C, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 1.557$, p > 0.05 BTBR vs. C57BL/6J, Fig. 4D Unpaired *t*-test, p = 0.7523 BTBR vs. C57BL/6J). In addition, the time spent performing environmental exploration was not significantly different between the two strains (Fig. 4E, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 3.444$, p > 0.05 BTBR vs. C57BL/6J, Fig. 4F, Unpaired *t*-test, p = 0.1572 BTBR vs. C57BL/6J). Moreover, the frequency of environmental exploration was significantly increased in BTBR mice during day 1 (Fig. 4G, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 0.4863$, p < 0.001 BTBR vs. C57BL/6J), although no differences were detected in the overall frequency of environmental exploration between the two strains (Fig. 4H, Unpaired *t*-test, p = 0.4913 BTBR vs. C57BL/6J). Furthermore, we found that BTBR spent significantly more time performing avoidance behavior during day 1 (Fig. 4I, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 0.01953$, p < 0.001 BTBR vs. C57BL/6J), while no differences were found in the overall avoidance duration Fig. 4J, Unpaired *t*-test, p = 0.8899 BTBR vs. C57BL/6J). These results were confirmed with the avoidance frequency that was significantly increased in BTBR mice compared to

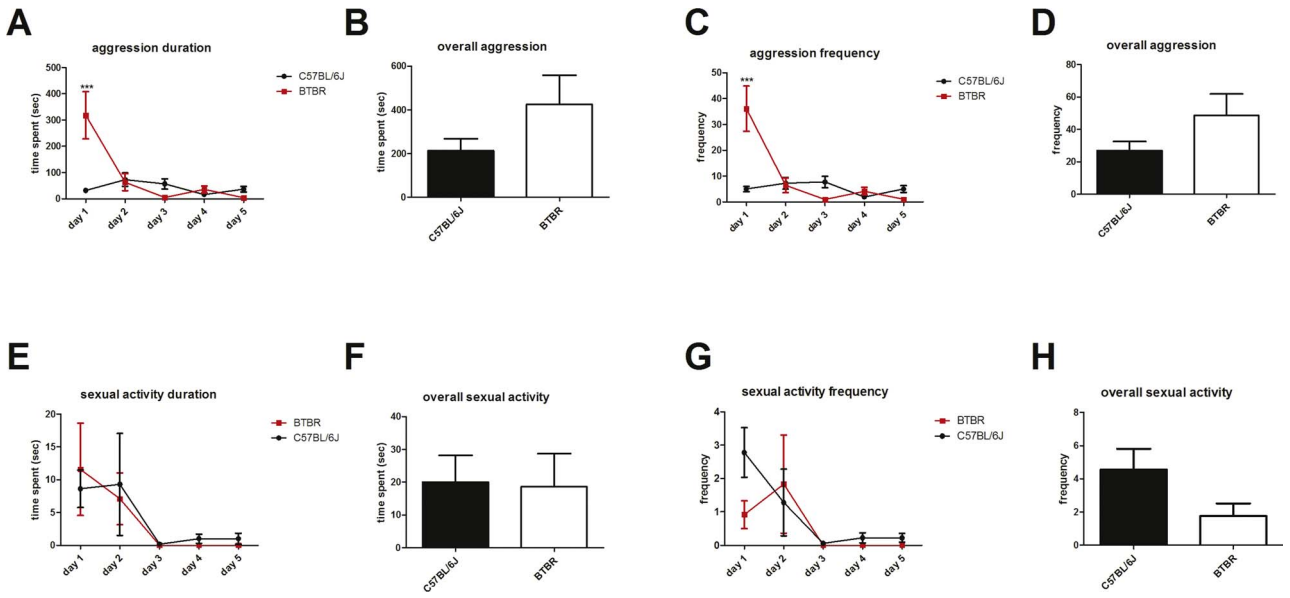

Fig. 5. Novelty-induced aggressive behavior in BTBR colonies.
Duration and frequency of aggression and sexual activity in BTBR and C57BL/6J mice. Time spent performing aggression per day (A) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, ***p < 0.001 vs. C57BL/6J. Overall time spent performing aggression (B) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Frequency of aggression per day (C) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, ***p < 0.001 vs. C57BL/6J. Overall frequency of aggression (D) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Time spent performing sexual activity per day (E) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall time spent performing sexual activity (F) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Frequency of sexual activity per day (G) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall frequency of sexual activity (H) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Data are expressed as mean ± SEM (n = 12 for BTBR group, n = 18 for C57BL/6J group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

controls only during day 1 (Fig. 4K, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 1.429$, $p < 0.001$ BTBR vs. C57BL/6J), and not in the overall avoidance frequency (Fig. 4L, Unpaired *t*-test, $p = 0.2419$ BTBR vs. C57BL/6J). Ultimately, time spent performing passive/receiving social contact behavior did not differ between the two strains (Fig. 4M, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 0.6164$, $p > 0.05$ BTBR vs. C57BL/6J, Fig. 4N, Unpaired *t*-test, $p = 0.2174$ BTBR vs. C57BL/6J). Also daily frequency of passive/receiving social contact behavior was not significantly different between BTBR and control animals (Fig. 4O, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 1.266$, $p > 0.05$ BTBR vs. C57BL/6J), while BTBR showed significantly more passive/receiving social contact frequency compared to controls (Fig. 4P, Unpaired *t*-test, $p < 0.05$ BTBR vs. C57BL/6J).

3.3. BTBR mice showed novelty-induced aggressive behavior in VBS colony housing

We scored daily aggressive behavior for both frequency and duration. We found that BTBR showed significantly more time spent performing aggression during day 1 (Fig. 5A, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 2.782$, $p < 0.001$ BTBR vs. C57BL/6J), while there were no differences in the overall aggressive behavior duration between the two strains (Fig. 5B, Unpaired *t*-test, $p = 0.1065$ BTBR vs. C57BL/6J). In addition, frequency of aggression was increased in BTBR during day 1 (Fig. 5C, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 2.907$, $p < 0.001$ BTBR vs. C57BL/6J), while there were no differences detected for the total aggression frequency (Fig. 5D, Unpaired *t*-test, $p = 0.0993$ BTBR vs. C57BL/6J). Since aggressive behavior could be influenced from sexual activity, we scored also sexual activity for both frequency and duration and we did not find any differences per day or in the total sexual activity between BTBR and control mice for both duration (Fig. 5E, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 0.1330$, $p > 0.05$ BTBR vs. C57BL/6J, Fig. 5F, Unpaired *t*-test, $p = 0.9150$ BTBR vs. C57BL/6J) and frequency (Fig. 5G, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 1.041$, $p > 0.05$, Fig. 5H, n. s. BTBR vs. C57BL/6J, Unpaired *t*-test, $p = 0.1036$ BTBR vs. C57BL/6J).

3.4. BTBR mice showed increased grooming behavior in VBS colony housing

We scored grooming behavior, which includes both allogrooming and autogrooming. We found that BTBR spent significantly more time performing grooming compared to controls during day 3 and 5 (Fig. 6A, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 3.687$, $p < 0.05$, $p < 0.01$ BTBR vs. C57BL/6J) and in the overall grooming (Fig. 6B, Unpaired *t*-test, $p < 0.05$ BTBR vs. C57BL/6J). Regarding frequency, BTBR showed significantly lower grooming frequency during day 1 compared to control mice (Fig. 6C, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 2.043$, $p < 0.05$ BTBR vs. C57BL/6J), while the overall grooming frequency was not significantly different between the two strains (Fig. 6D, Unpaired *t*-test, $p = 0.0703$ BTBR vs. C57BL/6J).

To investigate whether the alteration in social activities in BTBR strain were due to alterations in general activity, we scored also the total activity of BTBR and C57BL/6J colonies, pooling all the active behaviors (social exploration, alone exploration, avoidance, passive, aggressive behavior, sexual activity and grooming). Our results showed that there were no differences in time spent performing total activity in BTBR compared to controls during the daily scoring (Fig. 6E, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 1.336$, $p > 0.05$ BTBR vs. C57BL/6J) and in the overall duration of total activity (Fig. 6F, Unpaired *t*-test, $p = 0.7457$ BTBR vs. C57BL/6J). Furthermore, we found an increase in total activity frequency during day 1 and a decrease during day 3 in BTBR mice compared to controls (Fig. 6G, Two-way ANOVA RM followed by Bonferroni multiple comparison test, $F_{(1,28)} = 5.306$, $p < 0.05$ BTBR vs.

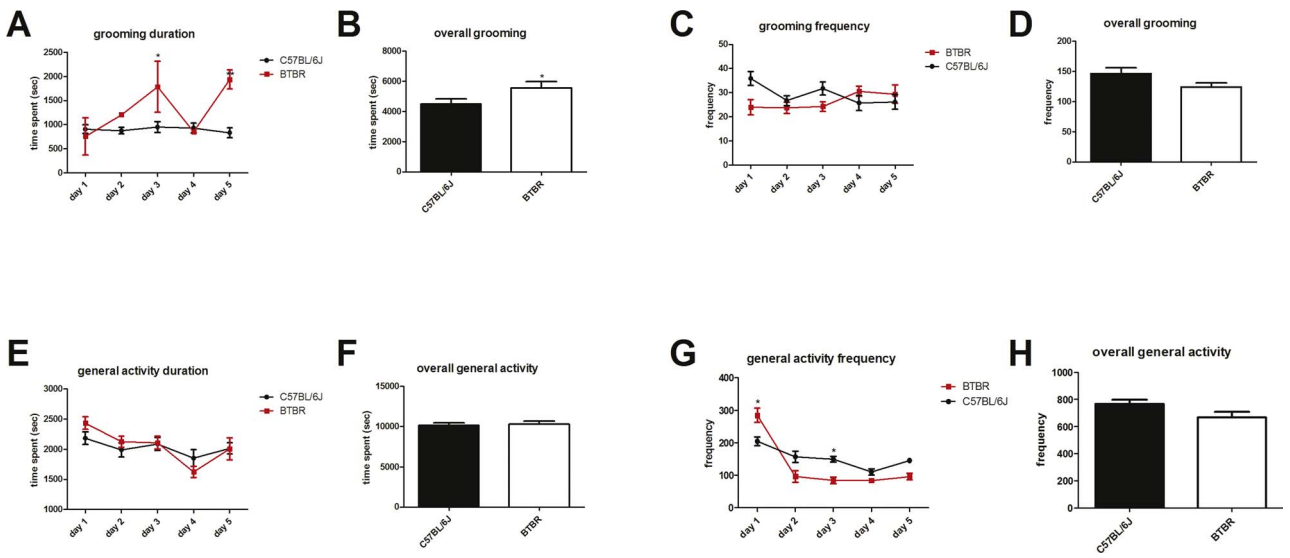

Fig. 6. Increased grooming behavior in BTBR colonies.
Duration and frequency of grooming and general activity in BTBR and C57BL/6J mice. Time spent performing grooming per day (A) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, *p < 0.05, ***p < 0.001 vs. C57BL/6J. Overall time spent performing grooming (B) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, *p < 0.05 vs. C57BL/6J. Frequency of grooming per day (C) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, *p < 0.05 vs. C57BL/6J. Overall frequency of grooming (D) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Time spent performing general activity per day (E) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, n.s. Overall time spent performing general activity (F) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Frequency of general activity per day (G) in BTBR (*red line*) and C57BL/6J (*black line*) mice. Two-way ANOVA RM followed by Bonferroni, *p < 0.05 vs. C57BL/6J. Overall frequency of general activity (H) in BTBR (*white bar*) and C57BL/6J (*black bar*) mice. Unpaired *t*-test, n.s. Data are expressed as mean ± SEM (n = 12 for BTBR group, n = 18 for C57BL/6J group). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

C57BL/6J), while there were no differences in the overall frequency of total activity between the two strains (Fig. 6H, Unpaired *t*-test, p = 0.0555 BTBR vs. C57BL/6J).

3.5. Modulation of GABA and glutamate following VBS colony housing is impaired in BTBR mice

In order to investigate the effect of the VBS colony housing on neurochemical outcomes, we quantified cortical and amygdaloidal GABA and glutamate levels in standard-housed animals and in VBS-housed animals in BTBR and C57BL/6J strains. We found an increase in GABA levels in both PFC and amygdala in VBS-housed C57BL/6J compared to the standard-housed C57BL/6J (Fig. 7A, Two-way ANOVA followed by Bonferroni multiple comparison test, p < 0.05 C57BL/6J VBS-housed vs. C57BL/6J standard-housed; Fig. 7B, Two-way ANOVA followed by Bonferroni multiple comparison test, p < 0.05 C57BL/6J VBS-housed vs. C57BL/6J standard-housed). Moreover, our post-hoc analysis indicated a strain-specific effect, since we found a decrease in GABA levels in BTBR compared to control colonies in PFC and amygdala only in VBS-housed animals (Fig. 7A, Two-way ANOVA followed by Bonferroni multiple comparison test, p < 0.01 BTBR VBS-housed vs. C57BL/6J VBS-housed; Fig. 7B, Two-way ANOVA followed by Bonferroni multiple comparison test, p < 0.05 BTBR VBS-housed vs. C57BL/6J VBS-housed).

Moreover, VBS-housed C57BL/6J showed a decrease in cortical glutamate levels compared to standard-housed C57BL/6J (Fig. 7C, Two-way ANOVA followed by Bonferroni multiple comparison test, p < 0.05 C57BL/6J VBS-housed vs. C57BL/6J standard-housed), while no difference was detected in amygdala (Fig. 7D, Two-way ANOVA followed by Bonferroni multiple comparison test, p > 0.05 C57BL/6J VBS-housed vs. C57BL/6J standard-housed). In addition, we found a significant increase in cortical and amygdaloidal glutamate levels in BTBR VBS-housed compared to C57BL/6J VBS colonies (Fig. 7C, Two-way ANOVA followed by Bonferroni multiple comparison test, p < 0.001 BTBR VBS-housed vs. C57BL/6J VBS-housed; Fig. 7D, Two-way ANOVA followed by Bonferroni multiple comparison test, p < 0.01 BTBR VBS-housed vs. C57BL/6J VBS-housed).

Ultimately, housing condition had no effect in BTBR strain, indeed no differences were detected in GABA and glutamate levels, in both PFC and amygdala, in VBS-housed compared to standard-housed mice (Fig. 7A–D, Two-way ANOVA followed by Bonferroni multiple comparison test, p > 0.05 BTBR VBS-housed vs. BTBR standard-housed).

3.6. Positive correlation between social exploration and amygdaloidal GABA in C57BL/6J but not BTBR mice colonies

In order to investigate the presence of correlations between social and non-social behaviors and GABA and glutamate tissue levels, we performed Pearson correlation for each mouse for 2 C57BL/6J and 2 BTBR colonies. We did not find any correlation among non-social behaviors and cortical and amygdaloidal GABA and glutamate levels and among social behaviors and cortical and amygdaloidal GABA and glutamate levels for both strains (data not shown), except for social explorative behavior and amygdaloidal GABA. Indeed, we found a significant positive correlation between social exploration and GABA in amygdala in C57BL/6J mice (Fig. 8A, Pearson correlation, r^2 = 0,6093, p < 0.01), while the correlation was not significant in BTBR mice (Fig. 8B, Pearson correlation, r^2 = 0,2509, p = 0.0971).

4. Discussion

In the present study, we investigated social dynamics and studied social withdrawal features in BTBR and C57BL/6J colonies in the VBS paradigm. Our results showed that BTBR mice performed less social behaviors and have a preference for non-social behaviors compared to C57BL/6J mice. The lack of sociability in BTBR was further accompanied by reduced GABA and increased glutamate concentrations in PFC and amygdala.

In our study, we implemented a modified version of an earlier used VBS paradigm, namely by adding two additional chambers in the burrow, enabling animals to have more nests and thus mimic the natural environment as much as possible. Moreover, we used mixed-sex

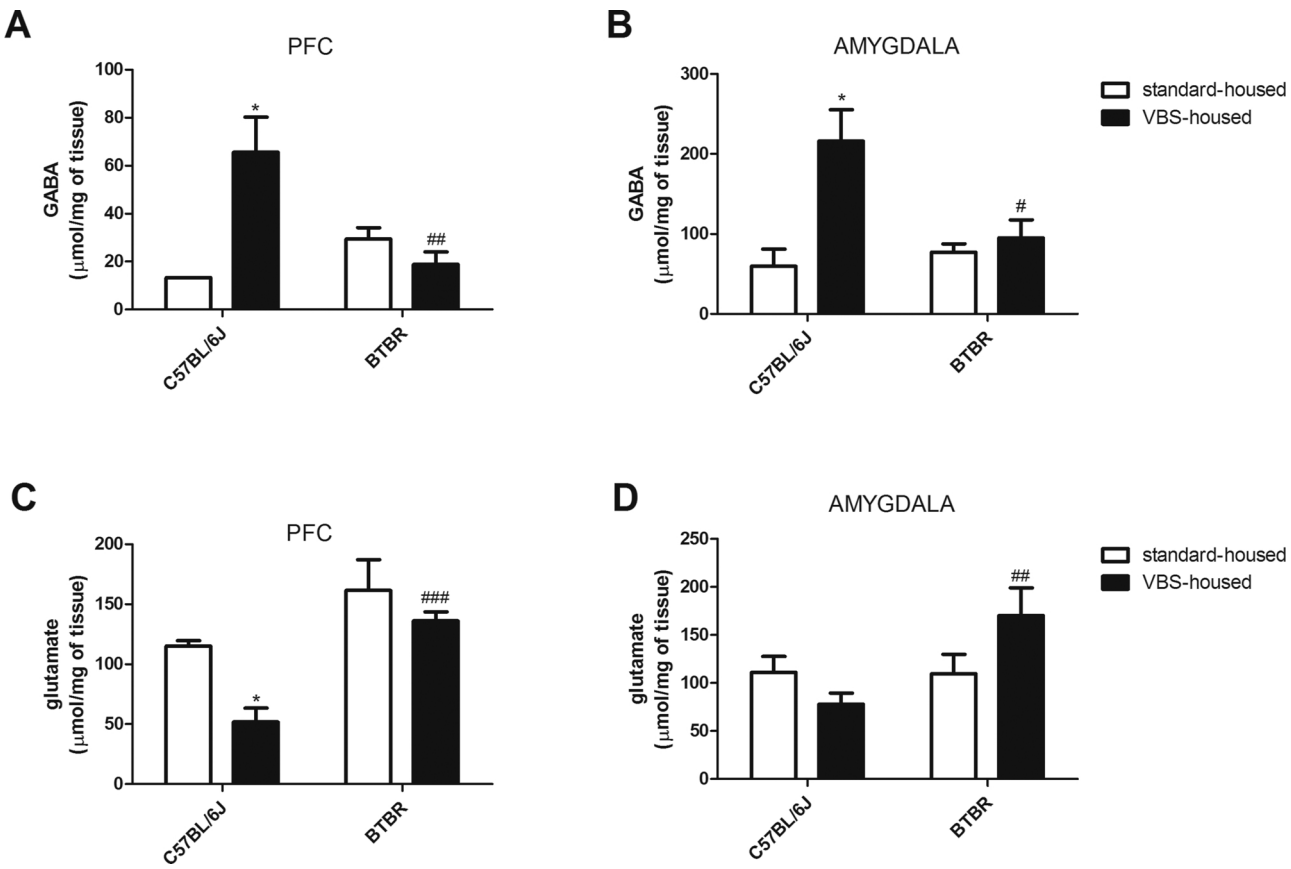

Fig. 7. GABA and glutamate concentrations in PFC and amygdala of BTBR and C57BL/6J mice VBS-housed or standard-housed.
Effect of VBS colony housing on cortical and amygdaloidal GABA and glutamate levels in BTBR and C57BL/6J mice. GABA levels in PFC (A) and amygdala (B) in C57BL/6J and BTBR mice housed in standard cages (*white bar*) and VBS-housed (*black bar*) C57BL/6J and BTBR mice. Two-way ANOVA followed by Bonferroni multiple comparisons test, *p < 0.05 C57BL/6J VBS-housed vs. C57BL/6J standard-housed; #p < 0.05, ##p < 0.01 BTBR VBS-housed vs. C57BL/6J VBS-housed. Glutamate levels in PFC (C) and amygdala (D) in C57BL/6J and BTBR mice housed in standard cages (*white bar*) and VBS-housed (*black bar*) mice. Two-way ANOVA followed by Bonferroni multiple comparisons test, *p < 0.05 C57BL/6J VBS-housed vs. C57BL/6J standard-housed; ##p < 0.01, ###p < 0.001 BTBR VBS-housed vs. C57BL/6J VBS-housed.

colonies to better reproduce the group-housed social dynamics that naturally occur in rodents [10].

The currently available dyadic tests can be carried out with no more than two animals at the same time. In addition, a battery of behavioral tests, such as the 5 short-term tests performed by Avraham and colleagues [35], is able to define a behavioral phenotype and to display short-term behavioral deficits, however it does not take into account the novelty effect and it is a short-term analysis.

The VBS is able to investigate social dynamics in a complex social environment, allowing interactions of up to eight mice at the same time. This adds translational value to the the model, since it allows the observation and quantification of social withdrawal, which is less convincingly observed in dyadic tests. Interestingly, this paradigm is suitable to study different pharmacological, environmental and genetic manipulations. In this regard, beyond the study of animal models displaying phenotypic variation in social behavior (such as BTBR mice and BALB/c mice), also animal models with genetic alterations affecting the social sphere, such as the oxytocin system, might be properly studied with the VBS paradigm. Indeed, the VBS can be used as a tool to study behavioral dysfunctions and might be further used as a behavioral paradigm to test pharmacological treatments aiming at restoring social dysfunctions commonly occurring in several neuropsychiatric disorders, such as the social withdrawal mentioned above. Moreover, the VBS allows to study social behavior longitudinally under baseline conditions, avoiding the novelty effect that commonly occurs in the social tests. As an example, BTBR mice showed very high levels of aggressive behaviors during the first day of novelty-induced social interactions that subsided in the following days. However, the VBS paradigm still has to be scored manually. This big disadvantage does not allow to track all the behaviors over the full period of time and thus the throughput is low. Further studies are currently being conducted to develop an automatic tracking system. The automatic system would also be helpful to investigate social networks, dominance and hierarchy within colonies.

Our results showed the total burden of social and non-social behaviors, displaying a clear picture of BTBR and C57BL/6J behaviors in colony. In these regards, we found a decrease in time spent performing social exploration in BTBR mice compared to controls during day 2 and in the overall duration. The decrease in time spent performing social exploration in BTBR was accompanied by a decrease also in the frequency during day 2, 3, 5 and in the overall social exploration frequency. As widely known, BTBR mice are studied as an ASD model, because of their reduced sociability compared to the commonly used as control C57BL/6J strain [13,36]. Indeed, the most important features of ASD phenotype consist of social deficits and high levels of repetitive grooming [13,37]. However, the most used behavioral test to assess sociability is the three chamber test, in which social preference is tested towards only one stimulus animal [17]. Hence, in our group-housed environment, we measured time spent and frequency of social behaviors for each component of the colony towards the other five males and two females, in order to untangle the social dynamics typical of the two studied strains. In this regard, we found a decrease of the overall huddling frequency in BTBR mice compared to controls, while no differences were detected in terms of duration. Our results are in line with previous studies from Blanchard group in which they found a decrease in huddling frequency in BTBR compared to control mice [13], as well

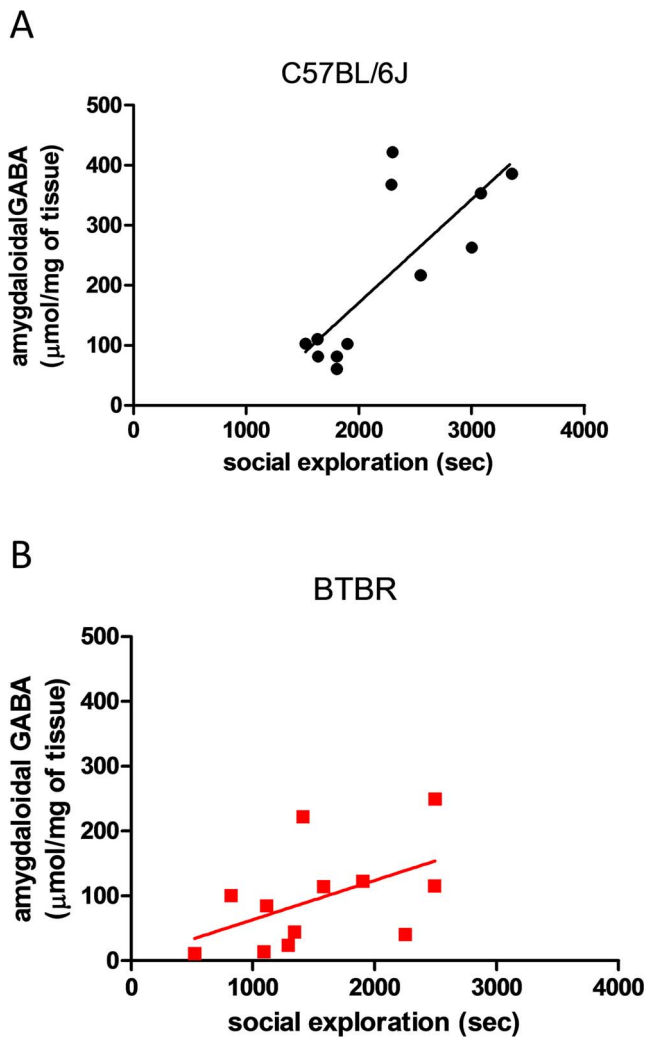

Fig. 8. Correlation between social exploration and GABA in C57BL/6J but not BTBR colonies.
Correlation between social exploration and amygdaloidal GABA in C57BL/6J and BTBR colonies. Positive correlation between social exploration and amygdaloidal GABA in C57BL/6J mice housed in VBS colonies (A). Pearson correlation, $r^2 = 0{,}6093$, $^{**}p < 0.01$. Correlation between social exploration and amygdaloidal GABA in BTBR mice housed in VBS colonies (B). Pearson correlation, $r^2 = 0{,}2509$, n.s.

as with the Kimchi group that consistently found lower social interactions in BTBR compared to C57BL/6 × 129 Sv control mice [4]. Huddling is commonly considered a social inactive behavior with an important thermoregulatory function. Factors such as social dominance, gender, ambient temperature and thermogenic needs can all have an influence on the amount of huddling (see [38] for review).

As regarding non-social behaviors, BTBR showed a general preference compared to C57BL/6J. In particular, we found a significant increase in the overall duration of alone inactivity in BTBR mice, while no differences were detected in terms of frequency. Conversely, BTBR showed an increase in the frequency of environmental exploration during day 1, but no differences in time spent performing environmental exploration. In addition, avoidance behavior was significantly increased in BTBR mice during day 1 for both frequency and duration. Ultimately, we found an increase of passive/receiving social contact behavior in BTBR mice only in terms of overall frequency, but not overall duration.

Taken together, our results confirm that the BTBR strain display less sociability and a preference for non-social behaviors, also in a semi-natural mixed-sex housing condition.

Although our results are in line with previous literature regarding BTBR strain and decrease of sociability [39], this is the first study showing also an effective increase in non-social behaviors. In particular, our results reported a trend towards social withdrawal behaviors in BTBR mice, opening to a deep investigation of the underlying neurobiology that gives rise to these behaviors.

Furthermore, we found an increase in aggressive behavior in BTBR compared to C57BL/6J mice during day 1, in terms of both frequency and duration. Interestingly, after the first day, the aggressive behavior in BTBR almost disappeared, suggesting a novelty-induced effect due to the new group housing condition. In this regard, very little is known about BTBR aggressiveness traits. Little aggressive behavior was observed during social interaction test [40] and resident-intruder paradigm (unpublished observations). Although aggression is not one of the core symptoms of ASD, ASD children display high levels of irritability, sometimes including aggressiveness towards others [37], and caregiver surveys reported some episodes of aggression in ASD patients towards others ASD patients [41,42]. However, to fully evaluate aggressive behavior features, hierarchy should to be taken into account. Dominance hierarchies are important aspects of animals living in social groups [10]. Here, we wanted to investigate strain differences and validate the suitability of VBS as a paradigm to study social and non-social behaviors. Future studies will be conducted to analyze individual animal behaviors and hierarchy formation within the colonies.

Since sexual activity is an important trigger for aggressive behavior, we decided to use mixed-sex colonies and analyze their sexual activity. Our results showed that there were no strain differences in sexual activity duration and frequency. Accordingly with aggressive behavior results, sexual activity was performed only during day 1 and 2 in BTBR colonies. Considering that females were monitored before the beginning of the experiment avoiding to start the experiment during the sexual receptivity phase, these results further confirm the novelty-induced effect due to the new housing condition in BTBR mice.

As regarding grooming behavior, we found an increase during day 3 and day 5 and in the overall duration of grooming in BTBR compared to C57BL/6J mice, while no differences were reported in terms of frequency. These data suggest that BTBR performed more grooming for a more prolonged time compared to C57BL/6J mice, indicating a reduced initiation of the behavior. As widely reported, BTBR strain display high levels of repetitive behaviors, such as persistent self-grooming and murble-burying [13,15,16,43–45]. In line with the previous literature, our increase in time spent performing grooming behavior might be interpreted as repetitive behavior that BTBR mice perform towards themselves (self-grooming) and towards others (allo-grooming). For this reason, we decided to pool together self- and allo- grooming due to the their repetitive features and not to consider allo-grooming as a social behavior, as differently reported in other VBS studies [13]. However, we are aware that not including allo-grooming in social behavior scores might represent a limitation of our study. Further investigations are currently being performed to better elucidate allo-grooming behavior and its possible correlations with social status and hierarchy. In this regard, it has been reported that allo-grooming behavior might serve multiple functions, including agonistic, affiliative or neutral, and that it is correlated to the social status of the animals and other contextual factors [9,46].

Finally, we also checked general activity to assess whether social and non-social strain differences were due to alteration in activity and we did not find any differences between the two strains. In support of this findings, Silverman and colleagues demonstrated that BTBR have the same response of C57BL/6J in terms of activity and locomotion [47].

From a neurochemical point of view, we found a significant decrease of GABA levels in PFC and amygdala in BTBR compared to C57BL/6J animals. The decrease in GABA was accompanied by an increase in glutamate levels, respectively in PFC and amygdala of BTBR mice. Recently, GABA involvement in sociability pathways is receiving great interest. In this regard, our results are consistent with those of

Paine and colleagues, who demonstrated that a decrease in GABA function in PFC and basolateral amygdala lead to a decrease in the social interaction and in the social preference tests, without affecting general anxiety, reward or locomotion [22]. However, different social factors contribute to sociability dysfunctions, such as social motivation, social anxiety and social cognition [48], hence future studies will be conducted to assess the involvement of these different social components in the sociability impairment.

Furthermore, it has been widely demonstrated that imbalances in the excitatory and inhibitory synaptic transmission might be responsible for severe neuropsychiatric-related symptoms [49–52]. In an elegant study, Yizhar and colleagues found a reduction in social interactions and social preference when activating optogenetically cortical pyramidal neurons [49]. Moreover, it has been reported that lesions in the medial PFC increased social behavior in the social interaction test [53]. In conclusion, the decrease in GABA and the corresponding increase in glutamate in PFC and amygdala might be responsible of the decrease in social behavior and increase in social withdrawal characteristics in BTBR strain. Thus, enhancing GABA neurotransmission could be a possible therapeutic strategy to treat social withdrawal symptoms that primarily occur in many neuropsychiatric and neurodegenerative diseases.

Intriguingly, Avraham and colleagues reported the involvement of oxytocin system in social behavior and particularly they showed that the increase of oxytocin secretion, acting through the induction of its ectoenzyme regulator CD38, was able to improve social interactions in BALB/c and BTBR mice [35]. In this regard, it has been demonstrated that a highly selective oxytocin receptor agonist produced inhibitory effects that led to an increase in GABA release in the medial sector of the central amygdala [54]. Hence, future studies will be conducted to better explore mechanisms between oxytocin and GABA systems and to elucidate the role of oxytocin and its possible therapeutical application to rescue social withdrawal symptoms.

In addition, we evaluated the effect of VBS colony housing condition on GABA and glutamate neurotransmission in the two studied strains. Interestingly, we found that GABA was increased in both PFC and amygdala in C57BL/6J housed in VBS compared to C57BL/6J housed in standard cages. Moreover, we found a glutamate decrease in PFC of C57BL/6J VBS-housed compared to C57BL/6J standard-housed, denoting a GABAergic and glutamatergic response to the highly social environment. Otherwise, BTBR did not follow the same trend of C57BL/6J; indeed, no differences were detected in GABA and glutamate levels between the VBS colonies and the standard cage housing condition. Thus, the neurochemical response to the highly social housing conditions in C57BL/6J mice was not found in the BTBR strain. The BTBR neurochemical non-response to VBS housing conditions might explain their tendency to perform social withdrawal behaviors in the colony. Our findings are consistent with a preclinical study from Crawley group, in which they showed that BTBR mice have poor abilities to modulate their responses to different social partners, resembling social cognition deficits in ASD patients [55].

To further corroborate neurochemical data with behavioral outcomes, we searched for correlations between social exploratory behavior and GABA levels in amygdala for each individual mouse within every colony. We found a significant positive correlation between social exploratory activity and amygdaloidal GABA in C57BL/6J, but not in BTBR colonies. These results endorse the hypothesis that GABA neurotransmission deeply affect sociability and that in control C57BL/6J mice, GABAergic tone is able to modulate the response to different social environments.

5. Conclusion

Our study validated the use of the VBS as a behavioral paradigm to deeply analyze sociability and social withdrawal behaviors, investigating mixed-sex group-housed dynamics in rodents. In conclusion, the VBS can be used as a tool to study behavioral dysfunctions and their underlying neurobiology, ultimately helping to design effective treatments for behavioral symptoms observed across neuropsychiatric diseases.

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Conflict of interests

The authors declare no conflict of interests.

References

[1] M.F. Green, Impact of cognitive and social cognitive impairment on functional outcomes in patients with schizophrenia, J. Clin. Psychiatry 77 (Suppl. 2) (2016) 8–11.
[2] C.A. Wilson, J.I. Koenig, Social interaction and social withdrawal in rodents as readouts for investigating the negative symptoms of schizophrenia, Eur. Neuropsychopharmacol. 24 (5) (2014) 759–773.
[3] L. Ricceri, A. Moles, J. Crawley, Behavioral phenotyping of mouse models of neurodevelopmental disorders: relevant social behavior patterns across the life span, Behav. Brain Res. 176 (1) (2007) 40–52.
[4] A. Weissbrod, A. Shapiro, G. Vasserman, L. Edry, M. Dayan, A. Yitzhaky, L. Hertzberg, O. Feinerman, T. Kimchi, Automated long-term tracking and social behavioural phenotyping of animal colonies within a semi-natural environment, Nat. Commun. 4 (2013) 2018.
[5] Y. Shemesh, Y. Sztainberg, O. Forkosh, T. Shlapobersky, A. Chen, E. Schneidman, High-order social interactions in groups of mice, Elife 2 (2013) e00759.
[6] J. Freund, A.M. Brandmaier, L. Lewejohann, I. Kirste, M. Kritzler, A. Kruger, N. Sachser, U. Lindenberger, G. Kempermann, Association between exploratory activity and social individuality in genetically identical mice living in the same enriched environment, Neuroscience 309 (2015) 140–152.
[7] S.J. Melhorn, C.T. Elfers, K.A. Scott, R.R. Sakai, A closer look at the subordinate population within the visible burrow system, Physiol. Behav. 178 (2017) 110–116.
[8] J.P. Herman, K.L. Tamashiro, The visible burrow system: a view from across the hall, Physiol. Behav. 178 (2017) 103–109.
[9] H. Arakawa, D.C. Blanchard, R.J. Blanchard, Colony formation of C57BL/6J mice in visible burrow system: identification of eusocial behaviors in a background strain for genetic animal models of autism, Behav. Brain Res. 176 (1) (2007) 27–39.
[10] B. Buwalda, J.M. Koolhaas, S.F. de Boer, Trait aggressiveness does not predict social dominance of rats in the visible burrow system, Physiol. Behav. 178 (2017) 134–143.
[11] D.C. Blanchard, E.B. Defensor, K.Z. Meyza, R.L. Pobbe, B.L. Pearson, V.J. Bolivar, R.J. Blanchard, BTBR T+tf/J mice: autism-relevant behaviors and reduced fractone-associated heparan sulfate, Neurosci. Biobehav. Rev. 36 (1) (2012) 285–296.
[12] D.C. Blanchard, R.L. Spencer, S.M. Weiss, R.J. Blanchard, B. McEwen, R.R. Sakai, Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates, Psychoneuroendocrinology 20 (2) (1995) 117–134.
[13] R.L. Pobbe, B.L. Pearson, E.B. Defensor, V.J. Bolivar, D.C. Blanchard, R.J. Blanchard, Expression of social behaviors of C57BL/6J versus BTBR inbred mouse strains in the visible burrow system, Behav. Brain Res. 214 (2) (2010) 443–449.
[14] B.S. McEwen, C.R. McKittrick, K.L. Tamashiro, R.R. Sakai, The brain on stress: insight from studies using the visible burrow system, Physiol. Behav. 146 (2015) 47–56.
[15] H.G. McFarlane, G.K. Kusek, M. Yang, J.L. Phoenix, V.J. Bolivar, J.N. Crawley, Autism-like behavioral phenotypes in BTBR T+tf/J mice, Genes Brain Behav. 7 (2) (2008) 152–163.
[16] R.T. Molenhuis, L. de Visser, H. Bruining, M.J. Kas, Enhancing the value of psychiatric mouse models; differential expression of developmental behavioral and cognitive profiles in four inbred strains of mice, Eur. Neuropsychopharmacol. 24 (6) (2014) 945–954.
[17] S.S. Moy, J.J. Nadler, N.B. Young, A. Perez, L.P. Holloway, R.P. Barbaro, J.R. Barbaro, L.M. Wilson, D.W. Threadgill, J.M. Lauder, T.R. Magnuson, J.N. Crawley, Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains, Behav. Brain Res. 176 (1) (2007) 4–20.
[18] M. Yang, M.L. Scattoni, V. Zhodzishsky, T. Chen, H. Caldwell, W.S. Young, H.G. McFarlane, J.N. Crawley, Social approach behaviors are similar on conventional versus reverse lighting cycles, and in replications across cohorts, in BTBR T+

+ tf/J, C57BL/6J, and vasopressin receptor 1B mutant mice, Front. Behav. Neurosci. 1 (2007) 1.
[19] S.K. Sanders, A. Shekhar, Regulation of anxiety by GABAA receptors in the rat amygdala, Pharmacol. Biochem. Behav. 52 (4) (1995) 701–706.
[20] A.C. Felix-Ortiz, A. Burgos-Robles, N.D. Bhagat, C.A. Leppla, K.M. Tye, Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex, Neuroscience 321 (2016) 197–209.
[21] T.J. Sajdyk, A. Shekhar, Excitatory amino acid receptors in the basolateral amygdala regulate anxiety responses in the social interaction test, Brain Res. 764 (1–2) (1997) 262–264.
[22] T.A. Paine, N. Swedlow, L. Swetschinski, Decreasing GABA function within the medial prefrontal cortex or basolateral amygdala decreases sociability, Behav. Brain Res. 317 (2017) 542–552.
[23] D.A. Lewis, Inhibitory neurons in human cortical circuits: substrate for cognitive dysfunction in schizophrenia, Curr. Opin. Neurobiol. 26 (2014) 22–26.
[24] B. Romeo, W. Choucha, P. Fossati, J.Y. Rotge, Meta-analysis of central and peripheral gamma-aminobutyric acid levels in patients with unipolar and bipolar depression, J. Psychiatry Neurosci. 42 (6) (2017) 160228.
[25] M.J. Minzenberg, A.J. Firl, J.H. Yoon, G.C. Gomes, C. Reinking, C.S. Carter, Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia, Neuropsychopharmacology 35 (13) (2010) 2590–2599.
[26] M. Mainardi, S. Landi, L. Gianfranceschi, S. Baldini, R. De Pasquale, N. Berardi, L. Maffei, M. Caleo, Environmental enrichment potentiates thalamocortical transmission and plasticity in the adult rat visual cortex, J. Neurosci. Res. 88 (14) (2010) 3048–3059.
[27] F. Cirulli, F. Capone, L.T. Bonsignore, L. Aloe, E. Alleva, Early behavioural enrichment in the form of handling renders mouse pups unresponsive to anxiolytic drugs and increases NGF levels in the hippocampus, Behav. Brain Res. 178 (2) (2007) 208–215.
[28] F. Cirulli, A. Berry, L.T. Bonsignore, F. Capone, I. D'Andrea, L. Aloe, I. Branchi, E. Alleva, Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels, Neurosci. Biobehav. Rev. 34 (6) (2010) 808–820.
[29] D.S. Albeck, C.R. McKittrick, D.C. Blanchard, R.J. Blanchard, J. Nikulina, B.S. McEwen, R.R. Sakai, Chronic social stress alters levels of corticotropin-releasing factor and arginine vasopressin mRNA in rat brain, J. Neurosci. 17 (12) (1997) 4895–4903.
[30] D.C. Blanchard, P. Cholvanich, R.J. Blanchard, D.W. Clow, R.P. Hammer Jr., J.K. Rowlett, M.T. Bardo, Serotonin, but not dopamine, metabolites are increased in selected brain regions of subordinate male rats in a colony environment, Brain Res. 568 (1–2) (1991) 61–66.
[31] D.C. Blanchard, R.R. Sakai, B. McEwen, S.M. Weiss, R.J. Blanchard, Subordination stress: behavioral, brain, and neuroendocrine correlates, Behav. Brain Res. 58 (1–2) (1993) 113–121.
[32] C.R. McKittrick, D.C. Blanchard, R.J. Blanchard, B.S. McEwen, R.R. Sakai, Serotonin receptor binding in a colony model of chronic social stress, Biol. Psychiatry 37 (6) (1995) 383–393.
[33] D.C. Blanchard, G. Griebel, R.J. Blanchard, Gender bias in the preclinical psychopharmacology of anxiety: male models for (predominantly) female disorders, J. Psychopharmacol. 9 (2) (1995) 79–82.
[34] K.L. Tamashiro, M.M. Nguyen, T. Fujikawa, T. Xu, L. Yun Ma, S.C. Woods, R.R. Sakai, Metabolic and endocrine consequences of social stress in a visible burrow system, Physiol. Behav. 80 (5) (2004) 683–693.
[35] Y. Avraham, E.M. Berry, M. Donskoy, W.A. Ahmad, L. Vorobiev, A. Albeck, D. Mankuta, Beta-carotene as a novel therapy for the treatment of "Autistic like behavior" in animal models of Autism, Behav. Brain Res. (2017) (in press, Epub ahead of print).
[36] S. Ryan, J. Fraser-Thomas, J.A. Weiss, Patterns of sport participation for youth with autism spectrum disorder and intellectual disability, J. Appl. Res. Intellect. Disabil. (2017) (in press, Epub ahead of print).
[37] J.L. Silverman, S.S. Tolu, C.L. Barkan, J.N. Crawley, Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP, Neuropsychopharmacology 35 (4) (2010) 976–989.
[38] C. Gilbert, D. McCafferty, Y. Le Maho, J.M. Martrette, S. Giroud, S. Blanc, A. Ancel, One for all and all for one: the energetic benefits of huddling in endotherms, Biol. Rev. Camb. Philos. Soc. 85 (3) (2010) 545–569.
[39] K. Meyza, T. Nikolaev, K. Kondrakiewicz, D.C. Blanchard, R.J. Blanchard, E. Knapska, Neuronal correlates of asocial behavior in a BTBR T (+) Itpr3(tf)/J mouse model of autism, Front. Behav. Neurosci. 9 (2015) 199.
[40] V.J. Bolivar, S.R. Walters, J.L. Phoenix, Assessing autism-like behavior in mice: variations in social interactions among inbred strains, Behav. Brain Res. 176 (1) (2007) 21–26.
[41] S.M. Kanne, M.O. Mazurek, Aggression in children and adolescents with ASD: prevalence and risk factors, J. Autism Dev. Disord. 41 (7) (2011) 926–937.
[42] L.B. Pouw, C. Rieffe, P. Oosterveld, B. Huskens, L. Stockmann, Reactive/proactive aggression and affective/cognitive empathy in children with ASD, Res. Dev. Disabil. 34 (4) (2013) 1256–1266.
[43] J.E. Moller, D. Raahave, Gastric localization of endodermal sinus tumour, Acta Pathol. Microbiol. Scand. A 82 (2) (1974) 179–181.
[44] D.M. Jones-Davis, M. Yang, E. Rider, N.C. Osbun, G.J. da Gente, J. Li, A.M. Katz, M.D. Weber, S. Sen, J. Crawley, E.H. Sherr, Quantitative trait loci for interhemispheric commissure development and social behaviors in the BTBR T(+) tf/J mouse model of autism, PLoS One 8 (4) (2013) e61829.
[45] D.A. Amodeo, J.H. Jones, J.A. Sweeney, M.E. Ragozzino, Differences in BTBR T+ + tf/J and C57BL/6J mice on probabilistic reversal learning and stereotyped behaviors, Behav. Brain Res. 227 (1) (2012) 64–72.
[46] A.V. Kalueff, A. Minasyan, T. Keisala, Z.H. Shah, P. Tuohimaa, Hair barbering in mice: implications for neurobehavioural research, Behav. Process 71 (1) (2006) 8–15.
[47] J.L. Silverman, B.A. Babineau, C.F. Oliver, M.N. Karras, J.N. Crawley, Influence of stimulant-induced hyperactivity on social approach in the BTBR mouse model of autism, Neuropharmacology 68 (2013) 210–222.
[48] D.P. Kennedy, R. Adolphs, The social brain in psychiatric and neurological disorders, Trends Cogn. Sci. 16 (11) (2012) 559–572.
[49] O. Yizhar, L.E. Fenno, M. Prigge, F. Schneider, T.J. Davidson, D.J. O'Shea, V.S. Sohal, I. Goshen, J. Finkelstein, J.T. Paz, K. Stehfest, R. Fudim, C. Ramakrishnan, J.R. Huguenard, P. Hegemann, K. Deisseroth, Neocortical excitation/inhibition balance in information processing and social dysfunction, Nature 477 (7363) (2011) 171–178.
[50] S.B. Nelson, V. Valakh, Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders, Neuron 87 (4) (2015) 684–698.
[51] R. Gao, P. Penzes, Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders, Curr. Mol. Med. 15 (2) (2015) 146–167.
[52] S. Sorce, S. Schiavone, P. Tucci, M. Colaianna, V. Jaquet, V. Cuomo, M. Dubois-Dauphin, L. Trabace, K.H. Krause, The NADPH oxidase NOX2 controls glutamate release: a novel mechanism involved in psychosis-like ketamine responses, J. Neurosci. 30 (34) (2010) 11317–11325.
[53] A.A. Shah, D. Treit, Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests, Brain Res. 969 (1–2) (2003) 183–194.
[54] D. Huber, P. Veinante, R. Stoop, Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala, Science 308 (5719) (2005) 245–248 New York, N.Y.
[55] M. Yang, D.N. Abrams, J.Y. Zhang, M.D. Weber, A.M. Katz, A.M. Clarke, J.L. Silverman, J.N. Crawley, Low sociability in BTBR T+tf/J mice is independent of partner strain, Physiol. Behav. 107 (5) (2012) 649–662.