Behavioural Brain Research 344 (2018) 9–19

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

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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ARTICLE INFO

Keywords:
Visible burrow system
BTBR mice
C57BL/6J mice
Sociability
Social withdrawal

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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https://doi.org/10.1016/j.bbr.2018.02.003
Received 9 November 2017; Received in revised form 1 February 2018; Accepted 2 February 2018
Available online 07 February 2018

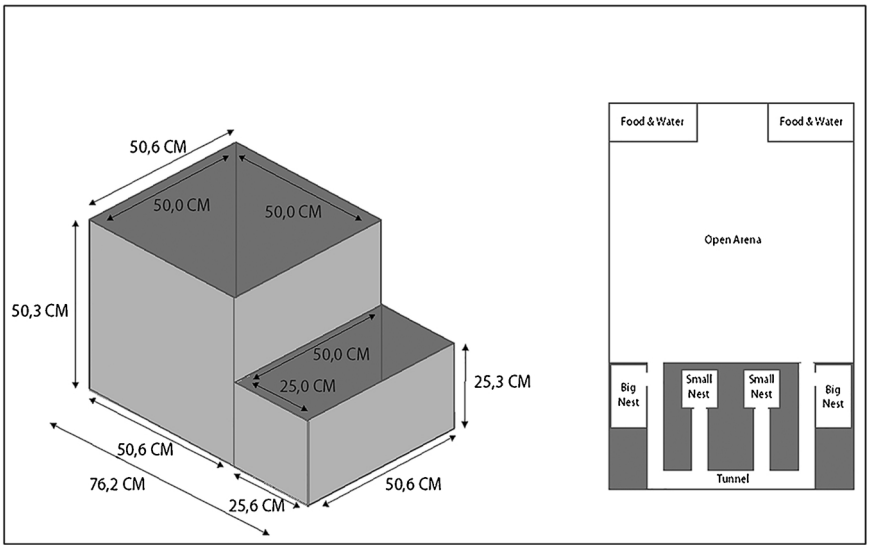

Fig. 1. The modified Visible Burrow System (VBS).

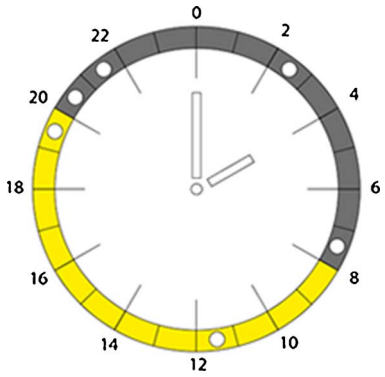

Fig. 2. Scoring schedule of the Visible Burrow System.
The system was kept under a light/dark cycle of 12L:12D with ZT0 at 8:00. Behavior was analyzed for the first 10 min of ZT3, 12–14, 18 and 23, denoted by small white circles.

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