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Research report

Behavioral assessments of BTBR T+Itpr3tf/J mice by tests of object attention and elevated open platform: Implications for an animal model of psychiatric comorbidity in autism

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ABSTRACT

Autism spectrum disorders (ASD) are diagnosed based on the behavioral criteria of impaired social interaction, defective communication and repetitive behaviors. Psychiatric comorbidities, such as anxiety and intellectual disability, are commonly present in ASD. The BTBR T+ Itpr3tf/J (BTBR) mice display a range of autistic phenotypes, yet whether this mouse model is appropriate to study psychiatric comorbidity in ASD remains unclear. We addressed this issue by subjecting the BTBR animals to three-chambered apparatus, open field, object attention test and elevated open platform. Compared to C57BL/6J control mice, the BTBR mice displayed hyperactivity in most of the tests. In the three-chamber assessment, they exhibited deficits in sociability. In the open field, more grooming and thigmotaxis and less rearing behaviors were observed. They also showed impaired object-based attention. On the elevated open platform, the BTBR animals stayed more to the edges than in the center of the platform. To further examine the properties of this test, naïve C57BL/6J mice were randomly administrated with saline or an anxiogenic substance, caffeine. The caffeine group demonstrated a similar behavioral pattern as the BTBR mice. When the saline group was re-exposed to the same platform, the time they stayed in the center substantially increased, likely due to reduced anxiety by habituation. These results indicate that the BTBR were more anxious than control mice on the open platform. Taken together, the BTBR strain exhibit emotional and cognitive impairments in addition to autistic behaviors, suggesting that they can be a valid model for ASD with psychiatric comorbidity.

1. Introduction

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by the core behavioral symptoms of defective social interactions, impaired verbal and nonverbal communication and restricted patterns of repetitive behaviors [1–3]. The etiology of ASD remains elusive, converging genetic, epigenetic and environmental impacts. Thus, the pathological phenotypes of ASD is highly heterogeneous. Besides the key symptoms, cognitive and psychiatric deficits are frequently present in ASD patients, including social anxiety disorder and attention-deficit/hyperactivity disorder [4,5]. ASD have been associated with anatomical alterations in the cerebral cortex, amygdala and cerebellum, as evidenced by neuroimaging and post-mortem studies [1,6,7].

The inbred BTBR T+ Itpr3tf/J (BTBR) mice, a phenotypic mouse model with face validity to ASD, have been employed to investigate pathogenesis of these disorders. They show reduced sociability [8,9], changes in ultrasonic vocalizations during development [10–12] and increased self-grooming behavior [8,13], which mimic the symptoms of deficient social interactions, impaired communication and repetitive behaviors seen in ASD. In addition, the BTBR model shares similarities of brain alternations with ASD. They display a complete agenesis of the corpus callosum, which is comparable to ASD patients with reduced volume of the corpus callosum [14–16]. Furthermore, volumetric differences in the frontal lobe, amygdala and cerebellum are highlighted in the BTBR animals [17,18]. As psychiatric deficits are widely comorbid in ASD [4,5], whether the BTBR mice exhibit additional psychiatric comorbidity is unclear. It has been shown that these mice have attentional deficits as tested by the five-choice serial reaction time task [19], while conflicting results on non-social anxiety assessed by the elevated plus maze have been reported [9,20,21]. To explore whether the BTBR strain is suitable as an animal model to study ASD with comorbid cognitive and emotional impairments, further behavioral tests targeting these functions are required.

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We first examined sociable behaviors with the three-chamber test, which are characteristic landmarks of the autistic mouse model [8,13,20,22]. The open field was then administered to assess distance traveled, rearing and self-grooming as repetitive behavior [8,13]. Finally, we investigated whether the BTBR mice had attentional deficits and high non-social anxiety/fear with the object-based attention [23,24] and elevated open platform tests, respectively. Compared to the C57BL/6J strain (commonly used as wild-type, WT), the BTBR animals exhibited a spatial pattern of staying more at the edges / less in the center of the elevated open platform. To validate the applicability of this test, naïve WT animals were randomly injected with saline- or caffeine, taken as an anxiogenic substance, before being evaluated on the platform. After a washout time, they were mixed, treated with saline and placed on the same platform, which functioned as re-exposure with reduce emotional arousal due to habituation. The caffeine-treated group showed a spatial pattern similar to the BTBR animals, while re-exposure group exhibited the opposite. Collectively, we revealed significant behavioral changes associated with attention and anxiety in addition to sociable deficits, hyperactivity and repetitive behaviors in the BTBR mice. Our results suggest that the BTBR strain is a compelling model for ASD with comorbid psychiatric symptoms.

2. Material and methods

2.1. Subjects

Male BTBR T+ Itpr3tf/J and C57BL/6J mice (8–12 months old) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Animals were grouped 3–5 per cage and food and water were provided *ad libitum*. The colonies were kept with controlled temperature and humidity and under a normal light-dark cycle (light on from 07:00 to 19:00). All procedures were in accord with the Institutional Animal Care and Use Committee, University of Minnesota, and the US Department of Agriculture Animal Welfare Act and Animal Welfare Regulations.

2.2. Apparatus

A transparent open field made of polyvinyl chloride (40 × 40 × 30 cm) was used for testing fear-like, locomotor and object exploration behaviors. A three-chambered apparatus made of transparent polyvinyl chloride (60 × 40 × 30 cm; composed of three 20 × 40 × 30 cm chambers) was applied for testing sociability and social novelty. The animal had free access to all the chambers through the passages (5 × 5 cm) within the walls that divided the chambers. Two pen cups (with mesh grids; diameter 10 cm, height 12 cm) were used for social contacts. A camera was set 1.5 m above the apparatus, connected to a computer for video recording and sending signals to tracking software (ANY-maze, Stoelting Co. IL, USA). Illumination was provided by a LED source and adjusted to dim light. For object exploration, various objects with different textures (smooth or rough), sizes (diameter 7–9 cm, height 14–17 cm) and shapes (column, irregular) were applied. The objects were over 500 g to ensure that the animal was unable to move them. The assignment of objects was counterbalanced to minimize exploration preference for object and/or place.

2.3. Behavioral testing

Behavioral performance of BTBR (n = 9) was compared to C57BL/6J (n = 10). Testing was conducted between 10:00 to 17:00. At least 30 min before testing, the animals were transported from their colony to a sound-attenuating room where the apparatus was located. One test was administered per day until the assessments were completed. Ethanol (70%) was used to clean the apparatus after each trial.

2.3.1. Sociability test

The sociability test was composed of three sessions: habituation, sociability and social novelty, which evaluated sociable behavior and recognition of social novelty [25]. For habituation, the animal was placed into the middle-chamber and allowed to freely explore all three chambers. In the sociable session, a stranger mouse (the same strain and gender but never contacted before) was put underneath the grid cup and placed in one of the two side-chambers, while another identical empty cup was placed on the other side. In the social novelty session, another stranger mouse was put inside the previously empty cup. Each session lasted eight minutes. If an animal did not explore sufficiently in the apparatus (over 5 min only in the middle chamber), it was excluded (n = 1 of BTBR). Distance traveled and physical contacts around the cups with the nose, head and forelimbs, defined as sniffing behavior, were recorded. Sniffing behavior was used as an index, rather than time spent in the different chambers, because only close proximity was counted as involving sociable behavior [26].

2.3.2. Open field

The open field test was a well-validated and commonly conducted test for general locomotion and exploratory behavior. The animal was placed in the center of the arena for 20 min. Distance traveled, rearing, grooming, thigmotactic behaviors, entries and time stayed at the center (virtually central square 13.3 × 13.3 cm) were analyzed. Time spent at the center were used as an index of anxiety [27]. Rearing behavior was considered as not only vertical locomotor and exploratory activity but also an index for non-selective attention [28], while thigmotaxis, defined as movement along the walls so that one side of the vibrissae could contact the wall, was a measurement for sensorimotor function [29,30].

2.3.3. Object attention test

This test consists of one learning trial and one test trial. The procedure was identical to the novel object preference test [31] except that no time interval was applied between the trials. In the learning trial, two different objects were located in the open field and the animal was put into the arena. In the test trial, the two objects were removed together, replaced by an object identical to either explored objects and by a novel one (Fig. 3A). The animal remained in the arena during object replacement. Each trial lasted for five minutes. Animals were excluded from analysis when the total amount of time for object exploration in the learning trial was less than 20 s (n = 2 of WT). This paradigm could be considered as a working and/or short-term memory test where little time lapse between the learning and test trial existed. Alternatively, this test was described as an “object-based attention test” by Alkam who presented evidence for its measurement of attentional-associated processes [23,24]. To minimize the individual difference, an index listed below was calculated:

Index = ([novel object exploration – old object exploration])/(total object exploration).

Positive values represent intact processing for object-based attention and/or short-term memory.

2.3.4. Elevated open platform

This test exploited the innate tendency of fear of open space in rodents [32,33]. The apparatus was 30 cm height and the animal was placed into the center of the platform for five min. The platform was virtually divided into a center area (26.7 × 26.7 cm) and edges (6.65 cm width around the edges; Fig. 4A). Distance traveled, time spent in the center and edges were recorded. This test was modified from the paradigm designed by Ennaceur et al. [34,35], but no steep slopes attached. We predict that animals with high anxiety/fear tended to stay more at the edges than in the center area.

2.3.5. Drug administration

To establish the applicability of elevated open platform, a new batch

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