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Water T-maze: A useful assay for determination of repetitive behaviors in mice

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HIGHLIGHTS

- There are few behavioral tasks for mice that can be used to study higher order repetitive behaviors.
- BTBR mice, a commonly used mouse model for autism, do not show higher order repetitive behaviors in commonly used assays.
- With use of the water T-maze, higher order repetitive behaviors become perceptible in BTBR mice.
- Water T-maze is sensitive, easy to perform and less time intensive than other tasks that can be used to study higher order repetitive behaviors.

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ABSTRACT

Background: Repetitive behavior is a term used to describe a wide variety of invariant and inappropriate behaviors that occur in many diverse conditions, including autism. It is necessary to utilize and/or design rodent behavioral assays that exploit individual types of repetitive behavior so that underlying pathology and therapeutic measures can be determined. A variety of high-throughput assays to investigate lower order repetitive behaviors are available for rodents, whereas, fewer assays are available to investigate higher order repetitive behaviors, such as perseverative behavior. BTBR *T*+*tf*/J (BTBR) mice, harbor behavioral deficits that share similarity to the core deficits found in autism, yet have not conclusively demonstrated deficits in conventional reversal learning tasks (i.e. Morris water maze (MWM), T-maze) which are typically used to examine perseverance.
New method: By combining elements of both the MWM and T-maze, we designed a water T-maze assay to determine if perseverative behavior could become perceptible in BTBR mice.
Results: We found that BTBR mice show a significant impairment in reversal learning as compared to C57BL/6J (B6) mice in our water-T-maze reversal learning assay.
Comparison of existing methods: Our water T-maze is sensitive, simple to perform, inexpensive and less time intensive than other tasks that can be used to measure higher order repetitive behaviors.
Conclusions: Our findings suggest that our water T-maze assay is effective for determining perseverance, which is not readily revealed by using conventional methods.

1. Introduction

Repetitive behavior is an all-inclusive term used to describe a variety of behaviors linked by repetition, rigidity, invariance, and inappropriateness (Turner, 1999). Repetitive behaviors can be grouped into lower order repetitive behaviors or higher order repetitive behaviors. Lower order repetitive behaviors include spontaneous dyskinesias, stereotyped movements, repetitive manipulation of objects, and repetitive self-injurious behavior. Higher order repetitive behaviors include specific object attachments, an anxiously obsessive desire for sameness (Kanner and Eisenberg, 1957), repetitive use of language, and narrow and circumscribed interests (Turner, 1999). Repetitive behaviors occur in many diverse conditions, which include developmental disabilities (e.g. autism, mental retardation), psychiatric disorders (e.g. schizophrenia, obsessive compulsive disorder [OCD]), and neurological conditions (e.g. Parkinson disease, Tourette syndrome (Bodfish et al., 2000)).

Many genetic and pharmacological studies performed on rodents have been used to examine lower order repetitive

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behaviors (Mehta et al., 2011; Peça et al., 2011; Silverman et al., 2010; Smith et al., 2011; Tanimura et al., 2010). However, given the variability of repetitive behaviors, it is important to utilize rodent behavioral assays that are appropriate to particular repetitive behaviors. A variety of high-throughput assays to investigate lower order forms of repetitive behaviors are available for rodents. Such assays include those that measure grooming, bar biting and marble burying (McFarlane et al., 2008; Pearson et al., 2011). Predictably, as behaviors become more complex, as in the case of the higher order forms of repetitive behaviors, fewer assays are available.

Higher order repetitive behaviors, such as perseverative behaviors, are often found in persons with Autism Spectrum Disorders (ASD). Perseverative behaviors are characterized by the continuation of a response in the absence of its purpose. Perseverative behaviors may be caused by poor mental flexibility or problems with set-shifting (Hill, 2004). In persons with ASD, perseverative behaviors are more evident when engaging in complex reversal learning tasks (Coldren and Halloran, 2003; Lionello-DeNolf et al., 2008; Yerys et al., 2009). It is thought that perseverative behaviors interfere with learning and adaptive behaviors.

To investigate ASD-relevant perseverative behavior, assays that employ reversal learning, have been utilized. In particular, the Morris water maze (MWM) and the appetitive T-maze have been used (Yang et al., 2012; Moy et al., 2007). In MWM, rodents use spatial cues to learn the location of a submerged underwater platform for escape from a circular pool. Once learning criterion is met, the location of the platform is switched to another location in the pool, and the animal is faced with the task of learning the new spatial location (Morris, 1984). The degree of perseverative behavior can be determined from measurement of time spent and/or distance traveled in the quadrant of the pool where the platform was originally located. Performance in the MWM is dependent upon both hippocampal and striatal contributions (Devan et al., 1996). In T-maze, rodents learn to select a particular arm of the T as to obtain a positive reinforcer, which is often an appetitive one. Once the position habit is acquired, the reinforcer is moved to the opposite arm of the T, and the animal must inhibit the initial learned response and learn the new location of the reinforcer. Tendency for perseverative behavior can be determined by counting the number of entries into the original location of the reinforcer, or by examination of the amount of training needed for the animal to learn the new location of the reinforcer (Moy et al., 2007; Chadman et al., 2006).

In both MWM and T-maze, a non-probabilistic reinforcement schedule is typical. In non-probabilistic assays the correct choice is reinforced 100% of the time. Interestingly, neither assay has been used to convincingly exploit perseverative behavior in BTBR mice (Moy et al., 2007; Yang et al., 2012), an increasingly popular mouse model for autism that behavioral deficits which share similarity to the core deficits found in persons affected by autism. BTBR mice have an unusual vocal repertoire and low sociablity. Furthermore, they groom excessively and bury a larger number of marbles in marble burying assays, which suggests that they harbor lower order repetitive behaviors. (Defensor et al., 2011; McFarlane et al., 2008; Pearson et al., 2011; Pobbe et al., 2010, 2011; Scattoni et al., 2008). Recently one group was successful in revealing higher order perseverance behaviors in BTBR mice by using a probabilistic assay (Amodeo et al., 2012). Probabilistic assays are thought to be more ethologically relevant (Tsuchida et al., 2010), as reinforcement is not supplied every time a correct choice is made, but non-probabilistic assays may be easier to perform and analyze. Other assays exist and have been used to show perseverative behavior in BTBR mice (Rutz and Rothblat, 2012; Karvat and Kimchi, 2012), in genetically manipulated mice as well as in C57BL/6 (B6) mice (Brigman et al., 2008; Izquierdo et al., 2006). Nonetheless, some of these assays require long training periods and the use equipment that may not readily be available to many labs.

With respect to some of the difficulties that have been encountered in ascertaining perseverative behavior in mice, we sought to design an assay that was easy to implement, high-throughput and could straightforwardly be utilized. We designed a water-T-maze and position habit schedule to assay perseverative behavior. To assess the validity of our water T-maze assay, we examined and compared the performance of the BTBR and B6 mouse strains in both acquisition and reversal in the water T-maze. Individual mice each had to meet highly restrictive but simple to learn criteria before moving to the reversal phase.

2. Methods

2.1. Subjects

Ten naïve BTBR *T*$^{+}$*tf*/J (BTBR) and ten naïve C57BL/6J (B6) were used in this task. Mice were between four to six months of age at time of testing. Each strain tested was represented by an equal number of males and females. Two cohorts of ten mice were trained and tested at a time and each of the cohorts had equal representation of BTBR and B6 mice. Mice were kept on a 12 h light-to-dark cycle, with the light cycle beginning at 6 am. Mice were tested between 3 pm and 7 pm every day. Testing started at 3 pm and finished before the onset of the dark cycle on all days except the initial training day for acquisition. All mice were housed with three or four littermates of the same gender and were provided with chow and water ad libitum. All experiments were approved by the New York State Institute for Basic Research in Developmental Disabilities IACUC.

2.2. Apparatus

The water-T-maze used was constructed from Plexiglas. Each arm of the T was 18.5 cm long and 5 cm wide. The T was placed in a circular pool with a diameter of 45 cm. The pool was filled with 23 °C (±1 °C) water to a depth of 13 cm, which was 1 cm above the surface of the platform. Water was made opaque with white Crayola Paint. The platform was a 5 cm × 5 cm square made from Plexiglas that was made to specially fit into the T maze. Two Plexiglas rectangles were placed on top of the arms of the T to prevent the mice from jumping out of the pool once the platform was reached (Fig. 1A). Data were collected manually by a single observer.

2.3. Position habit acquisition

Mice were placed in the water T-maze without a platform and were allowed to swim for 60 s for pre-training. The first arm entered by the mouse was noted. On all training days, the platform was placed in the arm opposite to the first arm selected during pre-training (Fig. 1B). Mice were given ten training trials per day. Mice were dried with a towel and allowed to rest in between trials for the amount of time it took for all others in the cohort to complete their trial, which was approximately 7–10 min. Mice were placed in the pool in the starting arm of the T and were allowed up to 60 s to find the location of the platform. Once the platform was found, they were trained to remain on the platform for 5 s. If they were unable to find the platform, they were gently guided to the location and forced to sit on the platform for 10 s. On each trial, mice were charged with errors if (1) they left the start arm and entered into the arm which did not contain the platform or (2) entered into the arm with the platform and left that arm. If a mouse was able to complete eight out of ten of training trials per day, it was scored as having met the criterion for that day. Once the mouse was able to reach daily criterion for four consecutive days, it was moved into training for reversal position learning.