Contents lists available at ScienceDirect

Behavioural Brain Research

Research report

Reversal learning in C58 mice: Modeling higher order repetitive behavior

Cristina M. Whitehouse[a], Lisa S. Curry-Pochy[b,*], Robin Shafer[a], Joseph Rudy[a], Mark H. Lewis[a,b]

[a] Department of Psychiatry, University of Florida, McKnight Brain Institute, 1149 Newell Drive, L4-100, Gainesville, FL 32611, USA
[b] Department of Psychology, University of Florida, 945 Center Drive, Gainesville, FL 32611, USA

ARTICLE INFO

ABSTRACT

Restricted, repetitive behaviors are diagnostic for autism and prevalent in other neurodevelopmental disorders. These behaviors cluster as repetitive sensory-motor behaviors and behaviors reflecting resistance to change. The C58 mouse strain is a promising model for these behaviors as it emits high rates of aberrant repetitive sensory-motor behaviors. The purpose of the present study was to extend characterization of the C58 model to resistance to change. This was done by comparing C58 to C57BL/6 mice on a reversal learning task under either a 100% or 80%/20% probabilistic reinforcement schedule. In addition, the effect of environmental enrichment on performance of this task was assessed as this rearing condition markedly reduces repetitive sensory-motor behavior in C58 mice. Little difference was observed between C58 and control mice under a 100% schedule of reinforcement. The 80%/20% probabilistic schedule of reinforcement generated substantial strain differences, however. Importantly, no strain difference was observed in acquisition, but C58 mice were markedly impaired in their ability to reverse their pattern of responding from the previously high density reinforcement side. Environmental enrichment did not impact acquisition under the probabilistic reinforcement schedule, but enriched C58 mice performed significantly better than standard housed C58 mice in reversal learning. Thus, C58 mice exhibit behaviors that reflect both repetitive sensory motor behaviors as well as behavior that reflects resistance to change. Moreover, both clusters of repetitive behavior were attenuated by environmental enrichment. Such findings, along with the reported social deficits in C58 mice, increase the translational value of this mouse model to autism.

1. Introduction

Restricted, repetitive behavior (RRB) is diagnostic for autism spectrum disorder (ASD), common in other neurodevelopmental disorders (e.g., Fragile X, Prader-Willi, non-syndromic intellectual disability), and manifests in a number of neurological conditions (e.g., Tourette syndrome, Parkinson's disease, fronto-temporal dementia) [1–5]. RRB includes multiple categories of responding including stereotyped movements, self-injury, repetitive object manipulation, repetitive speech, compulsions, rituals, and circumscribed interests and sameness behaviors [1,6]. Factor analytic studies, using repetitive behavior items from ASD diagnostic instruments, have generated two, or in one case three, behavioral clusters [7,8]. The first aggregate labeled repetitive sensory-motor behavior, often called lower order, includes stereotyped motor responses with or without objects and self-injury. A second cluster labeled resistance to change or insistence on sameness, often called higher order, includes compulsions, rituals, and routines. Lam and Aman have presented evidence for a third factor of circumscribed interests [9].

The behavioral inflexibility or rigidity at the core of resistance to change or insistence on sameness has been examined in the context of laboratory tasks (see Rodriguez & Thompson [10] for a recent review). For example, Baron-Cohen [11] used a hide-a-penny task to show that children with ASD were more likely to generate a simple, predictable pattern, such as repeatedly switching from left to right hands. Beyond restricted sequence variability [12], rigid or inflexible responding can also manifest as difficulty in making a transition from a preferred behavior to alternative behaviors that may be more adaptive. Cognitive tasks involving reversal learning [13,14] and set-shifting [15–17] have demonstrated that ASD subjects often exhibit insistence on sameness and inflexible behavior. High levels of perseveration, when an individual continues with a previously correct rule despite negative feedback, are often seen in individuals with ASD and are thought to be a measure of cognitive inflexibility [18].

A number of efforts have been made to model, in animals, the repetitive behavior observed in neurodevelopmental and related disorders [19]. Such models allow for investigation of inducing conditions, pathophysiology, and potential treatments. To a large degree, these

models preferentially reflect repetitive sensory-motor behaviors such as excessive grooming, vertical jumping, or backward somersaulting. Less focus has been on modeling higher order RRB such as resistance to change or insistence on sameness. Nonetheless, resistance to change has been assessed in animals exhibiting high levels of motor stereotypy using a variety of tasks including response extinction, reversal learning, and intra- and extra-dimensional set shifting (e.g., [20–23]). The presence of both motor stereotypy and a measure of higher order repetitive behavior in the same animals has been demonstrated by several of these studies.

The C58 mouse strain has been shown to be a useful model for studying repetitive motor behavior due to its expression of stereotypic hind limb jumping and backward somersaulting which develop as early as post-natal day 16 [24,25]. These mice have also been reported to exhibit social deficits as evidenced by their lack of preference for social novelty in the three chamber social approach task [26,24] and a lack of affinity for a socially transmitted food preference [24]. Extending to higher order repetitive behavior, Moy et al. [27] found that C58 mice showed restricted exploration of a novel holeboard, but these mice did not exhibit a resistance to change following familiarization to an appetitive stimulus. Additionally, Muehlmann et al. [25] showed that C58 mice had an increased number of nose-poke responses during general exploration of a hole-board, but they did not exhibit increased marble burying nor did they display reduced exploratory behavior in the holeboard task. Finally, Blick et al. [28] assessed C58 mice in a novel object exploration task but found little evidence of patterned exploratory behavior which might reflect higher order repetitive behavior. Thus, it is unclear whether this promising mouse model exhibits higher order as well as lower order repetitive behavior. Expression of both clusters of repetitive behavior in the same mouse model would significantly increase its translational value. Therefore, the purpose of this study was to extend characterization of the C58 model to include higher-order repetitive behavior by examining resistance to change and behavioral inflexibility using reversal learning of a positional discrimination task as well as extinction of a conditioned behavior. C58 mice were compared to control C57BL/6 mice on these measures using an appetitive operant task. In Study 1, a fixed ratio 1 (FR1) schedule of reinforcement was used for responses on the left or right side on a positional discrimination task, whereas the opposite side did not receive any reinforcement. In Study 2, a probabilistic schedule of reinforcement was used on the same task, now with 80% of responses reinforced on one side and 20% of responses reinforced on the other side. In addition, we assessed the effect of housing on reversal learning on the same task using the probabilistic schedule of reinforcement. This was of interest as we have shown that post-weaning environmental enrichment markedly reduces stereotyped motor behavior in C58 mice [25].

2. Materials and methods

2.1. Study 1 FR1 reinforcement

2.1.1. Animals

C58 and C57BL/6 mice of both sexes were bred and housed in a colony room at the University of Florida. The breeding colony of C57BL/6 mice were replenished at least every 5th generation with new mice from Jackson Labs. Both the humidity (50%–70%) and temperature (70–75°F) were controlled, and the room was maintained on a 12:12 light:dark cycle (lights off at 8:00 pm). Fourteen C58 mice (5 males, 9 females) and 8 C57BL/6 mice (4 males, 4 females) were used for motor stereotypy assessment and reversal learning tests. All mice were reared in standard housing following weaning at postnatal day 21 (see Study 2 for a description of environmental enrichment). Standard housing consisted of mice group housed with up to 5 same-sex, same-strain mice in standard laboratory cages (29 × 18 × 13 cm). Water was available ad lib, and two Nestlet squares were provided for nest

construction. Animal care and use was performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and approved by the University of Florida Institutional Animal Care and Use Committee.

2.1.2. Equipment

Vertical hindlimb jumping and backward somersaulting, the primary motor stereotypies in C58 mice, were quantified using photobeam arrays (Columbus Instruments). Vertical displacement resulted in photobeam interruptions which were recorded as counts with accompanying time stamps [25]. The apparatus was set so rearing or other non-stereotyped vertical activity did not result in photobeam interruptions. All test sessions were video-recorded to identify the topography of stereotypy and verify the accuracy of the automated counters.

Operant chambers (Med Associates model ENV-307W, 21.6 × 17.8 × 12.7 cm; St. Albans, VT) were enclosed in sound attenuating cabinets, and were equipped with two nose-poke holes which could be illuminated with cue lights (ENV-314W with a diameter of 1.3 cm and a depth of 1 cm). The food hopper was located between the two nose-poke holes. Programming was written and data were collected using Med-PC IV software (Med Associates, St. Albans, VT). Operant chambers were vacuumed and cleaned with 70% ethanol between each session.

2.1.3. Motor stereotypy assessment

All C58 and C57BL/6 mice were assessed for their repetitive motor behaviors at postnatal day 53 using the apparatus previously described. Mice were placed in individual chambers (28 × 22 × 25 cm) 1 h before testing began. Following habituation, each animal was assessed for the 12 h dark cycle with food and water available (see [25])

2.1.4. Acquisition, reversal learning, and extinction of a positional discrimination task

Starting at postnatal day 63, access to food was adjusted to reduce the animals' weights to 85–90% of their individual ad lib. feeding weight, after which, testing began. None of the mice had previous experience with operant testing, and all operant testing occurred during the light cycle between 9:00 am and 4:00 pm. Before each session, mice were given a 10 min habituation session in the operant chamber with the house light off prior to each 60 min session. Mice were randomly assigned to be reinforced on a FR1 schedule for nose pokes on either the right or left side during acquisition and were reinforced with a 14 mg Dustless Precision Pellet®: Rodent, Purified (Bio-Serv®). Upon a correct response, the yellow cue lights illuminating the two nose-poke holes turned off to signify that they were inactive, a reinforcer was delivered to the food hopper, and a click tone sounded to signify the delivery of the reinforcer. Once the mouse entered the hopper to retrieve the reinforcer, the nose-poke holes returned to their active, illuminated state. No formal shaping was conducted; the animals were left to explore the chamber and contact the reinforcement contingency, the nose-poke hole, on their own. If after 3 days the animal was not responding during the 60 min session, a food pellet from the home cage was rubbed around the nose poke hole to increase the probability of coming into contact with the reinforcement contingency. One female C58 mouse and 1 male C57BL/6 mouse received this intervention and responding increased in later sessions.

The acquisition criterion was 80% correct responses for 4 consecutive sessions. If after 32 sessions a mouse had not met this criterion, it was dropped from the study. Two female C58 mice did not meet this criterion, and 1 female C58 mouse was not included in the results section due to a technical error. After meeting the acquisition criterion, the mice were exposed to a reversal learning contingency in which responses were reinforced at the nose-poke hole opposite to the one at which they were reinforced during acquisition. Reversal learning criterion for each strain was the same as the acquisition criterion. Once the reversal learning criterion was met, an extinction condition was