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Reversal learning in C58 mice: Modeling higher order repetitive behavior

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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.

Keywords

stereotypy; environmental enrichment; autism spectrum disorders; neurodevelopmental disorders; resistance to change

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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) [1,2,3,4,5]. 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 [15,16,17] 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., [20,21,22,23]). 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). Thirteen C58 mice (5 males, 8 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, $21.6 \times 17.8 \times 12.7$ 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 1.3cm and a depth of 1cm). 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 ($28 \times 22 \times 25$ 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 initiated in which nose-pokes at either position were not reinforced. Extinction was carried out for 4 sessions.

2.2 Study 2 80%/20% Probabilistic Reinforcement

2.2.1 Animals—In Study 2, a separate group of 8 standard housed C58 mice (2 males, 6 females), 4 environmentally enriched C58 mice (4 females), and 8 standard housed C57BL/6 mice (4 males, 4 females) were used for motor stereotypy assessment and reversal learning tests. Room conditions and animal care and use were the same as described in section 2.1.1. All animals in Study 2 were assessed for motor stereotypy and used the same behavioral testing equipment as described in sections 2.1.2 and 2.1.3, respectively.

2.2.2 Environmental Enrichment—C57BL/6 mice and C58 mice assigned to standard housing were reared as described in section 2.1.2. C58 mice assigned to environmental enrichment were group housed with 3 other same-sex mice weaned at postnatal day 21 in a large dog kennel (121.9 × 81.3 × 88.9 cm) customized with two extra levels and connected ramps constructed from galvanized wire [24]. A running wheel, a large opaque shelter, and Habitrail tubes were kept in the kennel throughout the 6-week enrichment period. Various objects (e.g., plastic toys, domes) were also present in the kennel, but they were rotated weekly to maintain novelty. During the weekly rotation of objects, approximately 2 oz. of bird seed was scattered into the kennel to promote foraging. Water was available *ad lib*, and 4 Nestlet squares were provided for nest construction. The kennel was kept in the same room as the standard housed animals.

After 5 weeks in enrichment and following assessment of motor stereotypy, mice were placed into a modified enriched environment to facilitate handling during testing. The modified enrichment cage is the same size as the standard housing cage (29 × 18 × 13 cm) and included a running wheel, 2 plastic toys that were rotated weekly, and 2 oz. of bird seed scattered weekly. Water was available *ad lib*, and two Nestlet squares were provided. The modified enrichment cages were kept in the same room as the standard housed animals

2.2.3 Acquisition and Reversal Learning of a Positional Discrimination Task—Food restriction and FR1 acquisition procedures were the same as described in Section 2.6. Once the criterion was met for the FR1 acquisition (80% correct responses for 4 consecutive sessions), nose-pokes on the same side were now reinforced 80% of responses rather than 100%. Nose-pokes to the opposite side, previously unreinforced, were reinforced 20% of responses.

Allocation of at least 80% of total responses to the 80% reinforced side during the first 20 min of 4 consecutive sessions was used as the criterion for acquisition. This session duration was selected as C58 mice were observed in Study 1 to respond markedly fewer times over the course of the last 40 min of the session than did the C57BL/6 mice.

After meeting the acquisition criterion, mice were exposed to a reversal learning condition in which 80% of responses were reinforced at the nose-poke hole opposite to that reinforced at 80% during acquisition. The criterion for completing reversal learning was the same as in

acquisition. As only 1 C58 mouse met criterion for reversal learning, an extinction condition was not included in this study.

2.3 Statistical Analyses

Independent sample t-tests were conducted to compare strain and housing differences in the frequency of motor stereotypy, the number of responses emitted to reach acquisition and reversal learning criterion, and the frequency of responses emitted when no contingency was in effect during reversal learning. The strain and housing group differences in the number of responses to criterion during acquisition and reversal learning were plotted by the Kaplan and Meier method and compared by the two-tailed log-rank test using Chi-Square [29]. This analysis allows for investigation of differing survival times to an event, when not all subjects remain in the study for the same duration (e.g., drop out when performance criterion is met) [31]. In the present study, the number of responses needed to meet criterion was analogous, in conventional survival curves, to time to reach an event. This was because of the strain differences in level of responding during a session. Also, as in survival analyses, we could employ censoring if an animal did not complete the study. In the present study that was reflected in terminating sessions for any mouse failing to reach criterion after a lengthy period of training. In Study 1, group differences of the proportion of responses made on the previously reinforced side to total responses for the first 4 sessions of extinction were analyzed using a General Linear Model Repeated Measures Analysis of Variance model (RM-ANOVA).

Group differences in pattern of nose-pokes during reversal learning were also analyzed. Responses on the low reinforcement density side as a proportion of total responses were examined across reversal learning sessions using RM-ANOVA. The RM-ANOVA analyses for these responses included the first 5 days of reversal learning for Study 1, whereas Study 2's analysis used the first 10 days of reversal learning. These time periods were selected as after day 5 (or day 10), because individual animals had met criterion and no additional reversal learning sessions were run on these mice..

The association between motor stereotypy and measures of the positional discrimination task were analyzed by Pearson correlation or Spearman rank correlation where applicable. The measures of positional discrimination included the number of responses for the acquisition and reversal learning sessions, the number of responses to criterion (for acquisition and reversal), responses on the low reinforcement density side as a proportion of total responses in reversal learning, and the proportion of responses on the previously reinforced side in extinction for Study 1.

Strain $\times$ sex interactions and sex main effects were not significant for any strain comparison analyses, and therefore the reported results did not include sex in the model. For all analyses, differences were considered significant when $p < 0.05$.

3. RESULTS

3.1 Study 1 FR1 Reinforcement: Strain Comparison

The probability of C58 and C57BL/6 mice meeting acquisition criterion as a function of responses emitted was comparable, $\chi^2(1) = 0.05$, $p = 0.83$ (see Fig. 1A). The number of responses needed to meet criterion also did not differ between strains, $t(19) = 0.16$, $p = 0.87$. All C57BL/6 mice tested met acquisition criterion, whereas 2 female C58 mice did not meet acquisition criterion in 32 sessions. The animals not meeting acquisition criterion, though included in these analyses, did not move on to the reversal learning and extinction conditions.

During the reversal learning condition, no differences were found in the probability of meeting criterion as a function of the number of total responses emitted during reversal learning, $\chi^2(1) = 0.07$, $p = 0.80$ (see Fig. 1B). Moreover, the strains did not differ in the number of responses required to meet criterion during reversal learning, $t(17) = 0.34$, $p = 0.74$. All mice met reversal learning criterion.

An analysis of the pattern of nose-pokes during reversal learning determined that C58 and C57BL/6 mice emitted a similar proportion of responses on the non-reinforced side across reversal learning sessions, $F(1, 17) = 1.38$, $p = 0.26$.

During extinction, C58 mice initially (sessions 1 and 2) showed a greater but non-significant proportion of responses on the previously reinforced side, but this difference was not apparent by third and fourth sessions of extinction, $F(1, 17) = 0.34$, $p = 0.57$ (see Fig. 1C).

With regard to motor stereotypy, C58 mice displayed significantly more hindlimb jumping and backward somersaulting than C57BL/6 mice, $t(12.05) = 7.02$, $p < .001$ (see Fig. 2A). The motor stereotypy of C58 mice that met acquisition criteria was positively correlated with the number of responses emitted to meet reversal learning criterion, $r_s = .62$, $n = 11$, $p = 0.04$ (see Fig. 2B). No other measure of operant responding analyzed was significantly correlated with motor stereotypy.

3.2 Study 2 80%/20% Probabilistic Reinforcement: Strain Comparison

During probabilistic reinforcement, the probability of meeting acquisition criterion as a function of the number of total responses was not significantly different between strains, $\chi^2(1) = 2.62$, $p = 0.11$ (see Fig. 3A). The number of responses needed to meet criterion also did not differ between strains, $t(14) = 1.52$, $p = 0.15$. All mice met acquisition criterion by session 10.

The probability of meeting reversal learning criterion as a function of total responses differed markedly between strains, $\chi^2(1) = 9.74$, $p = .002$. The survival curves revealed that C58 mice needed a significantly greater number of responses than C57BL/6 mice to meet reversal learning criterion (see Fig. 3B). Moreover, only 1 of the 8 (12.5%) C58 mice met reversal learning criterion, whereas 7 of the 8 (87.5%) C57BL/6 mice met reversal learning criterion. This was a significant difference using Fischer's exact test ($p = 0.01$). Due to the

small number of C58 mice meeting criterion, a t-test could not be performed to assess the number of responses emitted to meet criterion.

There was a significant interaction between session and strain on the proportion of responses on the low reinforcement density side across reversal learning sessions, $F(3.9, 54.8) = 6.41$, $p < .001$, with a main effect of both strain, $F(1, 14) = 37.36$, $p < .001$, and session, $F(3.91, 54.79) = 8.30$, $p < .001$. Both C58 and C57BL/6 mice emitted the majority of their responses on the low reinforcement density side during the first two sessions of reversal learning. After the third session, however, C58 mice continued to make the majority of their responses on the low reinforcement density side whereas the C57BL/6 mice transitioned to the high reinforcement density side (Fig. 3C). Both strains made a small number of non-contingent responses (i.e., nosepokes made when cue lights were turned off) during reversal learning sessions. This measure did not differ significantly between strains.

As in Study 1, C58 mice displayed significantly more motor stereotypy than C57BL/6 mice, $t(7.0) = 4.05$, $p = .005$ (see Fig. 3D). These jumping and somersaulting counts were not correlated with any operant responding measure analyzed.

3.3 Study 2 80%/20% Probabilistic Reinforcement: Housing Comparison

During probabilistic reinforcement, no differences between standard housing (SH) and environmental enrichment (EE) C58 mice were found in the probability of reaching acquisition criterion as a function of the number of total responses emitted during acquisition, $\chi^2(1) = 0.01$, $p = 0.91$ (see Fig. 3A). The number of responses emitted to reach criterion was also not significantly different between housing groups, $t(10) = 0.09$, $p = 0.93$.

The survival distributions for meeting reversal learning criterion as a function of total responses differed significantly between housing conditions. C58 mice reared in EE needed fewer responses to reach reversal learning criterion than SH C58 mice, $\chi^2(1) = 4.46$, $p = 0.04$ (see Fig. 3B). Criterion for reversal learning was achieved by 12.5% (1/8) of SH C58 mice and by 75% (3/4) of EE C58 mice, but this was not a significant difference using Fischer's exact test ($p = 0.07$).

SH C58 mice performed significantly more of their total responses on the low reinforcement density side across reversal learning sessions compared to EE C58 mice, $F(1, 10) = 4.88$, $p = 0.05$ (see Fig. 3C). There was no significant housing $\times$ session interaction or main effect of session.

C58 mice reared in standard housing displayed significantly more motor stereotypy than EE C58 mice, $t(7.03) = 3.97$, $p = .005$ (see Fig. 3D).

4. DISCUSSION

The present experiments were designed to determine if C58 mice, that have been shown to exhibit a highly robust lower order repetitive behavior, would also display higher order repetitive behavior. To assess higher order repetitive behavior in C58 mice, resistance to change was assessed during reversal learning and extinction of a positional discrimination task using two different reinforcement contingencies.

Consistent with previous studies [24, 25], standard housed C58 mice exhibited high rates of motor stereotypy characterized by hindlimb jumping and backward somersaulting. C58 mice performed similarly to control C57BL/6 mice in the acquisition, reversal learning, and extinction phases under a FR1 schedule of reinforcement. Use of an 80%/20% probabilistic schedule of reinforcement resulted in a very different outcome, however. Importantly, and as in Study 1, C58 and C57BL/6 mice performed comparably during acquisition. During reversal learning, however, C58 mice were markedly impaired in their ability to reverse their pattern of responding from the low reinforcement density side to the high reinforcement density side. C57BL/6 mice achieved this reversal much more readily. C58 mice persisted, then, in engaging in a response, the reward for which had now been markedly de-valued. This is a widely used operational definition of habit (e.g., [31,32]). Thus, one potential interpretation of the marked impairment of C58 mice in reversal learning is that these mice were more likely to develop habitual responding over the training period than control mice. Interestingly, rats with a preference for a habitual learning strategy have been shown to develop repetitive motor behavior (schedule-induced polydipsia) more readily than rats using a place-learning strategy [33]. Disorders such as Tourette syndrome, obsessive-compulsive disorder (OCD), and addiction have been linked to an imbalance in goal-directed vs. habitual responding, but the literature related to ASD is lacking. Future studies will assess independently whether differences in habit formation exist between these strains.

During both Study 1 and Study 2, C58 mice responded markedly fewer times over the course of the last 40 min of the session than did the C57BL/6 mice. We altered our Study 2 criterion to reflect the first 20 min of responding to have comparable levels of responding for analysis. C58 mice could have decreased responding for multiple reasons such as satiety or competing motor tasks such as jumping and backward somersaulting, but we do not have the empirical data from this experiment to support these hypotheses.

The motor stereotypy of C58 mice was positively correlated with the number of responses needed to reach FR1 reversal learning criterion. This association of reversal learning performance and repetitive behavior is similar to that reported by Judge et al. [34] who showed a positive correlation between errors on a reversal learning task and self-directed behaviors (e.g., self-grooming, self-scratching) using animals from three different non-human primate species. In addition, our lab [22] showed a positive correlation between measures of repetitive motor behavior and reversal learning errors in a T-maze using deer mice.

The strain differences obtained here between C58 and C57BL/6 mice are similar to those reported by Amodeo et al. [21] who compared BTBR mice, which exhibit excessive grooming, to C57BL/6 control mice on a different experimental task than used in our study. As in the present study, these investigators found no strain difference in acquisition or reversal of a spatial discrimination task with 100% reinforcement of correct trials but clear reversal learning deficits in BTBR mice when using an 80%/20% probabilistic reward schedule. No attempt was made to correlate reversal learning performance with the excessive grooming phenotype.

The results of the present study and those of Amodeo et al. [21] are consistent with findings in the ASD literature. D'Cruz et al. [14] used a probabilistic reversal learning task and showed that although ASD participants did not differ from controls in acquisition performance, they required more trials to meet reversal learning criterion. Using the Wisconsin Card Sort Task (WCST) to test for executive function, many studies consistently report that individuals with ASD show impairment characterized by increased perseveration [18].

Environmental enrichment also altered the repetitive behavior phenotype of C58 mice. The marked attenuation by enrichment of repetitive motor behavior in C58 mice replicated the larger study of such effects by Muehlmann et al. [25]. Environmental enrichment was not used in Study 1 under a FR1 schedule of reinforcement due to the lack of strain differences in behavior. Under probabilistic reinforcement, however, standard housed (SH) and environmentally enriched (EE) mice performed similarly in acquisition, but EE C58 mice needed significantly fewer responses than SH C58 mice to meet reversal learning criterion. Moreover, 3 of 4 EE C58 mice met reversal learning criterion whereas only 1 of 8 SH C58 did and, over time, EE C58 mice emitted markedly fewer responses on the low reinforcement density side than SH C58 mice. The small number of EE C58 mice warrant some caution, however, and replication is certainly needed. The reduction of both higher order and lower order repetitive behavior in C58 mice by environmental enrichment has not been previously described in the literature. We have shown, however, that deer mice performed better on both acquisition and reversal learning in a T-maze when reared in environmental enrichment. Moreover, stereotypy scores in these animals were significantly correlated with the number of errors made in reversal learning [22].

Our study did not find any effects of sex in these strain comparison studies. This may reflect a Type II error as we likely did not have enough power to find these effects, particularly interactions, if present. In Study 2 the effects of environmental enrichment were confounded with sex. The translational value of sex effects to the field of ASD research is highly relevant, and future work should aim to reexamine the role of sex in an animal model of repetitive behavior. However, we do not expect that sex is responsible for the higher order repetitive behavior because previous experiments in C58 mice have found no significant sex effects driving lower order stereotypy [25].

The findings of the present study suggest that C58 mice exhibit behaviors that reflect both lower order and higher order repetitive behavior. Moreover, both repetitive behavior types 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 ASD. Behavioral characterization of both lower order and higher order repetitive behavior in C58 mice sets the stage for future studies examining pharmacological and experiential interventions as well as the neurobiological basis for both lower order and higher order repetitive behavior.

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Highlights

- C58 mice are significantly impaired on a probabilistic reversal learning task.
- C58 mice exhibit both repetitive sensory-motor behaviors and resistance to change.
- Environmental enrichment reduces both repetitive behavior domains in C58 mice.

mice ($n=8$), but this difference was not apparent by latter sessions of extinction, $F(1, 17) = 0.34$, $p = 0.57$. All values are group means $\pm$ SEM.

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Figure 2.

Strain comparison of motor stereotypy in Study 1 (FR1 schedule of reinforcement). (A) C58 mice ($n=13$) performed significantly more vertical repetitive behaviors than C57BL/6 mice ($n=8$), $t(12.05) = 7.02$, $p < .001$. (B) The amount of repetitive motor behaviors emitted by C58 mice that met acquisition criterion ($n=11$) was positively correlated with the number of responses needed to reach reversal learning criterion, $r_s = .62$, $n = 11$, $p = 0.04$. All values are group means $\pm$ SEM.

Figure 3.

Study 2 strain and housing comparison of acquisition and reversal learning of a positional discrimination task (80%/20% probabilistic schedule of reinforcement). (A) The probability of standard housed (SH) C58 mice ($n=8$), environmentally enriched (EE) C58 mice ($n=4$), and C57BL/6 mice ($n=8$) meeting acquisition criterion as a function of responses emitted did not differ between strains, $\chi^2(1) = 2.62$, $p = 0.11$, or between housing conditions, $\chi^2(1) = 0.01$, $p = 0.91$. (B) During probabilistic reinforcement, the probability of meeting reversal learning criterion differed significantly between strains, $\chi^2(1) = 9.74$, $p = .002$. Only 12.5% (1/8) of SH C58 mice met reversal learning criterion, whereas 87.5% (7/8) of C57BL/6 mice met reversal learning criterion. The probability of SH C58 mice and EE C58 mice meeting reversal learning criterion as a function of responses emitted differed significantly between housing conditions, $\chi^2(1) = 4.46$, $p = 0.04$. Only 12.5% (1/8) of SH C58 mice met reversal learning criterion, whereas 75% (3/4) of EE C58 mice met reversal learning criterion. (C) There was a significant strain $\times$ session interaction on the proportion of responses on the low reinforcement density side across reversal learning sessions, $F(3.91, 54.79) = 6.41$, $p < .001$, with a main effect of both strain, $F(1, 14) = 37.36$, $p < .001$, and session, $F(3.91, 54.79) = 8.30$, $p < .001$. Both SH C58 and C57BL/6 mice initially emitted the majority of their responses on the low reinforcement density side. After the third session, however, SH C58

mice continued to make the majority of their responses on the low reinforcement density side whereas the C57BL/6 mice transitioned to the high reinforcement density side. Moreover, SH C58 mice emitted a greater proportion of responses on the low reinforcement density side across reversal learning sessions than EE C58 mice, $F(1, 10) = 4.88$, $p = 0.05$. (D) C57BL/6 mice performed significantly fewer vertical repetitive behaviors than SH C58 mice, $t(7.0) = 4.05$, $p = .005$. SH C58 mice, however, performed significantly more vertical repetitive behaviors than EE C58 mice, $t(7.03) = 3.97$, $p = .005$. All values are group means $\pm$ SEM.