Received: 9 January 2021 | Revised: 1 May 2021 | Accepted: 4 May 2021

DOI: 10.1111/gbb.12741

ORIGINAL ARTICLE

Genes, Brain and Behavior

Progression of behavioral deficits during periadolescent development differs in female and male DISC1 knockout rats

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Funding information
National Center for Research Resources, Grant/Award Number: 5P20RR016463-12; National Institute of General Medical Sciences, Grant/Award Number: 8P20GM103423-12

Abstract

Mutations in the disrupted in schizophrenia-1 (DISC1) gene are associated with an increased risk of developing psychological disorders including schizophrenia, bipolar disorder, and depression. Assessing the impact of knocking out genes, like DISC1, in animal models provides valuable insights into the relationship between the gene and behavioral outcomes. Previous research has relied on mouse models to assess these impacts, however these may not yield as reliable or rich a behavioral analysis as can be obtained using rats. Thus, the goal of the present study was to characterize the behavioral effects of a biallelic functional deletion of the DISC1 gene in the Sprague Dawley rat. Female and male wild type and DISC1 knockout rats were assessed beginning just prior to weaning and during the post-weaning periadolescent period. The primary outcomes evaluated were activity, anxiety, responses to novel objects and conspecifics, and prepulse inhibition. These behaviors were selected as analogous indices of psychological dysfunction in humans. The DISC1 knockout had significant effects on behavior, although the kind and magnitude of deficits was different for females and males: in females, effects included hyperactivity, aversion to novelty, and a modest prepulse inhibition deficit; in males, effects in anxiety and neophobia were mild but their prepulse inhibition deficit was large. These data confirm that the DISC1 knockout rat model is an excellent way to reproduce and study symptoms of psychological disorders and provides compelling evidence for differential consequences of its dysfunction for females and males in the progression and emergence of specific behavioral deficits.

KEYWORDS
anxiety, locomotion, schizophrenia, sensorimotor gating, sex differences, Sprague Dawley rats

1 | INTRODUCTION

Recent efforts to uncover the genetic bases to psychological disorders have identified genes that are associated with increased incidence of and risk for them (reviewed in Refs. 1,2). Among these is the gene, disrupted-in-schizophrenia-1 (DISC1). This gene was first identified in a Scottish family sustaining unusually high rates of psychiatric illness.[3] In this family, balanced translocations between chromosomes 1 and 11 (T(1;11)(q42;q14) were highly associated with psychiatric diagnosis, including schizophrenia but also bipolar and major depressive disorder. These are devastating and unremitting psychological disorder that affect over 250 million people worldwide.[4] Although descriptions

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and characterizations of these disorders are plentiful and much is known about the biological mechanisms that give rise to their features, there is much less clarity about the etiological bases for them. Animal models that investigate the impacts of gene mutations and knockouts are a valuable tool in gaining insights into the gene's functions and role in behaviors associated with psychological conditions.

DISC1 encodes a scaffolding protein with multiple functions associated with embryonic and adult neurogenesis, neurite growth and migration, and synaptic transmission and plasticity.[5–9] It interacts with several signaling molecules and is an essential mediator of their roles in developmental plasticity.[10] These findings align well with the kinds of early, abnormal developmental profiles that are suspected to contribute to the later in life emergence of schizophrenia (see Ref. 11) as well as the stress-diathesis models of major depressive and bipolar disorder (e.g., Ref. 12). However, despite significant strides in our understanding of the genetic basis of psychological disorders, it remains unclear how and why a genetic predisposition, such as a mutation in the DISC1 gene, may eventually precipitate psychological disorders in some individuals, but not others.

Most etiological hypotheses rest on the idea that perturbations to brain development early in life are central to disease etiology. For schizophrenia, the neurodevelopmental hypothesis posits that a genetic mutation or gene variant could trigger the abberant neural development that accumulates and escalates until the disorder is fully expressed.[13] In the two-hit hypothesis (including stress-diathesis), a genetic mutation or gene variant could serve as the first, early life hit making an individual vulnerable to a second hit later in life.[14,15] Thus, understanding the kinds of genetic backgrounds that may produce these susceptibilities has the potential to advance significantly our understanding of what causes psychological disorders and ways to circumvent or correct these risks. In this way, the improper functioning of the DISC1 gene could perturb normal neural developmental processes that lead to the eventual emergence of behavioral deficits in late adolescence or early adulthood or interact with significant life events occurring beyond the developmental period, placing individuals at an increased risk for developing a psychological disorder. In either case, the prediction addressed in the present study is that a functional deletion in the DISC1 gene in a rat model would increase the likelihood of a symptom profile in the social, emotional and cognitive domains and a short-term longitudinal design was used to assess the early appearance of deficits in female and male rats from before weaning to mid-adolescence.

2 | MATERIAL AND METHODS

2.1 | Animals – wild type and DISC1 knockout female and male rats

Subjects in the study were the female and male offspring of 6 breeding pairs: 3 pairs were wild type (WT) and 3 were DISC1 knockout (DISC1-KO) rats (Sage® Labs; Horizon Discovery, Cambridge, MA). The specific knockout was a result of a CRISPR-Cas9 20 base deletion in the DISC1 gene on chromosome 19, causing an early stop codon which causes termination at exon 6 out of 14. All rats in the study, including parents and offspring, were housed on a 12:12 h light: dark cycle with lights on at 0800 under colony conditions of 20°C ± 1 and 30%–50% humidity; all procedures were carried out during the light phase of the cycle. Access to rat chow and water was ad libitum and corncob bedding was used to line cages. Breeding pairs were housed together in $26 \times 47.6 \times 20.3$ cm^3 polycarbonate cages for 1 week for mating, after which pregnant females were removed and housed singly in $30.80 \times 30.80 \times 18.72$ cm^3 clear polycarbonate cages. All 6 dams gave birth within 48 h of each other and cross-fostering occurred on postnatal day (PD) 2: all pups were collected, toe-marked for genotype, and returned to dams in mixed litters of 10–12 female, male, wild type, and DISC1 knockout pups. Dams and litters remained undisturbed during rearing except for routine colony husbandry. Litter size and composition were similar for WT and DISC1-KO dams (data not shown) with the exception of WT dams giving birth to fewer female pups; the conditions and sample sizes were: female-WT ($n = 8$); female-DISC1-KO ($n = 19$); male-WT ($n = 18$), and Male-DISC1-KO ($n = 16$). Maternal behaviors were observed on PD 3 through 10 and no detectable impacts of the knockout were observed (data not shown). Pups were weaned into same-sex, same genotype pairs on postnatal day 23 and housed in the clear polycarbonate cages that were individually-ventilated on mobile cage rack systems (Thoren Caging Systems, Inc., Hazelton, PA). A schematic representation of these procedures is shown in Figure 1. Methods were approved by the Institutional Animal Care and Use Committee of Colby College and conducted in compliance with federally regulated standards and the Office of Laboratory Animal Welfare of the National Institutes of Health.

2.2 | Offspring behavioral tests

An overview of the experimental timeline is shown in Figure 2.

2.2.1 | PD21-22 – small open field test of activity and exploration

Prior to weaning, on PD21 and 22, female and male WT and DISC1 KO rats were assessed for their tendency to explore a novel environment as indexed by their individual activity in a small open field. The field was a 60×70 cm^2 wooden box with 90 cm high walls that was painted black and lined with a thin layer of corn cob bedding. Soiled bedding from each litter's cage was collected and added to the bedding in the field. Along the center of one wall of the field there was a small $6 \times 6 \times 6$ cm^3 wooden box with a single opening that faced the center. Pups were tested singly with females from litters being evaluated on PD21 and males on PD22. Each pup began their 5-min test in the shelter and the latency to emerge was recorded as a marker of low exploration. If the pup emerged, total distance traveled in the field outside of the enclosure was recorded, as well as number of returns

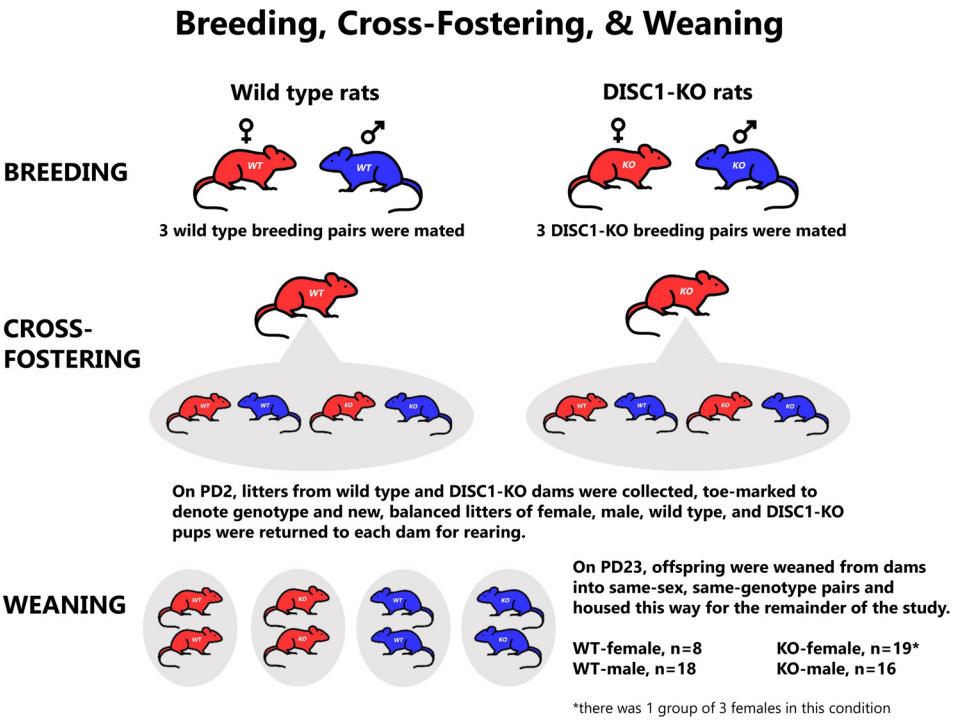

FIGURE 1 Schematic representation of the breeding, cross-fostering, and weaning/housing procedures

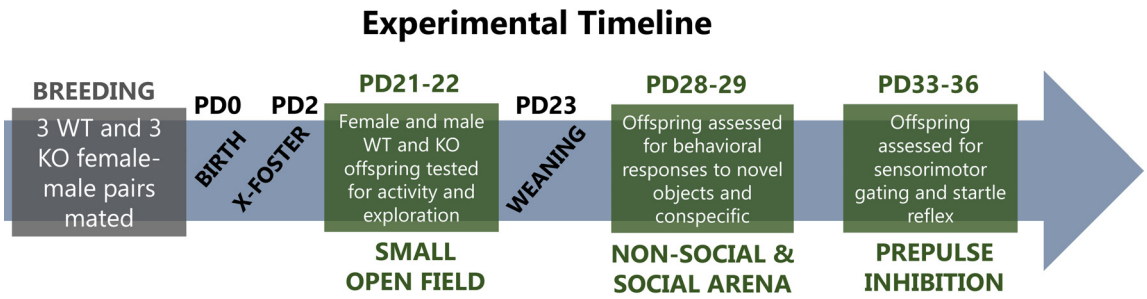

FIGURE 2 Experimental timeline of the procedures

to and duration of time in the enclosure. These measures gauged overall activity levels in the pups. All tests were recorded via an overhead camera (Logitech Quickcam Pro 9000 with 2MP, 720p HD, and 1600 × 1200 at 30fps) connected to AnyMaze tracking software (Stoelting Co., Wood Dale, IL) to gather and summary rats' behavior.

2.2.2 | PD28-29 – behavioral response to non-social or social novelty

One week after the first test and 5 days following weaning, rats were assessed in a large open field for their response to novelty in a non-social or social context. Thus, each rat was tested twice on the same day: once in the non-social arena to measure their reaction to novel objects and a second time in the social arena to measure their response to a novel conspecific.[16] As above, rats were tested over 2 days—in this case, males were tested first on PD28 and females were tested on PD29. The procedures described below were the same for female and male test days. The large open field was a 100 × 100 cm^2 wooden box with 30 cm high walls and the floor was covered with a thin layer of corn cob bedding. Here too, a digital camera was suspended over the field and each test was recorded for archiving and tracked for behavioral analysis using AnyMaze.

Non-social novelty – Objects

Rats were placed singly in the field for 5 min with two different, novel objects positioned in adjacent corners. Object investigation was recorded when the rat's head was oriented toward the object within 2 cm. The primary dependent measure was the latency of rats to investigate each object; also collected was the duration of time spent investigating objects, number of investigations and the average duration of investigatory bouts.

Social novelty – A stimulus rat

Approximately 4 h after the non-social assessment, rats were returned to the field for 5 min with a novel conspecific enclosed in one corner.

To do this, adjacent corners were modified with an 8 × 8 cm^2 thin mesh enclosure. For each test a stimulus rat, novel to the test subject, was placed inside one the enclosure and the other remained empty; the position of the stimulus rat was counterbalanced across conditions. Once tested, rats were queued up to serve as the stimulus rat for another rat in the study. This meant that the last rat tested served as stimulus for the first rat tested and thus first served as a stimulus rat before its own test. There was a minimum of 3 h between their test and serving as a stimulus rat for another rats' test. The primary dependent measure was rats' latencies to investigate the conspecific, the duration of time spent investigating the conspecific, number of investigations, and the average duration of investigatory bouts.

2.2.3 | PD33-36 – prepulse inhibition

In the third week, rats began a 3-day procedure to assess prepulse inhibition to acoustic stimuli. These procedures were conducted using the SR-LAB startle response system (San Diego Instruments, Inc., San Diego, CA)—a sound attenuated cabinet (38.1 × 35.56 × 45.72 cm^3) equipped with an animal enclosure (20.32 cm long and 8.89 cm internal diameter) centered upon the accelerometer that senses and records the rat's displacement magnitude in Hz, and LED house lights and a puretone generator to produce the acoustic stimuli. The 3-day procedure began on PD33 for males and PD34 for females.

Habituation

On the first day, rats were placed inside the enclosure in the cabinet for 10 min with only the fans and house LED lights on. On the second day, rats were exposed to the acoustic stimuli that would be used in the test. They were placed in the enclosure in the cabinet and allowed to acclimate for 5 min. This time 70 dB background noise was continuously present during acclimation and for the entire session. Following acclimation, there were 24 trials of acoustic stimuli presented in a pseudorandom order to ensure that each trial type occurred at least once in an 8-trial block. Of the 24 trials, 15 were a 40-ms presentation of the acoustic startle stimulus of 120 dB and 9 trials were a 20-ms presentation of the acoustic prepulse stimuli—3 each of 75, 80, and 85 dB. The intertrial interval (ITI) averaged 30 s with a range of 15–45 s.

Prepulse inhibition test

The third day was the prepulse inhibition (PPI) test day. Rats were again allowed to acclimate for 5 min before trials began and continuous background noise was present during this period and the entire session. The test session consisted of a pseudorandom presentation of 60 trials of 6 types with each type occurring at least once in a 10-trial block. The trial types were 12 "no stimulus" trials in which no acoustic stimulus was presented but accelerometer data were recorded, 12 startle trials in which a 120 dB acoustic stimulus was presented for 40 ms, and 36 prepulse trials in which one of the prepulse stimuli (75, 80, or 85 dB) were presented for 20 ms and followed 100 ms later by a 40-ms presentation of the 120 dB startle stimulus. Each of the 3 prepulses (5, 10, and 15 dB over background) were used 12 times each and the ITI during this session was also an average of 30 s with a range of 15–45 s. Startle responses were measured in Hz by the accelerometer and averaged for each trial type. These averages were then used to calculate the percentage of prepulse inhibition (% PPI) for each rat for each prepulse intensity, as follows: % PPI = 100 × ([AS-PP]/AS), where AS is the average startle reaction of that rat to the trials when just the acoustic startle stimulus was presented and PP is the average startle reaction to the acoustic startle stimulus when it was preceded by the prepulse stimulus.

2.3 | Statistical analyses

Means and standard error of means were calculated for dependent measures and are shown in figures. Dependent measures were analyzed using 2 × 2 analyses of variance (ANOVA) with the between-subjects factors of sex (females vs. males) and Genotype (WT vs. DISC1-KO). When applicable, post hoc Tukey tests were conducted to follow up significant interactions. However, for the PPI test, a 2 × 2 × 3 mixed factorial ANOVA with the between-subjects factors of sex and genotype and the within-subjects factor of prepulse (5, 10, and 15 dB above background) was used to analyze % PPI. Rats that did not leave the shelter on the small open field test were exclude from analyses: 2 female WT, 4 female DISC1-KO, 7 male WT, and 6 male DISC1-KO. Similarly, one male WT rat was excluded from the arena analyses due to a failure to investigate any stimuli. Significance levels were set at $p < 0.050$ and effect sizes were reported as partial eta squared (η_p^2). The data that support the findings of this study are available from the corresponding author upon request.

3 | RESULTS

3.1 | PD21-22 – small open field test

Figure 3 shows the results of the open field behavioral analysis of female and male WT and DISC1-KO rats conducted prior to weaning from dams. The ANOVA conducted on latencies for rats to emerge from the shelter in the field showed a significant main effect of sex ($F[1,36] = 4.125$, $p = 0.050$, $\eta_p^2 = 0.103$); female rats, overall, emerged from the shelter more quickly than males (see Figure 3(A)). The main effect of Genotype and the interaction between sex and genotype were not statistically significant (ps > 0.05). By contrast, there was a statistically significant interaction of sex and genotype in the analysis of time in the shelter ($F[1,36] = 3.004$, $p = 0.015$, $\eta p2 = 0.307$; Figure 3(B)). In this case, female DISC1-KO rats spent significantly less time in the shelter than the other groups. This pattern was also evident on distance and speed measures of rats when they were outside the shelter, evident by significant interactions of sex and genotype for distance ($F[1,36] = 35.009$, $p = 0.001$, $\eta_p^2 = 0.272$; Figure 3(C)) and

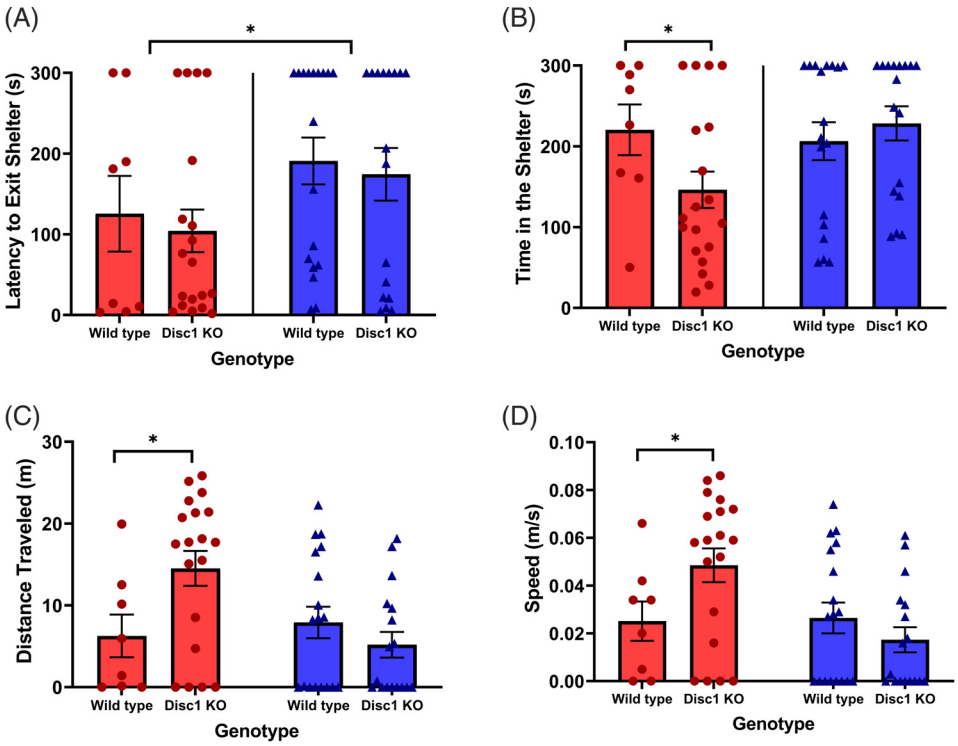

FIGURE 3 Results from the small open field test conducted on PD21-22. Shown are the latencies to leave a shelter in the field (A), the total time in the shelter (B), and the distance traveled (C) and speed (D) outside the shelter. *$p < 0.05$; in B, C, and D *$p < 0.05$ WT versus DISC1-KO

speed (F[1,36] = 11.428, $p = 0.002$, $\eta_p^2 = 0.241$; Figure 3(D)). As above, female DISC1-KO rats traveled farther and more quickly than the other groups, which did not differ from each other (ps > 0.05). The main effects of sex and genotype were not statistically significant in either analyses, nor in any that followed (ps > 0.05).

3.2 | PD28-29 – non-social and social arena tests

3.2.1 | Non-social arena – response of rats to novel objects

As with the small open field test, rats' overall activity in the larger field was marked by distance traveled and speed. There were statistically significant main effects of sex on distance (F[1,54] = 5.659, $p = 0.021$, $\eta_p^2 = 0.095$; Figure 4(A)) and speed (F[1,54] = 5.606, $p = 0.022$, $\eta_p^2 = 0.094$; Figure 4(B)) but the main effect of Genotype and the interaction of sex and genotype were not statistically significant (ps > 0.05). Analyses of rats' reaction to the novel objects showed that the main effect of Genotype on the latencies to investigate the first object (Figure 4(C)) and total time spent investigating objects (data not shown) were not significant. However, there were significant main effects of genotype on the latencies to investigate the second object (F[1,54] = 9.232, $p = 0.004$, $\eta_p^2 = 0.146$; Figure 4(D)), the duration between investigating the first and second object (F[1,54] = 6.819, $p = 0.012$, $\eta_p^2 = 0.112$; Figure 4(E)) and the average duration of object investigatory bouts (F[1,54] = 7.981, $p = 0.007$, $\eta_p^2 = 0.129$; Figure 4 (F)). No other significant effects were found (ps > 0.05).

3.2.2 | Social arena – response of rats to a novel conspecific

During rats' second time in the arena, distance traveled and speed were once again assessed and this time it was observed that the male DISC1-KO rats traveled less distance (see Figure 5(A)) and had slower speeds (see Figure 5(B)) in comparison to the other groups. However, the interaction between sex and genotype was not statistically significant in either case (ps > 0.05). The main effect of Genotype neared statistical significance for distance (F [1,57] = 3.142, $p = 0.082$; $\eta_p^2 = 0.052$) and speed (F [1,57] = 3.106, $p = 0.083$, $\eta_p^2 = 0.052$), indicating that the effect of the DISC1-KO in male rats was contributing to DISC1-KO rats, overall, having lower distance values. The same pattern was evident in the main effect of sex: there was a non-significant trend for distance (F[1,57] = 3.950, $p = 0.052$, $\eta_p^2 = 0.065$) and a significant effect for speed (F[1,57] = 4.042, $p = 0.049$, $\eta_p^2 = 0.066$). Here too, the male DISC1-KO rats was contributing to lower values for males, overall, compared with females. Analyses of rats' reaction to the novel conspecific showed no significant effects in the analyses of latencies to investigate the stimulus rat (see Figure 5(C)) or total investigation time (see Figure 5(D)). However, there were significant main effects of sex on the number of investigatory bouts (F [1,57] = 6.634, $p = 0.013$, $\eta_p^2 = 0.104$; see Figure 5(E)), the average duration of these bouts (F[1,57] = 10.597, $p = 0.002$, $\eta_p^2 = 0.157$; see Figure 5(F)). The main effect of genotype and the interaction between sex and genotype in these analyses were not significant (ps > 0.05).

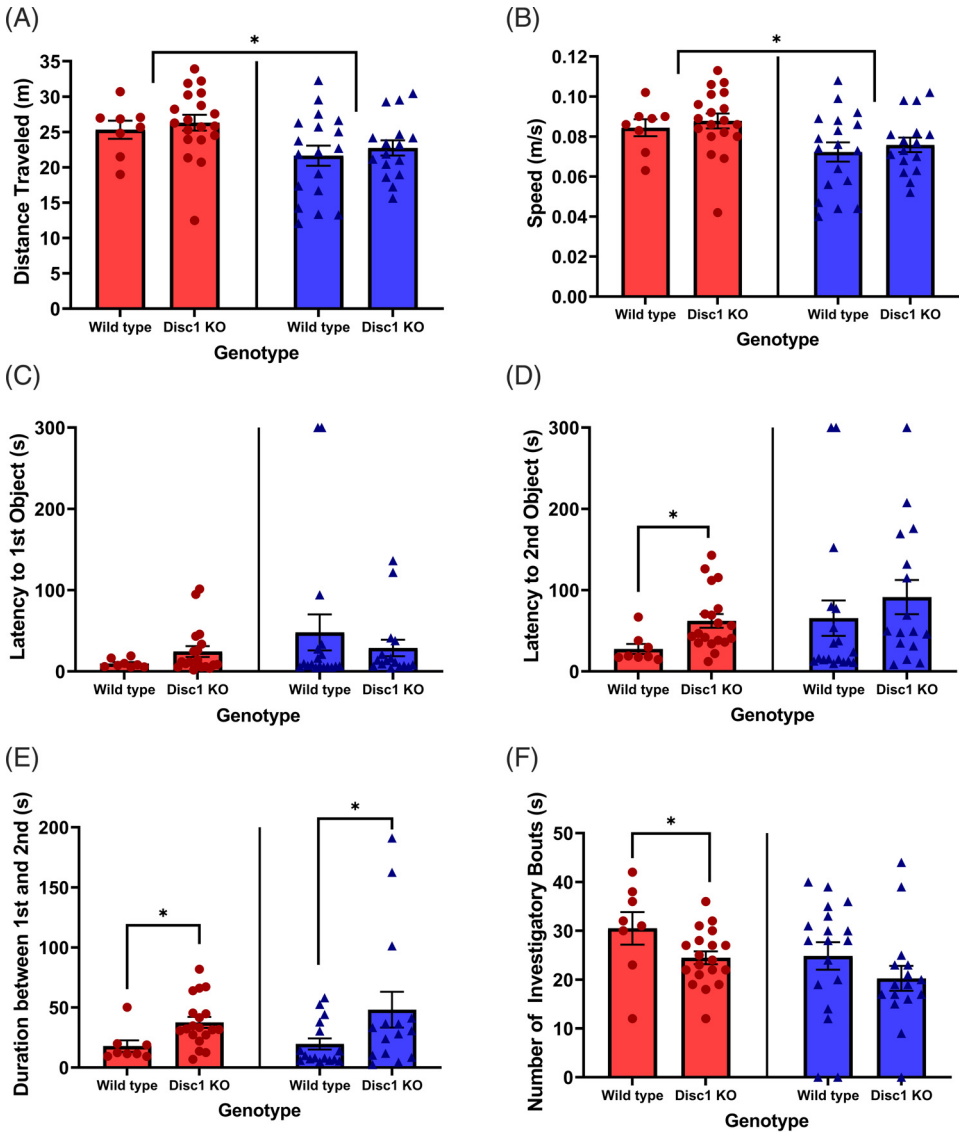

FIGURE 4 Results from the non-social arena test conducted on PD28-29. Shown are the distance (A) and speed (B), as well as the latency to investigate the first (C) and second (D) objects, the average duration between the two investigations (E), and the total number of investigatory bouts (F). *$p < 0.05$; in D, E, and F *$p < 0.05$ WT versus DISC1-KO

3.3 | PD35-36 – prepulse inhibition test

Figure 6 shows the results of the prepulse inhibition test. The analyses of % PPI showed significant main effects of prepulse ($F[2114] = 54.858$, $p < 0.001$, $\eta_p^2 = 0.490$), sex ($F[1,57] = 4.935$, $p = 0.030$, $\eta_p^2 = 0.080$), and genotype ($F[1,57] = 23.570$, $p < 0.001$, $\eta_p^2 = 0.293$); these results are shown in Figure 6(A) for females and Figure 6(B) for males. Overall, inhibition increased with prepulse intensity, males showed less inhibition than females, and DISC1-KO rats showed less inhibition than WT (ps < 0.05). Further, male DISC1-KO rats showed less inhibition than female DISC1-KO rats ($p < 0.05$; see Figure 6(C)). No other main effects or interactions were significant (ps > 0.05). Analysis of startle amplitude on acoustic stimulus trials showed a significant main effect of Genotype ($F[1,57] = 7.892$, $p = 0.007$, $\eta_p^2 = 0.122$; see Figure 6(D)), but no other effects were significant (ps > 0.05). There were also no significant effects in the analyses of amplitude on trials when no acoustic stimuli were presented (ps > 0.05). However, DISC1-KO males displayed higher amplitudes on no stimulus trials in comparison to other groups (see Figure 6(E)).

4 | DISCUSSION

The goal of the present research was to investigate the emergence of behavioral deficits that are consistent with human psychological conditions, including schizophrenia, during the periadolescent period in female and male rats. The hypotheses under investigation were that the neurodevelopmental perturbations induced by the DISC1 knockout would result in behavioral effects consistent with emotional, social, and sensorimotor gating, including hyperactivity, anxiety, and social and prepulse inhibition deficits, respectively, and the magnitude and timing of behavioral effects would differ for female and male rats. A short-term longitudinal design was used with activity and anxiety assessments just prior to weaning (PD21-22) and in early adolescence (PD28-29), a social assessment in early adolescence, and a

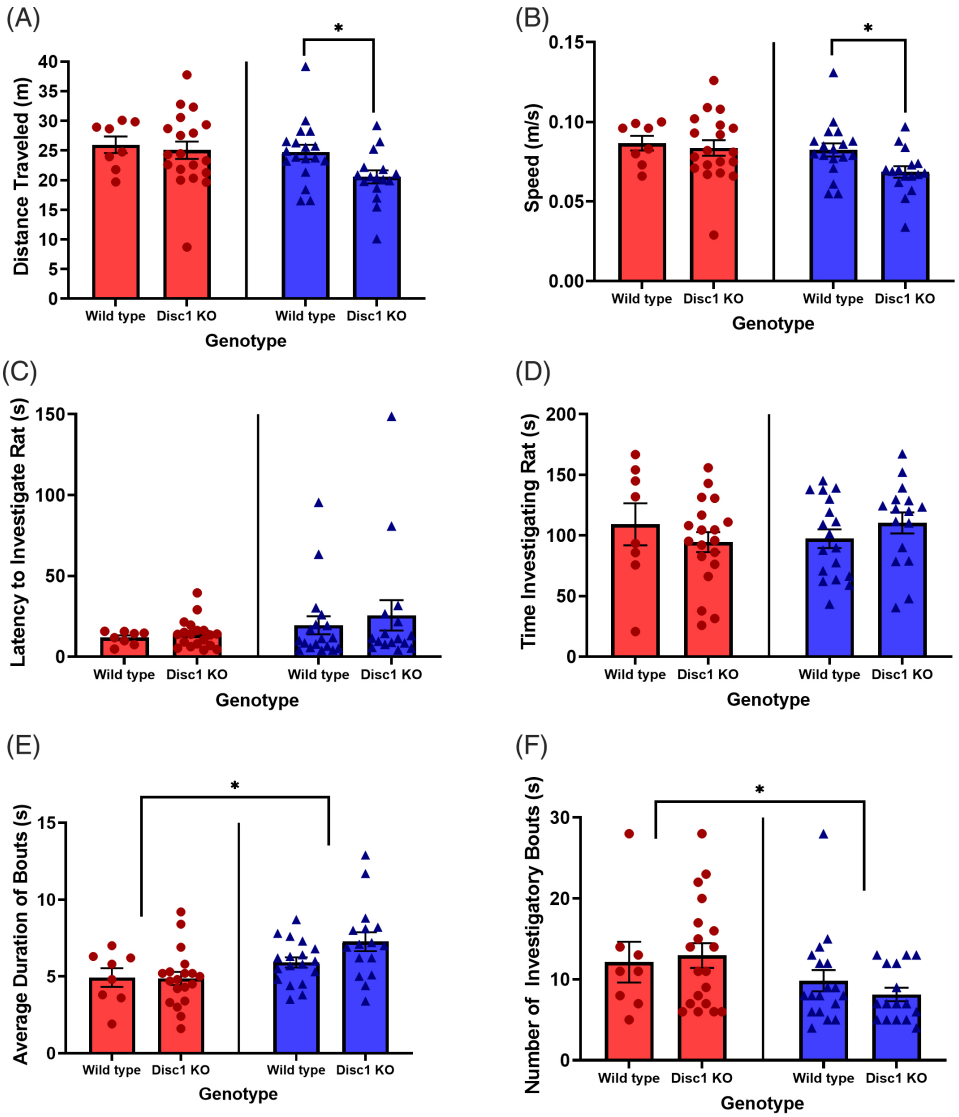

FIGURE 5 Results from the social arena test conducted on PD28-29. Shown are the distance (A) and speed (B), as well as the latency to investigate the stimulus rat (C), the total time investigating the stimulus rat (D), the number of investigatory bouts (E) and average duration of bouts (F). *$p < 0.05$; in A and B *$p < 0.05$ WT versus DISC1-KO

sensorimotor gating assessment in mid-adolescence (PD35-36). Overall, the DISC1 knockout produced widespread impacts on rats' behavior and did so in a distinct manner in females and males. In females, the DISC1 knockout produced marked hyperactivity—although only in early life, longer latencies to investigate novel objects and fewer investigatory bouts, increased startle to an acoustic stimulus, and sensorimotor gating deficits. By contrast, the effects of the DISC1 knockout were, in some assessments, difficult to determine in males due to their lower levels of activity and investigation compared with females. That said, there was evidence that the DISC1 knockout in males, as in females, produced longer latencies to investigate objects and sensorimotor gating deficits. However, the sensorimotor gating deficits in DISC1-KO males were notably more pronounced than in females. In general, the results of this study are consistent with the role of the DISC1 gene in the regulation of behaviors and point to its value in studying the behavioral and neurological outcomes associated with DISC1 dysfunction.

The marked and persistent impacts of the DISC1 knockout in female rats align well with the patterns of risk and prognosis in psychiatric illness, particularly in terms of mood disorders.[17] Although men may be at an elevated risk for and have a higher incidence and earlier age of onset of schizophrenia,[18–20] women's symptoms are less severe and more depressive and affective.[21] It is also notable, then, that in the present study females had more and varied outcomes from the gene knockout than males. In the small open field test conducted prior to weaning, female rats, overall, left the shelter more quickly than males and once out in the field the effects of the DISC1 knockout were highly evident: DISC1-KO females traveled at nearly double the speeds of the wild type females and, accordingly, covered twice as much distance. Of the males that emerged from the shelter, fewer than females overall, there was, in contrast, a tendency for the DISC1-KO males to travel slower and cover less distance. Although these differences were not statistically significant, they do stand in stark contrast to the opposite effects in females. Interestingly, when activity levels were assessed in the non-social and social arena testing conducted post-weaning and a week later, an effect of the knockout in females was no longer evident. In males, the DISC1 knockout did not affect activity in the first arena test, however, in the

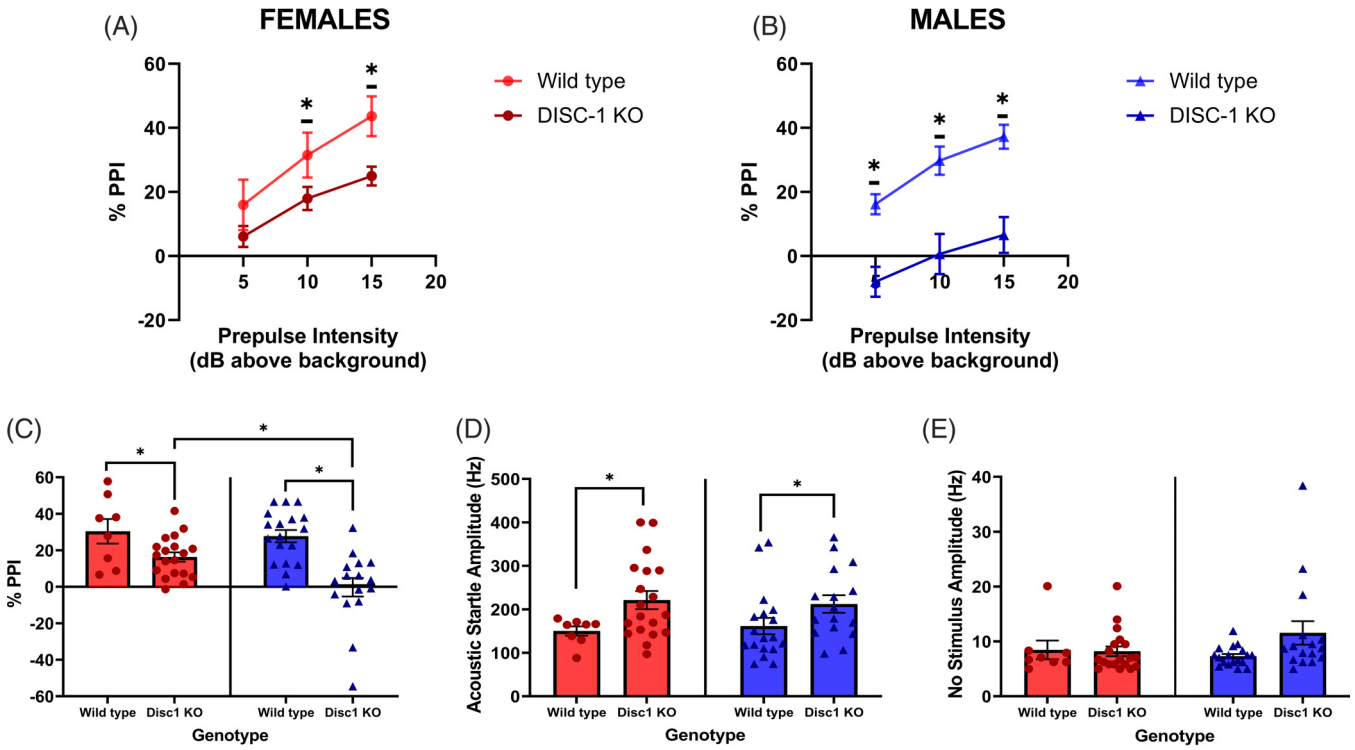

FIGURE 6 Results from the prepulse inhibition test conducted on PD36. Shown are the % PPI values for female (A) and male (B) rats at each of the three prepulse intensities, as well as the average % PPI (C), startle amplitudes to the 120 dB acoustic stimulus (D), and amplitudes on trials without stimulus presentation (E). * $p < 0.05$ WT versus DISC1-KO; in C * $p < 0.05$ female versus male DISC1-KO

second test later that day, DISC1-KO males traveled significantly less distance and were slower than wild type males. In this case, wild type, but not DISC1-KO, male rats increased their activity in the second test, likely the result of habituation. This finding suggests that the DISC1 knockout may be slowing male rats' adaptation to novel environments.

During early adolescence, rats' reactions to a "non-social" and "social" arena were assessed based on methods described by Ref. 16. In so doing, we aimed to seek evidence that more social declines would be evident in females and more non-social declines evident in males as a function of DISC1 gene status. Here too, the results were unexpected in that the impacts of the DISC1 knockout in female rats in the non-social arena, compared with males, were more marked. DISC1-KO females took longer to investigate each object, had longer durations between their first and second object investigation, and fewer investigatory bouts. For males, the DISC1 knockout increased the duration between the first and second object investigation only. Also contrary to our hypothesis, the DISC1 knockout had very little affect on rats' interest in a novel conspecific in the social arena. As others have observed,[16] the females in the present study, overall and compared with males, investigated the stimulus rat more times, although the duration of their investigations were shorter and thus there were no sex differences in total investigation times. It is possible that the inability of rats to physically interact with the stimulus rat (which was enclosed behind a mesh barrier) limited the potential to detect DISC1 knockout effects. However, it is also possible that sociality is not impacted in our model and/or at the age assessed. Future investigations of social interactions in adult DISC1-KO rats would help elucidate the relative weight of these options.

The use of PPI to assess pre-attentional processes involved in sensorimotor gating and deficits in it as a symptom of schizophrenia are well documented in humans[22] and rodents[23]; also see Ref. 24. Consistent with other reports and complementing prior research with other preclinical schizophrenia models, the DISC1 knockout produced significant deficits in PPI in female and male rats. As with the other behavioral measures, the effect of the DISC1 knockout differed for female and male rats. However, in this case, the effect on females was lesser than that in males. DISC1-KO females showed significant decreases in PPI at only the two highest prepulse intensities and not at the lowest. DISC1-KO males, on the other hand, were significantly impaired at all three prepulse intensities and the magnitude of their deficits were more marked than those in females. This was confirmed by a statistically significant difference between female and male DISC1-KO rats in % PPI averaged across prepulse intensities. PPI testing also allowed for the assessment of startle reactivity by marking the amplitude of rat's response to the acoustic stimulus alone. On this measure, female and male DISC1-KO rats had significantly augmented startle reactivity, which is consistent with other reports[23] and may be interpreted as an indicator of heightened anxiety.[25,26] These findings also point to the need to collect ample behavioral data around a given construct, such as anxiety and cognition, to increase the richness and accuracy of our animal models. In the present study, we elected to

target different behaviors across periadolescent development and aimed to understand some symptom types (activity and anxiety) from different perspectives and in different contexts. In so doing, we lacked the capacity to know how or when a behavioral deficit or a specific type might emerge in females and males in our model. A vital next step for this type of research would be to characterize specific behaviors over development and into adulthood and studies of this sort are ongoing in our laboratory.

The functions of the DISC1 gene are instrumental in numerous processes that pertain to neuron growth (reviewed in Ref. 5) and synaptic plasticity.[27] These functions fit well with the hypothesis that schizophrenia and other, related disorders, such as bipolar and depression, may arise from failures in neural plastic mechanisms[28] and may account for the early life behavioral effects reported in the present study. A potential site for failed neural plasticity is the hippocampus and evidence for a role of DISC1 in hippocampal function,[29] and its expression in the hippocampus through development[30] additionally aligns with the contribution of the hippocampus to affective and cognitive functioning. DISC1 is also a prominent regulatory factor in dopamine signaling,[31] which may underlie some of the effects reported here, particularly PPI deficits.[23] Additional studies with DISC1-KO rats are poised to offer substantive insights into the biological basis of psychopathology induced in the model. Rats have been widely used in behavioral research for over a century and our understanding of their cognitions and motivations is vast. The parallels of their physiological and behavioral interactions to that of humans may be more analogous than other rodent models.[32,33] That said, the present results are consistent with research using DISC1 mice[34,35]; also see Ref. 36. Further, an analysis of DISC1 isoforms and a measure of protein levels were not conducted in the present study and would be a necessary future direction. Similar models in mice[35] show a truncated and unstable form of protein along with similar behavioral deficits.

In conclusion, the present study offers novel insights into the role of the DISC1 gene in the early emergence of behavioral deficiencies in female and male rats. In so doing, a pattern emerges in which female DISC1-KO rats exhibited early indicators of hyperactivity, anxiety-like effects and mild sensorimotor gating deficits, whereas males exhibited few early indicators of activity changes or anxiety-like effects but had marked sensorimotor gating deficits. These biological sex differences reinforce the importance of studying females and males, accounting for their baselines differences, and attending to the suitability of different test parameters for each. The impetus for attending to these matters is bolstered by related findings that rat behavior in tests of anxiety may only reflect anxiety-like states in males, not females.[37] More specific to the present study, the sex differences reported here point to the need for further research into the kind, magnitude, and timing of behavioral outcomes in females and males in genetic studies, as well as in preclinical animal models of psychopathology. Overall, the results provided support for the usefulness of the DISC1 model for studying behavioral effects and future studies on the biological underpinnings of those effects will be of great value.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Ju-Young Park with the summary of portions of the collected data. This research was supported by funding from the National Center for Research Resources (5P20RR016463-12) and the National Institute of General Medical Sciences (8P20GM103423-12).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Glenn MJ, Batallán Burrowes AA, Yu W, Blackmer-Raynolds L, Norchi A, Doak AL. Progression of behavioral deficits during periadolescent development differs in female and male DISC1 knockout rats. *Genes, Brain and Behavior*. 2022;21(1):e12741. https://doi.org/10.1111/gbb.12741