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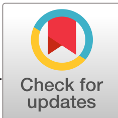

ORIGINAL ARTICLE

Genes, Brain and Behavior

Diminished social interaction incentive contributes to social deficits in mouse models of autism spectrum disorder

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Abstract

One of the core symptoms of autism spectrum disorder (ASD) is impaired social interaction. Currently, no pharmacotherapies exist for this symptom due to complex biological underpinnings and distinct genetic models which fail to represent the broad disease spectrum. One convincing hypothesis explaining social deficits in human ASD patients is amotivation, however it is unknown whether mouse models of ASD represent this condition. Here we used two highly trusted ASD mouse models (male Shank3-deficient [Shank3$^{+/\Delta C}$] mice modeling the monogenic etiology of ASD, and inbred BTBR mice [both male and female] modeling the idiopathic and highly polygenic pathology for ASD) to evaluate the level of motivation to engage in a social interaction. In the behavioral paradigms utilized, a social stimulus was placed in the open arm of the elevated plus maze (EPM), or the light compartment of the light-dark box (LDB). To engage in a social interaction, mice were thus required to endure innately aversive conditions (open areas, height, and/or light). In the modified EPM paradigm, both Shank3$^{+/\Delta C}$ and BTBR mice demonstrated decreased open-arm engagement with a social stimulus but not a novel object, suggesting reduced incentive to engage in a social interaction in these models. However, these deficits were not expressed under the less severe aversive pressures of the LDB. Collectively, we show that ASD mouse models exhibit diminished social interaction incentive, and provide a new investigation strategy facilitating the study of the neurobiological mechanisms underlying social reward and motivation deficits in neuropsychiatric disorders.

KEYWORDS
autism spectrum disorder, behavior model, BTBR, elevated plus maze, incentive, light-dark box, motivation, Shank3, sociability, social deficits

1 | INTRODUCTION

Autism spectrum disorder (ASD) is a complex heterogeneous neurodevelopmental disorder characterized by impaired communication and social interaction, repetitive behaviors, and restricted interests. ASD is a collection of clinically described disorders that affects 1 in every 59 children in the United States[1] and presents an urgent public health need, with the annual estimated cost of treatment and care for individuals with ASD in the US reaching $11.5-60.9 billion.[2,3] Though social-skill interventions have been used among Asperger's syndrome and high functioning ASD patients,[4,5] no effective pharmacotherapeutic strategies exist for the social deficits in ASD[6] due to the broad genetic etiology and resulting limitations of existing animal models.

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Strong genetic factors contribute to the etiology of ASD.[7] Large-scale genetic studies have revealed several susceptibility genes or copy number variations that are highly associated with the diagnosis of ASD cases, such as SHANK-family genes[8,9] and 16p11.2 deletions.[10] These studies promote our understanding of the genetic basis of autism and facilitate the development of animal models that reflect genetic polymorphisms linked to autism.[11,12] Many animal models focus on the monogenic heritable ASD conditions caused by loss-of-function mutations, such as the *SHANK3* gene that encodes a scaffolding protein at glutamatergic synapses.[13] SHANK3 haploinsufficiency is one of the most penetrant monogenic causes of autism.[14] Mice lacking the murine ortholog of the human *SHANK3* gene exhibit selective deficits in social interactions and repetitive behaviors reminiscent of ASD in humans. In the 3-chamber social preference test, Shank3-deficient (Shank3$^{+/\Delta C}$) mice demonstrate a significantly lower preference for social stimuli than wild-type (WT) mice.[15-17] Additionally, the BTBR T^{+}Itpr3^{tf}/J (BTBR) inbred mouse strain exhibits a variety of behavioral abnormalities that model ASD symptoms,[18,19] including impaired social behavior and pronounced repetitive behaviors. Though the genetic background of BTBR mice is complex and poorly understood, BTBR mice carry single nucleotide polymorphisms in several autism candidate genes,[18] and are a trusted ASD model which imitates the idiopathic and highly polygenic pathology for ASD.

While both Shank3$^{+/\Delta C}$ and BTBR mice exhibit decreased social interaction, it remains unclear whether this is related to changes in the hedonic impact of social reward, or rather impaired incentive motivation, which has been implicated in reward processing.[20] In the current study, we aimed to assess whether the incentive of social interaction is impaired in these ASD mouse models by merging the elevated plus maze (EPM) and light-dark box (LDB) behavioral tests with elements of social interaction tests. As rodents prefer dark, enclosed areas, the elevated and well-lit open arms of the EPM and the light-chamber of the LDB represent highly aversive environments for mice. Thus, mice typically exhibit a strong preference for the enclosed arms in the EPM, and the dark compartment of the LDB.[21,22] Here, we placed a social stimulus (age- and sex-matched WT mouse) into the aversive component of each behavioral paradigm—the open arm of the EPM, or the light chamber of the LDB—to assess whether WT mice and ASD mouse models were equally motivated to enter the aversive environment in order to engage in a social interaction. We found that both Shank3$^{+/\Delta C}$ and BTBR mice exhibited selective reductions in social engagement in the modified EPM protocol, suggesting that the widely observed social deficits in these ASD models are partially mediated by a reduction of social interaction incentive. Furthermore, these deficits were not expressed in Shank3$^{+/\Delta C}$ or BTBR mice in the modified LDB protocol, which presents a less aversive barrier to interaction, indicating that the relative aversive strength of the interaction barrier is critical for revealing deficits in social interaction incentive in ASD models.

2 | MATERIALS AND METHODS

2.1 | Animals

Mice expressing C-terminal (exon 21)-deleted Shank3 (*Shank3$^{tm1.1Pfw}$*/J) with significant loss of full-length Shank3 expression were purchased from the Jackson Laboratory (Bar Harbor, ME, Stock #018398). These mice were backcrossed at least five generations to C57BL/6J mice at the Jackson Laboratory. Upon arrival at our laboratory, these mice were backcrossed three more generations to C57BL/6J mice (Jackson Laboratory, Stock #: 000664) before any experimental use. All subsequent breeding, genotyping, and colony maintenance were performed in-house as previously described.[15] Heterozygous Shank3$^{+/\Delta C}$ mice were crossed to produce litters containing WT, heterozygous Shank3$^{+/\Delta C}$, and homozygous Shank3$^{+/\Delta C}$ offspring, which were identified via in-house genotyping. Genotyping of these mice was determined by polymerase chain reaction (PCR) on their tail genomic DNA. The primers used in genotyping were the wild-type reverse primer (5′-ATG TCC TGT TGC AGG TAG GG-3′), the common forward primer (5′-GTG TCC CCT CAT TGA TGT TG-3′), and the mutant reverse primer (5′-CTC TGC CAC CTT CTG CCT AC-3′). Only heterozygous Shank3$^{+/\Delta C}$ mice (6-8 weeks old, male) and age-matched WT littermates (male) were used in this study. Female Shank3$^{+/\Delta C}$ mice lack autism-like social deficits, so they (along with female WT animals) were not used. BTBR T^{+}Itpr3^{tf}/J (BTBR) mice were obtained from The Jackson Laboratory (Stock #: 002282) and bred in house. Both male and female BTBR mice (6-8 weeks old) were used for all experiments described. Mice of all genotypes were group-housed (2-4 per cage) with littermates of the same gender. Shank3$^{+/\Delta C}$ mice were housed with littermates of any genotype (WT or Shank3$^{+/\Delta C}$); BTBR mice were housed only with BTBR littermates. All mice were maintained on a 12-hour light/dark cycle. All the behavior tests were conducted during light cycle. Researchers were blind to genotypes during experiments. All animal studies were performed with the approval of the Institutional Animal Care and Use Committee of the State University of New York at Buffalo.

2.2 | Brain tissue lysate preparation and western blot

Mice were sacrificed by rapid decapitation, and brains were removed and sliced into 1-mm-thick sections using a brain matrix (Zivic instruments, Pittsburgh, PA, stock #: BSMAS001-1), and 2-mm-diameter tissue punches from the prefrontal cortex were collected and rapidly frozen on dry ice. Then punched tissues were homogenized in 25 mM Tris (pH 8.0) and 0.25 M sucrose buffer as previously described.[23] Total protein was extracted, and 30 μg samples was loaded onto 7.5% Tris-SDS polyacrylamide gels for electrophoresis separation, then transferred to nitrocellulose membranes and blocked with 5% nonfat milk in PBS. Membranes were incubated overnight at 4°C with primary antibodies diluted in blocking buffer (5% skim milk, Sigma, 1153630500), including: mouse anti-shank3 (1:500, NeuroMab, Davis, CA, Cat. #: 75-344, clone N367/62), mouse anti-tubulin (1:10 000, Sigma, St. Louis, MO, Cat. #: T9026). Membranes were incubated with

ECL HRP-conjugated secondary antibodies (1:2000; GE healthcare Life Science, Pittsburgh, PA, stock #: NA9310) for 1 hour at room temperature. The blots were imaged using the Gel Dox system (Bio-Rad, Hercules, CA) and quantified by densitometry using Image J. Tubulin was used as a loading control.

2.3 | Behavioral testing

For all behavioral testing, each test animal was exposed to all three trial types described (baseline, object-, and social incentive trials) on three separate days, with a 24-hour interval. Animals were first tested in the baseline trial, and the order of the object and social incentive trials was counterbalanced over the subsequent 2 days to prevent practice effects. In order to avoid fatigue or anxiety-related differences in animal performance in the EPM and LDB tests, separate and independent groups of WT, Shank3$^{+/\Delta C}$ and BTBR mice were used in each respective model (one group for all three EPM trials, and a separate group for all three LDB trials).

For all social-incentive trials for both Shank3$^{+/\Delta C}$ and BTBR mice, an unfamiliar age- and sex-matched WT mouse (C57BL/6J) was used as the social stimulus. For all object-incentive trials, a 1.5″ × 1.5″ wooden block (square wood block by ArtMinds; Michaels.com) was used as the novel object. For all object- and social-incentive trials, either the object or social stimulus was placed under an inverted pencil cup (spectrum diversified galaxy pencil holder, chrome; Amazon.com) with a water bottle placed on top to prevent the test mouse from climbing the cup. The mice used as the social stimuli were randomized across experimental groups and between animals.

2.3.1 | Elevated plus maze

The test mouse was placed into the center (facing into a closed arm) of a plus-shaped apparatus with four arms (3″ wide × 15.5″ long), two of which were enclosed with 11″ tall opaque walls, while the two open arms did not have walls. Testing was conducted in a brightly lit room, with the open arms illuminated by overhead lighting (200 lx) and the closed arms/center shielded from light by upright walls (3 lx), consistent with suggested lighting levels for the EPM.[22,24] For the modified protocols included in this study (object incentive trial/social incentive trial), either a novel object (wooden block) or a social stimulus (randomly selected, unfamiliar age- and sex-matched WT mouse) was placed in one open arm of the maze under an inverted pencil cup with a water bottle placed on top to prevent the test mouse from climbing the cup. The other arm was kept empty for each trial to serve as an internal control sensitive to changes in the animal's baseline open arm entry. The open arm in which the stimulus was placed was alternated between tests to control for the potential preference for either open arm. For each trial type, the test animal was allowed to explore for 5 minutes, and the amount of time spent in each open arm was manually quantified by a genotype-blind researcher. For scoring purposes, animals were considered to be in the open arm when all four paws were touching the floor of that arm. Each test animal was exposed to all three trial types described (baseline, object-, and social incentive trials) on three separate days, with a 24-hour interval. Animals were first tested in the baseline trial, and the order of the object and social incentive trials was counterbalanced over the subsequent 2 days in order to avoid practice effects.

2.3.2 | Light/dark box

The LDB used in the current study is comprised of two 13.75″ × 10.375″ × 13.5″ compartments joined by a 3″ × 5″ doorway. The walls of one compartment (the light box) are made of transparent glass to allow the area to be illuminated by overhead lighting (200 lx), while the walls of the other compartment (dark box) are opaque black to prevent the transmission of light and ensure darkness within the compartment (3 lx). The lighting levels used here are consistent with reported experimental guidelines.[25] The test animal was placed into the dark box and allowed 5 minutes to explore the apparatus. The amount of time spent exploring the light box (defined as all four paws inside the light box) was manually quantified by a genotype-blind researcher. In the modified object incentive and social incentive trials, either a novel object (wooden block) or a social stimulus (randomly selected, unfamiliar age- and sex-matched WT mouse), respectively, was placed in the center of the light box, under an inverted pencil cup with a water bottle placed on top to prevent the test mouse from climbing the cup.

2.4 | Statistical analysis

Data analyses were performed with Graphpad 6 (Graphpad Software, Inc., La Jolla, CA) and Minitab 18. Experiments with two groups were analyzed using two-tailed Students *t* tests. Experiments with more than two groups were subjected to two-way ANOVA followed by post hoc Bonferroni tests for multiple comparisons. Experiments with three factors were analyzed by three-way ANOVA followed by post hoc Bonferroni tests for multiple comparisons. Sample sizes were based on power analyses with current sample size providing a power of 0.95 for $P < .05$, which were similar to those reported previously.[17] Data in figures are presented as mean ± SEM. *F* values, degrees of freedom, and *P* values for all ANOVAs, as well as *t* values and degrees of freedom for *t* tests, are included within the text for all statistical analyses performed.

3 | RESULTS

3.1 | Shank3$^{+/\Delta C}$ and BTBR mouse models of ASD exhibit diminished social interaction incentive in a modified EPM protocol

We first confirmed the genotype of Shank3-deficient (Shank3$^{+/\Delta C}$) mice (Figure 1A). WT mice showed a PCR product with a size of 399 bp, whereas homozygous Shank3-deficient mice showed a 500 bp PCR product. Both PCR products (399 bp and 500 bp) were present in Shank3$^{+/\Delta C}$ mice, and a clear decrease of Shank3 protein level was found (Figure. 1B, n = 4-5 per group, $t_{(7)} = 4.07$, $P = .005$,

t test). In order to evaluate whether the social deficits widely exhibited by the Shank3-deficient (Shank3$^{+/\Delta C}$) and BTBR mouse models of ASD are related to a reduction in the drive for social engagement, we designed a modified protocol of the EPM. In the original EPM testing format, a test mouse is placed into the center of the maze, and is allotted 5 minutes to explore any of the four arms (Figure 2A, baseline trial). Since rodents express an innate preference for dark, enclosed areas, the two open arms represent an aversive environment for mice. Thus, mice typically demonstrate a strong preference for the enclosed arms, and the amount of time spent exploring the open arms is thought to serve as a measure of anxiety-related behavior; specifically, reduced open-arm exploration time is thought to represent elevated anxiety-like behavior.[22] Since the open arms of the EPM represent an aversive environment, we wondered if we could gauge an animal's drive to interact with an object or social stimulus via their willingness to enter the open arm and endure exposure to an innately aversive environment. Therefore, animals were tested in two modified formats of the EPM in which one open arm contained either a novel object (wooden block) (Figure 2B, object incentive trial) or a social stimulus (age- and sex-matched WT mouse) (Figure 2C, social incentive trial). The opposing open arm was left empty for each trial, serving as an internal control for the animal's baseline drive to enter the open arm.

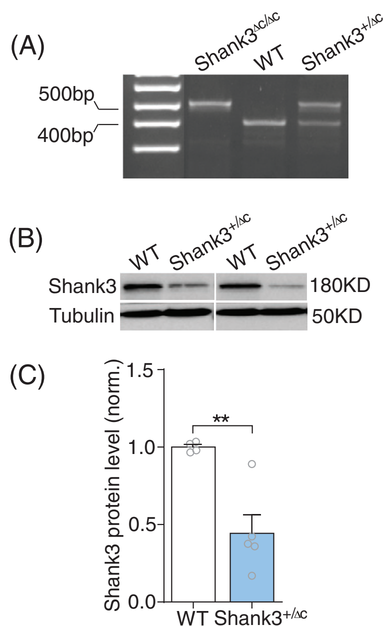

FIGURE 1 Genotype confirmation for Shank3$^{+/\Delta C}$ mice. (A) Representative genotyping results (PCR) for wild-type (WT), homozygous Shank3-deficient (Shank3$^{\Delta C/\Delta C}$) and heterozygous Shank3-deficient (Shank3$^{+/\Delta C}$) mice. (B,C) Representative western blot image and bar graph showing Shank3 protein level in total protein lysate from prefrontal cortex of WT and Shank3$^{+/\Delta C}$ mice

We first tested Shank3$^{+/\Delta C}$ and WT control mice in the three EPM trial types described. In the baseline trial, Shank3$^{+/\Delta C}$ did not differ from WT mice in the total amount of time spent exploring the open arms (Figure 3A, left, $t_{(23)} = 0.28$, $P = .78$, *t* test). Additionally, neither group demonstrated a preference for either open arm (Figure 3A, right, n = 12-13 per group, $F_{1,46\ (\text{interaction})} = 0.47$, $P = .50$, $F_{1,46\ (\text{arm side})} = 1.76$, $P = .19$, $F_{1,34\ (\text{genotype})} = 0.11$, $P = .74$, two-way ANOVA). To assess the incentive value of a novel object, WT and Shank3$^{+/\Delta C}$ animals were tested in the object incentive trial, in which a novel object was placed in one open arm. WT and Shank3$^{+/\Delta C}$ mice did not differ in the amount of time spent exploring either the object-containing arm or the empty arm (Figure 3B, n = 12 per group, $F_{1,44\ (\text{interaction})} = 0.83$, $P = .37$, $F_{1,44\ (\text{object vs empty arm})} = 3.06$, $P = .09$, $F_{1,34\ (\text{genotype})} = 1.3$, $P = .26$, two-way ANOVA). The difference between the amount of time spent exploring the object-containing arm relative to the empty arm (object arm time − empty arm time) also did not differ between WT and Shank3$^{+/\Delta C}$ mice (WT: 12.07 ± 4.8 seconds, Shank3$^{+/\Delta C}$: 3.8 ± 1.9 seconds, $t_{(22)} = 1.61$, $P = .12$). However, in the social incentive trial, in which a social stimulus was placed in one open arm of the EPM, WT mice spent significantly more time exploring the social-stimulus-containing arm than the empty arm, whereas Shank3$^{+/\Delta C}$ mice did not exhibit a preference for the arm containing the social stimulus, and spent significantly less time than WT animals exploring the social arm (Figure 3C, n = 12 per group, $F_{1,44\ (\text{interaction})} = 8.30$, $P = .006$, $F_{1,44\ (\text{social vs empty arm})} = 5.48$, $P = .02$, $F_{1,44}$

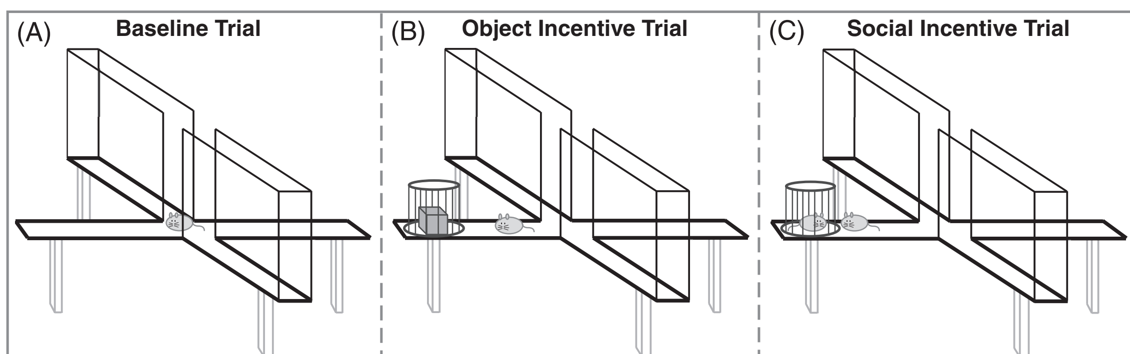

FIGURE 2 Graphic illustrating the three elevated plus maze (EPM) protocols used in the current study. (A) Baseline trial, in which both open arms are left empty. (B) Object incentive trial, in which a novel object (wooden block) is placed under an inverted pencil cup in one open arm of the EPM. (C) Social incentive trial, in which a social stimulus (age- and sex-matched WT mouse) is placed under an inverted pencil cup in one open arm of the EPM

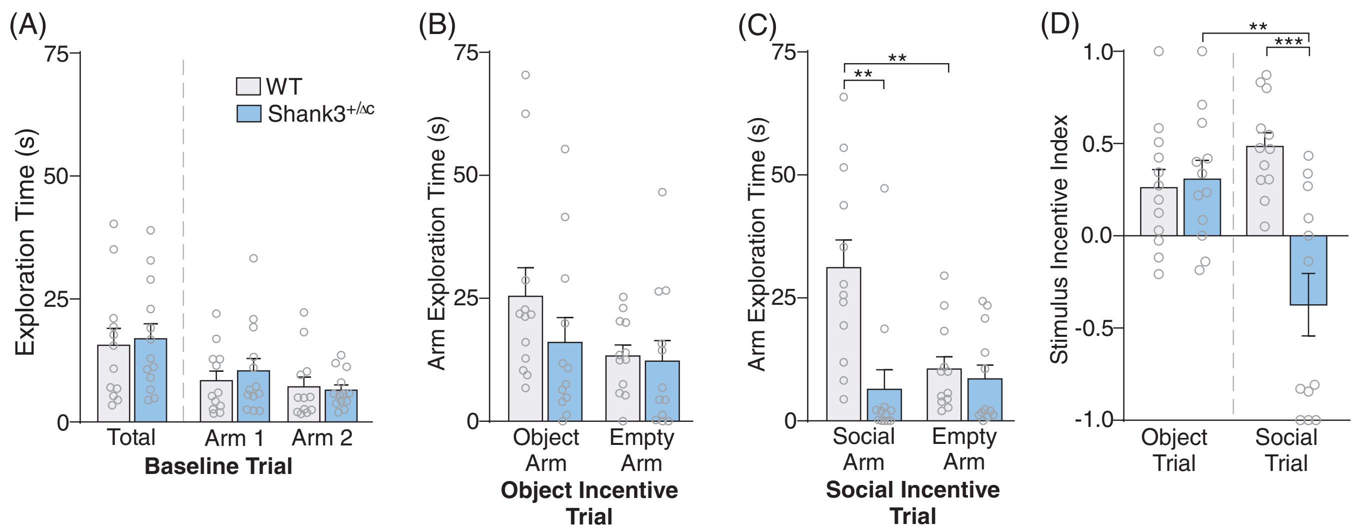

FIGURE 3 Shank3$^{+/\Delta C}$ mice exhibit diminished social interaction incentive in a modified elevated plus maze (EPM) protocol. (A) Bar graph (mean ± SEM) showing the total amount of time wild type (WT) and Shank3 deficient (Shank3$^{+/\Delta C}$) mice spent exploring both open arms of the EPM (left), and the amount of time spent exploring each individual arm (right) in the baseline trial. (B) Bar graph (mean ± SEM) showing the amount of time WT and Shank3$^{+/\Delta C}$ mice spent exploring the object-containing arm and the empty arm in the object incentive trial. (C) Bar graph (mean ± SEM) showing the amount of time WT and Shank3$^{+/\Delta C}$ mice spent exploring the social-stimulus-containing arm and empty arm in the social incentive trial. (D) Bar graph (mean ± SEM) showing the stimulus incentive index for WT and Shank3$^{+/\Delta C}$ mice in the object incentive trial (left) and the social incentive trial (right). ***P < .0001, **P < .01, two-way ANOVA post hoc t test

$_{(genotype)}$ = 11.44, P = .0015, two-way ANOVA). In addition, the difference between the amount of time spent exploring the social-stimulus-containing arm relative to the empty arm (social arm time – empty arm time) was significantly greater for WT animals (WT: 20.62 ± 4.7 seconds, Shank3$^{+/\Delta C}$: −2.14 ± 3.5 seconds, $t_{(22)}$ = 3.9, P = .0008), suggesting that WT animals have a significantly greater preference for the social-stimulus-containing arm over the empty arm than Shank3$^{+/\Delta C}$ mice.

To quantify the animal's level of motivation to explore the stimulus-containing-arm, we calculated a "stimulus incentive index" (SII) for each trial which represents the animal's preference for the stimulus-containing-arm vs the empty arm (calculated as: [time in stimulus-containing-arm – time in empty arm]/total time in both open arms). In the object incentive trial, WT and Shank3$^{+/\Delta C}$ mice exhibited a similar and positive SII, indicating that both groups display a preference for the object-containing-arm, and that the presence of the object enhances the drive for both WT and Shank3$^{+/\Delta C}$ mice to enter that arm of the maze relative to an empty open arm (Figure 3D, n = 12 per group). However in the social incentive trial, Shank3$^{+/\Delta C}$ mice demonstrated a negative SII that was significantly lower than that of WT mice, suggesting that the incentive to engage in a social interaction is significantly reduced in Shank3$^{+/\Delta C}$ mice (Figure 3D, n = 12 per group, $F_{1,44\ (interaction)}$ = 15.15, P = .0003, $F_{1,44\ (trial\ type)}$ = 3.86, P = .06, $F_{1,44\ (genotype)}$ = 12.14, P = .0011, two-way ANOVA). Notably, Shank3$^{+/\Delta C}$ mice demonstrated a significantly lower SII in the social incentive trial than in the object incentive trial, indicating that the incentive to interact with a novel object exceeds the incentive to interact with a social stimulus in Shank3$^{+/\Delta C}$ mice. Collectively, these data indicate that the aversive value presented by the open arm of the EPM is sufficient to deter Shank3$^{+/\Delta C}$ mice, but not WT mice, from pursuing a social interaction, and that the incentive of social engagement is diminished in Shank3$^{+/\Delta C}$ mice.

To determine whether the modified EPM paradigm used here was sensitive to social incentive deficits in other ASD models, we next attempted to replicate these findings in BTBR mice. Since ASD-like deficits in BTBR mice are caused by an unclear genetic background and BTBR mice do not have a distinct wild-type counterpart, experiments with BTBR mice were conducted without WT controls, and all statistical comparisons were performed between open-arm side and trial type. BTBR mice did not show any preference for either open arm of the EPM in the baseline trial (Figure 4A, n = 10 per group, $t_{(9)}$ = 0.53, P = .61, paired t test). In the object incentive trial, BTBR mice demonstrated a significant preference for the object-containing-arm (Figure 4B, n = 10 per group, $t_{(9)}$ = 2.94, P = .017, paired t test), however, BTBR mice did not show a preference for either arm in the social incentive trial (Figure 4C, n = 10 per group, $t_{(9)}$ = 0.35, P = .73, paired t test). Correspondingly, the SII was significantly lower in the social incentive trial than in the object incentive trial (Figure 4D, n = 10 per group, $t_{(9)}$ = 3.95, P = .003, paired t test), indicating that the incentive to engage in a social interaction is reduced in BTBR mice.

3.2 | Diminished social interaction incentive in ASD models is not revealed under less aversive barrier conditions

We next questioned whether the diminished incentive for social interaction in Shank3$^{+/\Delta C}$ and BTBR mice was selectively evoked by the aversive pressures of the modified EPM paradigm, or if this deficit

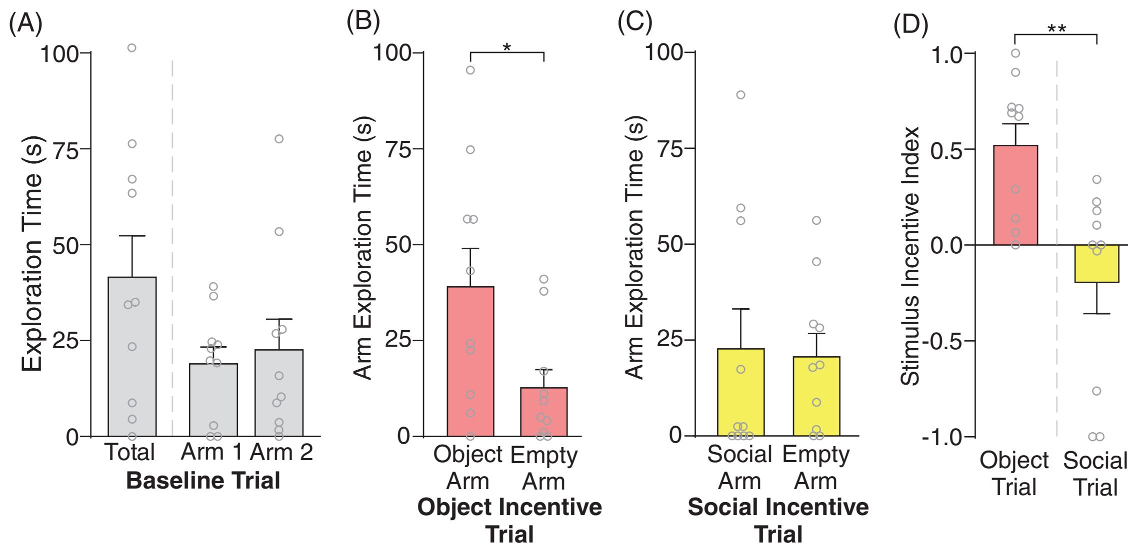

FIGURE 4 BTBR mice exhibit diminished social interaction incentive in a modified elevated plus maze (EPM) protocol. (A) Bar graph (mean ± SEM) showing the total amount of time BTBR mice spent exploring both open arms of the EPM (left), and the amount of time spent exploring each individual arm (right) in the baseline trial. (B) Bar graph (mean ± SEM) showing the amount of time BTBR mice spent exploring the object-containing arm and the empty arm in the object incentive trial. *$P < .05$, t test. (C) Bar graph (mean ± SEM) showing the amount of time BTBR mice spent exploring the social-stimulus-containing arm and empty arm in the social incentive trial. (D) Bar graph (mean ± SEM) showing the stimulus incentive index for BTBR mice in the object incentive trial (left) and the social incentive trial (right). **$P < .01$, t test

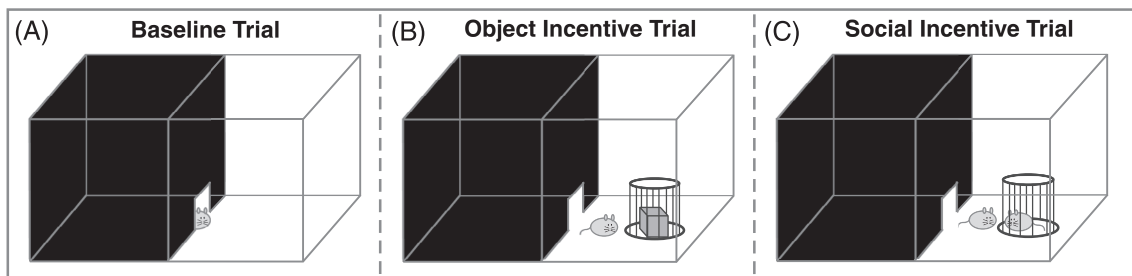

FIGURE 5 Graphic illustrating the three light-dark box protocols used in the current study. (A) Baseline trial, in which the light chamber is left empty. (B) Object incentive trial, in which a novel object (wooden block) is placed under an inverted pencil cup in the light chamber. (C) Social incentive trial, in which a social stimulus (age- and sex-matched WT mouse) is placed under an inverted pencil cup in the light chamber

was pervasive and present under all conditions. To determine if the deficit was dependent upon the severity of the aversive pressure imposed by the barrier to interaction (ie, open space/bright light/elevation in the open arm of the EPM), we designed a modified LDB paradigm which paralleled the modified EPM protocol. The LDB test measures anxiety-like behavior by gauging the animal's propensity to explore a brightly illuminated—and thus aversive—chamber relative to a dark compartment.[21] In the modified LDB test used here, the animal's intent to enter the aversive, brightly-lit light chamber of the LDB was used to measure the drive to interact with an object or social stimulus. Like the open arm of the EPM, the light chamber of the LDB is well-lit, however the light chamber of the LDB is not elevated and is enclosed by transparent walls, both of which are in contrast to the open arm of the EPM. Therefore, the light chamber lacks two key aversive features presented by the open arm (elevation and exposure), and thus presents a less aversive barrier to interaction relative to the EPM paradigm. In the three trial types used here, the light chamber contained either nothing (Figure 5A, baseline trial), a wooden block under an inverted pencil cup (Figure 5B, object incentive trial), or a social stimulus under an inverted pencil cup (Figure 5C, social incentive trial), and the amount of time spent exploring the light and dark chambers was measured.

We first compared WT and Shank3$^{+/\Delta C}$ mice in the modified LDB paradigm. In both the object- and social incentive trials, WT and Shank3$^{+/\Delta C}$ mice spent significantly more time in the light box (and less time in the dark box) than in the baseline trial, however WT and Shank3$^{+/\Delta C}$ mice did not differ in the amount of time spent in the light box or dark box during any of the three trials (Figure 6A, n = 10-11 per group, $F_{1,114\ (\text{genotype})} = 0.0$, $P > .999$, $F_{2,114\ (\text{trial type})} = 0.0$, $P > .999$, $F_{1,114\ (\text{box side})} = 4.03$, $P = .047$, three-way ANOVA). Additionally, the "stimulus enhancement index" (SEI)—which, due to the lack of an internal control such as the empty arm in EPM, was

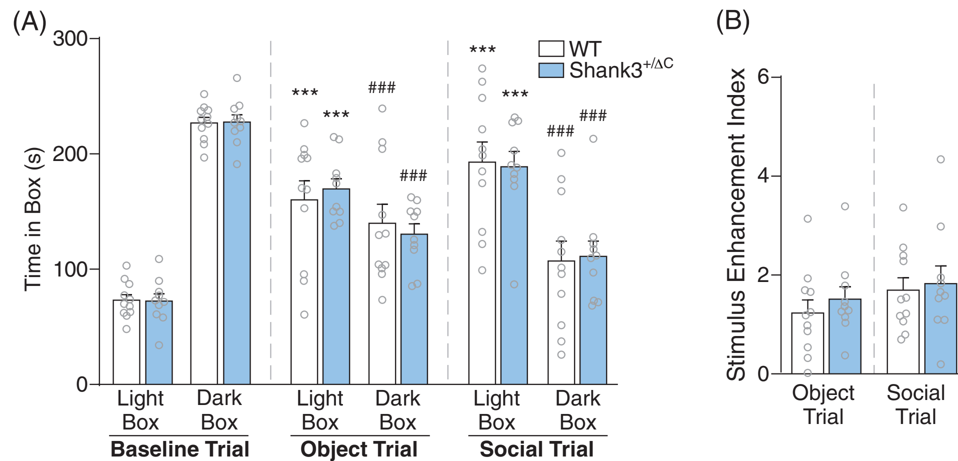

FIGURE 6 Shank3$^{+/\Delta C}$ mice do not exhibit reduced social interaction incentive when presented with a less aversive barrier to interaction. (A) Bar graph (mean ± SEM) showing the amount of time WT and Shank3$^{+/\Delta C}$ mice spent exploring the light and dark compartments of the light-dark box in the baseline trial (left), in the object incentive trial (middle), and in the social incentive trial (right). * indicates statistical difference from time in light-box during the baseline trial for each respective group. # indicates statistical difference from time in dark-box during the baseline trial for each respective group. ***$P < .0001$, ###$P < .0001$, three-way ANOVA post hoc *t* tests. (B) Bar graph (mean ± SEM) showing the stimulus enhancement index for WT and Shank3$^{+/\Delta C}$ mice in the object incentive trial (left) and social incentive trial (right)

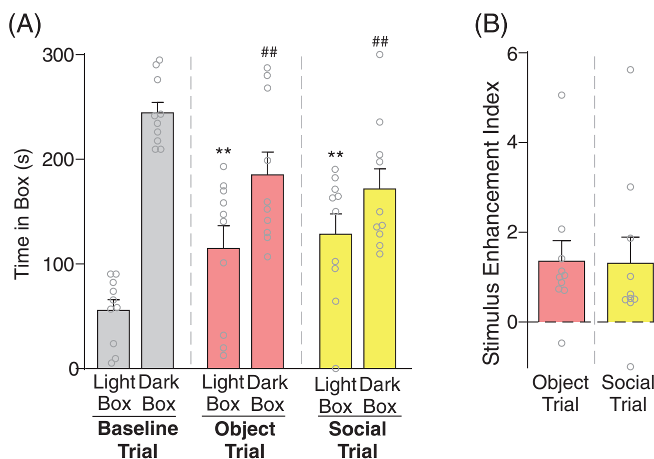

FIGURE 7 BTBR mice do not exhibit reduced social interaction incentive when presented with a less aversive barrier to interaction. (A) Bar graph (mean ± SEM) showing the amount of time BTBR mice spent exploring the light and dark chambers of the light-dark box in the baseline trial (left), in the object incentive trial (middle), and in the social incentive trial (right). * indicates statistical difference from time in light-box during the baseline trial. # indicates statistical difference from time in dark-box during the baseline trial. **$P < .001$, ##$P < .001$, two-way ANOVA post hoc *t* tests. (B) Bar graph (mean ± SEM) showing the stimulus enhancement index for BTBR mice in the object incentive trial (left) and social incentive trial (right)

calculated differently from the SII used prior (calculated as: [time in light-box during given trial type – time in light-box during baseline trial]/time in light-box in baseline trial) did not differ between WT and Shank3$^{+/\Delta C}$ mice in either the object- or social incentive trial (Figure 6B, n = 10-11 per group, $F_{1,38\ (\text{genotype})} = 0.53$, $P = .47$, $F_{1,38\ (\text{trial type})} = 1.86$, $P = .18$, two-way ANOVA), indicating that the reduced incentive of social engagement in Shank3$^{+/\Delta C}$ mice is veiled under less severe aversive conditions.

We next tested BTBR mice in the modified LDB paradigm. BTBR mice spent significantly more time exploring the light box in both the object- and social incentive trials than in the baseline trial (Figure 7A, n = 10 per group, $F_{2,18\ (\text{interaction})} = 22.52$, $P < .0001$, $F_{2,18\ (\text{trial type})} = 1.0$, $P = .39$, $F_{1,9\ (\text{box side})} = 9.23$, $P = .01$, two-way ANOVA). Additionally, the SEI for the object- and social incentive trials did not differ in BTBR mice (Figure 7B, n = 10 per group, $t_{(9)} = 0.05$, $P = .96$), indicating that BTBR mice were similarly driven by both an object and a social stimulus to explore the light chamber of the LDB.

Collectively, these data indicate that the incentive to engage in a social interaction in Shank3$^{+/\Delta C}$ and BTBR mice is selectively reduced in the modified EPM paradigm, but not in the modified LDB paradigm, suggesting that the expression of this behavioral phenotype in these ASD models is specific to the aversive pressures presented by the open arm of the EPM (bright light, open spaces, elevation) and that the deterrent pressure imposed by the more mild aversive environment of the light chamber of the LDB is insufficient to dissuade Shank3$^{+/\Delta C}$ or BTBR mice from exploring a compartment containing a social stimulus.

4 | DISCUSSION

Social deficits in Shank3$^{+/\Delta C}$[15-17] and BTBR[18,26,27] mice have been reported by our lab and other labs, indicated by a reduced preference for a social stimulus over a novel object in the 3-chamber social preference test. In order to determine whether reduced incentive of social engagement may contribute to the observed social deficits, we designed a new behavioral paradigm to quantitate a social incentive index. Through the use of this novel behavioral model, we determined that both Shank3$^{+/\Delta C}$ and BTBR mice exhibit diminished motivational

intent to interact with social stimuli, which may be an underlying factor contributing to the widely observed social deficits.

One way to evaluate the motivational value of a reward is to increase the effort required to obtain the reinforcer.[28,29] The progressive ratio operant task is a well-recognized behavioral paradigm which has been widely used to evaluate motivation for reinforcers like sucrose, food, and addictive drugs.[28] However, this paradigm requires long-term training and the success rate of learning the paradigm for mice with social deficits (such as BTBR mice) is only 50%,[30] which may be due to learning impairments in the context of social reward.[30] Conditioned place preference (CPP) is another widely used method of measuring motivation. However, socially deficient mice (BTBR[31]) are likewise unable to develop CPP, which may also be due to an impaired learning capacity.[32] In our paradigm, we used a simplified strategy in which the level of difficulty of obtaining a social reward was increased. Since rodents express an innate preference for enclosed and dark spaces, they typically avoid aversive environments such as the open arm of EPM and the light compartment of the LDB. When a novel object or social stimulus is introduced to such a nonpreferred environment, mice must actively overcome the aversive pressures to engage in a play- or social interaction. In the baseline EPM protocol, Shank3$^{+/\Delta C}$ mice showed no difference from WT mice, indicating that Shank3$^{+/\Delta C}$ mice do not express any anxiety phenotype which could alter the social incentive index. Furthermore, Shank3$^{+/\Delta C}$ and WT mice expressed comparable open-arm engagement time with the novel object in the object incentive trial, suggesting that Shank3$^{+/\Delta C}$ mice do not exhibit general incentivization deficits, but rather these deficits are specific to social reward. Future studies should focus on whether Shank3$^{+/\Delta C}$ mice also express reward processing deficits for other rewards such as food, water and drugs of abuse.

Our data also indicate diminished incentive for social interaction in BTBR mice. Unlike WT and Shank3$^{+/\Delta C}$ mice, BTBR mice showed a notably strong preference for the novel object, indicating increased novelty reward valence in BTBR mice which may correspond to the elevated interest in restricted domains in human ASD patients.[33] Other studies have indicated decreased motivation for other rewards such as food in BTBR mice,[30] suggesting a generalized decrease of motivation in BTBR mice. This discrepancy may be due to the complexity of test protocols and the impaired learning ability of the tested mice.

One may speculate that the reduction of social interaction incentive in Shank3$^{+/\Delta C}$ and BTBR mice in the modified EPM paradigm may be driven by social anxiety (or social aversion/avoidance). Following this explanation, the observed avoidance of social cues should be unaffected by altering the level of environmental aversiveness. In contrast, WT and Shank3$^{+/\Delta C}$ mice displayed a similar social enhancement index in the social incentive trial of the LDB, suggesting that Shank3$^{+/\Delta C}$ mice do not exhibit avoidance (or aversion) of social conspecifics under milder environmental conditions, and that the observed deficits are dependent upon specific aversive pressures and not resulting from intrinsic social aversion. We also hypothesize that in order to express the social incentive deficits, the aversive pressures imposed by the barrier to interaction must reach a certain threshold of severity which is sufficient to deter animals with reduced incentive of social engagement. Relative to the open arm of the EPM, the light compartment of the LDB represents a more mildly aversive environment, presenting light as the lone aversive stimulus, in contrast to the EPM open arm which is well-lit, elevated and exposed (without walls). We thus speculate that the aversive pressure imposed by light exposure alone in LDB is insufficient to suppress the drive for social reward in Shank3$^{+/\Delta C}$ and BTBR mice. There is an apparent contradiction as Shank3$^{+/\Delta C}$ mice display social withdrawal symptoms in the social approach test[17] where a social stimulus is presented in the middle of open chamber similar to the light compartment of LDB box, while this deficit is not shown here. This may be due to the fact that, unlike the LDB test, during the habituation phase of social approach test animals become very familiar with the open arena to avoid open space induced anxiety. Additionally, the social approach test quantifies the amount of time the test mouse spends directly interacting with the social cue, whereas here we quantified the amount of time spent in the chamber with the social cue. However, due to the complexity of social motivation, we do acknowledge the limitations of our experimental design, in that the motivational component of social interaction behavior cannot be thoroughly isolated and quantitated. More sophisticated (but straightforward) experiments are needed to directly measure the incentive value of social stimulus in mouse models of ASD.

In the modified EPM protocols, Shank3$^{+/\Delta C}$ and BTBR mice displayed a significantly lower SII in the social incentive trial than the object incentive trial, suggesting that in these ASD models, the incentive to engage in an interaction with a novel object may exceed that of a social stimulus. However, the SII for WT animals did not differ between the object- and social-incentive trials, which appears in contrast to 3-chamber social preference findings in which WT animals routinely demonstrate a significant preference for the social stimulus over a novel object.[16,17] We theorize that the lack of difference between the WT object- and social-stimulus incentive indexes is due to differences in testing parameters between the traditional 3-chamber paradigm and the EPM models used here. Principally, the 3-chamber paradigm exposes the test mouse to a social stimulus and a novel object simultaneously, forcing the test animal to actively choose to interact with one stimulus over the other, which typically results in a time distribution favoring the social stimulus over the object. Here, WT animals were exposed to novel- and social-stimuli at different times, precluding the need for the animal to establish preference of one stimulus over the other.

The biological mechanisms involved in social motivation rely on brain circuitry including the amygdala, ventral striatum, orbital, and ventromedial prefrontal cortex,[34] along with mesolimbic reward circuitry comprised of the nucleus accumbens and ventral tegmental area.[35] Synaptic dysfunction has been reported in the PFC[16,17] and VTA[36] of Shank3$^{+/\Delta C}$ mice, which may compromise social reward processing. In BTBR mice, changes in the shape and localization of many brain structures, including the hippocampus and amygdala[37] may also diminish the regulation of social motivation.

The molecular mechanisms underlying social motivation are closely related to the neuropeptide oxytocin and its interaction with dopamine.[38,39] Dopamine in the mesocorticolimbic system influences the assignment of motivational salience by impacting the drive toward such rewards, without affecting the pleasure derived from the reward itself.[40,41] Oxytocin, which shares receptor localization sites with the mesocorticolimbic dopamine system, acts with dopamine to specifically increase the salience of social stimuli.[38,42,43] Oxytocin treatment has been shown in clinical studies to improve social behavior in ASD patients,[44,45] and in preclinical studies to alleviate the social deficits in Shank3$^{+/\Delta C}$[46] and BTBR mice.[47] Therefore, the social motivation deficits observed here in these mouse models may be related to oxytocin and the dopamine system. Future studies could focus on examining this relationship.

In conclusion, we have used a simplified behavioral approach to assess the incentive of a social interaction in ASD mouse models (Shank3$^{+/\Delta C}$ and BTBR), identifying diminished social interaction incentive in both models, and providing a new strategy to facilitate the investigation of neurobiological mechanisms for social reward and motivation deficits underlying neuropsychiatric disorders.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

B.R. designed behavioral paradigms and experiments, performed behavioral tests, analyzed data, and wrote the paper. Z.Y. designed experiments and supervised the project. Z.J.W. designed behavioral paradigms and experiments, supervised the project and wrote the paper.

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REFERENCE

1. Christensen DL, Maenner MJ, Bilder D, et al. Prevalence and characteristics of Autism Spectrum disorder among children aged 4 years – early Autism and developmental disabilities monitoring network, seven sites, United States, 2010, 2012, and 2014. *MMWR Surveill Summ*. 2019;68(2):1-19.
2. Buescher AV, Cidav Z, Knapp M, Mandell DS. Costs of autism spectrum disorders in the United Kingdom and the United States. *JAMA Pediatr*. 2014;168(8):721-728.
3. Lavelle TA, Weinstein MC, Newhouse JP, Munir K, Kuhlthau KA, Prosser LA. Economic burden of childhood autism spectrum disorders. *Pediatrics*. 2014;133(3):e520-e529.
4. Andanson J, Pourre F, Maffre T, Raynaud JP. Social skills training groups for children and adolescents with Asperger syndrome: a review. *Arch Pediatr*. 2011;18(5):589-596.
5. Bonete S, Calero MD, Fernandez-Parra A. Group training in interpersonal problem-solving skills for workplace adaptation of adolescents and adults with Asperger syndrome: a preliminary study. *Autism*. 2015;19(4):409-420.
6. Sanchack KE, Thomas CA. Autism spectrum disorder: primary care principles. *Am Fam Physician*. 2016;94(12):972-979.
7. Grove J, Ripke S, Als TD, et al. Identification of common genetic risk variants for autism spectrum disorder. *Nat Genet*. 2019;51(3): 431-444.
8. Autism Genome Project Consortium, Szatmari P, Paterson AD, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nat Genet*. 2007;39(3):319-328.
9. Leblond CS, Nava C, Polge A, et al. Meta-analysis of SHANK mutations in autism spectrum disorders: a gradient of severity in cognitive impairments. *PLoS Genet*. 2014;10(9):e1004580.
10. Kumar RA, KaraMohamed S, Sudi J, et al. Recurrent 16p11.2 microdeletions in autism. *Hum Mol Genet*. 2008;17(4):628-638.
11. Kazdoba TM, Leach PT, Yang M, Silverman JL, Solomon M, Crawley JN. Translational mouse models of autism: advancing toward pharmacological therapeutics. *Curr Top Behav Neurosci*. 2016;28:1-52.
12. Kazdoba TM, Leach PT, Crawley JN. Behavioral phenotypes of genetic mouse models of autism. *Genes Brain Behav*. 2016;15(1):7-26.
13. Jiang YH, Ehlers MD. Modeling autism by SHANK gene mutations in mice. *Neuron*. 2013;78(1):8-27.
14. Betancur C, Buxbaum JD. SHANK3 haploinsufficiency: a "common" but underdiagnosed highly penetrant monogenic cause of autism spectrum disorders. *Mol Autism*. 2013;4(1):17.
15. Duffney LJ, Zhong P, Wei J, et al. Autism-like deficits in Shank3-deficient mice are rescued by targeting actin regulators. *Cell Rep*. 2015;11(9):1400-1413.
16. Qin L, Ma K, Wang ZJ, et al. Social deficits in Shank3-deficient mouse models of autism are rescued by histone deacetylase (HDAC) inhibition. *Nat Neurosci*. 2018;21(4):564-575.
17. Wang ZJ, Zhong P, Ma K, et al. Amelioration of autism-like social deficits by targeting histone methyltransferases EHMT1/2 in *Shank3*-deficient mice. *Mol Psychiatry*. 2019. [Epub ahead of print].
18. McFarlane HG, Kusek GK, Yang M, Phoenix JL, Bolivar VJ, Crawley JN. Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes Brain Behav*. 2008;7(2):152-163.
19. Meyza KZ, Blanchard DC. The BTBR mouse model of idiopathic autism – current view on mechanisms. *Neurosci Biobehav Rev*. 2017; 76(Pt A):99-110.
20. Berridge KC, Robinson TE, Aldridge JW. Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol*. 2009;9 (1):65-73.
21. Bourin M, Hascoet M. The mouse light/dark box test. *Eur J Pharmacol*. 2003;463(1–3):55-65.
22. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc*. 2007;2(2):322-328.
23. Wang ZJ, Martin JA, Mueller LE, et al. BRG1 in the nucleus accumbens regulates cocaine-seeking behavior. *Biol Psychiatry*. 2016; 80(9):652-660.
24. Komada M, Takao K, Miyakawa T. Elevated plus maze for mice. *J Vis Exp*. 2008;22.
25. Serchov T, Calker DV, Biber K. Light/dark transition test to assess anxiety-like behavior in mice. *Bio-protocol*. 2016;6(19).

26. Bolivar VJ, Walters SR, Phoenix JL. Assessing autism-like behavior in mice: variations in social interactions among inbred strains. *Behav Brain Res*. 2007;176(1):21-26.
27. Yang M, Zhodzishsky V, Crawley JN. Social deficits in BTBR T+tf/J mice are unchanged by cross-fostering with C57BL/6J mothers. *Int J Dev Neurosci*. 2007;25(8):515-521.
28. DeLeon IG, Iwata BA, Goh HL, Worsdell AS. Emergence of reinforcer preference as a function of schedule requirements and stimulus similarity. *J Appl Behav Anal*. 1997;30(3):439-449.
29. Der-Avakian A, Barnes SA, Markou A, Pizzagalli DA. Translational assessment of reward and motivational deficits in psychiatric disorders. *Curr Top Behav Neurosci*. 2016;28:231-262.
30. Martin L, Sample H, Gregg M, Wood C. Validation of operant social motivation paradigms using BTBR T+tf/J and C57BL/6J inbred mouse strains. *Brain Behav*. 2014;4(5):754-764.
31. Pearson BL, Bettis JK, Meyza KZ, Yamamoto LY, Blanchard DC, Blanchard RJ. Absence of social conditioned place preference in BTBR T+tf/J mice: relevance for social motivation testing in rodent models of autism. *Behav Brain Res*. 2012;233(1):99-104.
32. McTighe SM, Neal SJ, Lin Q, Hughes ZA, Smith DG. The BTBR mouse model of autism spectrum disorders has learning and attentional impairments and alterations in acetylcholine and kynurenic acid in prefrontal cortex. *PLoS One*. 2013;8(4):e62189.
33. McFayden TC, Albright J, Muskett AE, Scarpa A. Brief report: sex differences in ASD diagnosis – a brief report on restricted interests and repetitive behaviors. *J Autism Dev Disord*. 2019;49(4):1693-1699.
34. Chevallier C, Kohls G, Troiani V, Brodkin ES, Schultz RT. The social motivation theory of autism. *Trends Cogn Sci*. 2012;16(4):231-239.
35. McCall C, Singer T. The animal and human neuroendocrinology of social cognition, motivation and behavior. *Nat Neurosci*. 2012;15(5):681-688.
36. Bariselli S, Tzanoulinou S, Glangetas C, et al. SHANK3 controls maturation of social reward circuits in the VTA. *Nat Neurosci*. 2016;19(7):926-934.
37. Mercier F, Kwon YC, Douet V. Hippocampus/amygdala alterations, loss of heparan sulfates, fractones and ventricle wall reduction in adult BTBR T+ tf/J mice, animal model for autism. *Neurosci Lett*. 2012;506(2):208-213.
38. Gordon I, Martin C, Feldman R, Leckman JF. Oxytocin and social motivation. *Dev Cogn Neurosci*. 2011;1(4):471-493.
39. Love TM. Oxytocin, motivation and the role of dopamine. *Pharmacol Biochem Behav*. 2014;119:49-60.
40. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*. 1998;28(3):309-369.
41. Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD. Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. *Behav Neurosci*. 2005;119(1):5-15.
42. Averbeck BB. Oxytocin and the salience of social cues. *Proc Natl Acad Sci U S A*. 2010;107(20):9033-9034.
43. Bartz JA, Zaki J, Bolger N, Ochsner KN. Social effects of oxytocin in humans: context and person matter. *Trends Cogn Sci*. 2011;15(7):301-309.
44. Yamasue H, Okada T, Munesue T, et al. Effect of intranasal oxytocin on the core social symptoms of autism spectrum disorder: a randomized clinical trial. *Mol Psychiatry*. 2018. [Epub ahead of print].
45. Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proc Natl Acad Sci U S A*. 2010;107(9):4389-4394.
46. Harony-Nicolas H, Kay M, du Hoffmann J, et al. Oxytocin improves behavioral and electrophysiological deficits in a novel Shank3-deficient rat. *Elife*. 2017;6.
47. Yamasue H. Promising evidence and remaining issues regarding the clinical application of oxytocin in autism spectrum disorders. *Psychiatry Clin Neurosci*. 2016;70(2):89-99.

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