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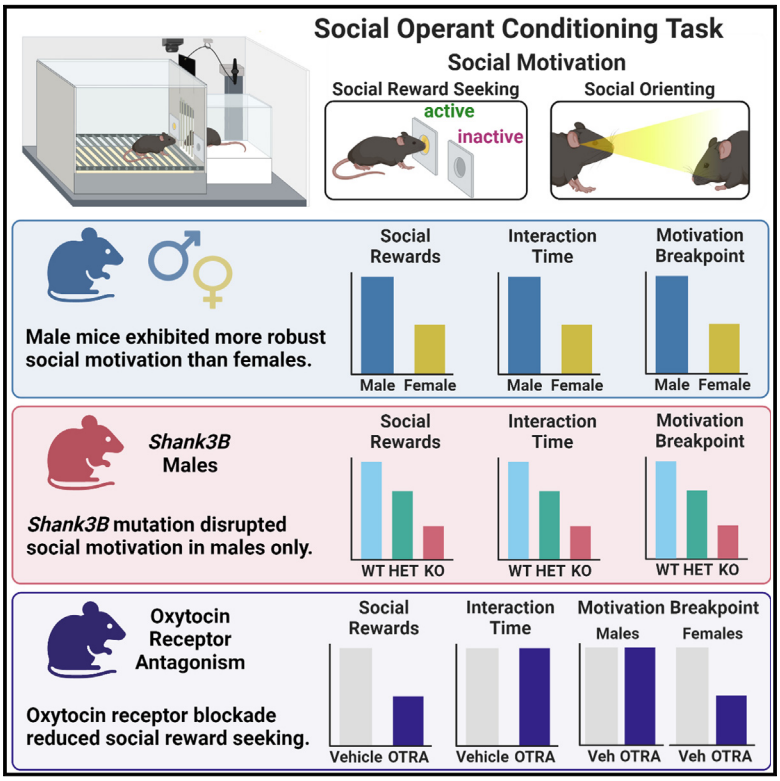
A comprehensive assay of social motivation reveals sex-specific roles of autism-associated genes and oxytocin

Graphical abstract



Highlights

- We developed a social motivation assay measuring social reward seeking and orienting
- Male mice exhibited a more pronounced response in the social motivation task
- *Shank3B* mutant male mice exhibited reduced motivation for a social interaction
- Oxytocin receptor blockade decreased social motivation

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In brief

Maloney et al. present a comprehensive assay of both social reward seeking and social orienting subcomponents of social motivation. This assay reveals innate sex-based differences in social motivation, male-biased deficits in social orienting and reward seeking in the *Shank3B* mutant model, and reduced social motivation in the presence of oxytocin receptor blockade, which was particularly pronounced in female mice.

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A comprehensive assay of social motivation reveals sex-specific roles of autism-associated genes and oxytocin

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MOTIVATION Social motivation is necessary for typical social development, and disruptions in social motivation have been associated with autism. Social motivation includes several subcomponents, including social seeking and social orienting. As rodent models are widely used to investigate the neural circuitry of sociability and autism, there exist numerous well-validated and valuable behavioral paradigms assaying general sociability (e.g., social approach and reciprocal social interaction) or social reward responses (social conditioned place preference). However, these tasks do not quantify social motivation directly (i.e., the amount of work an animal will exert for access to social interaction). To meet this need in the field, we developed an automated social reward operant conditioning task coupled with video tracking. Using the task, we can quantify effort for access to a social partner with concurrent social orienting behavior.

SUMMARY

Social motivation is critical to the development of typical social functioning. Social motivation, specifically one or more of its components (e.g., social reward seeking or social orienting), could be relevant for understanding phenotypes related to autism. We developed a social operant conditioning task to quantify effort to access a social partner and concurrent social orienting in mice. We established that mice will work for access to a social partner, identified sex differences, and observed high test-retest reliability. We then benchmarked the method with two test-case manipulations. *Shank3B* mutants exhibited reduced social orienting and failed to show social reward seeking. Oxytocin receptor antagonism decreased social motivation, consistent with its role in social reward circuitry. Overall, we believe that this method provides a valuable addition to the assessment of social phenotypes in rodent models of autism and the mapping of potentially sex-specific social motivation neural circuits.

INTRODUCTION

In social species such as humans and mice, social interactions are inherently rewarding and necessary for typical development. Social motivation, defined as the internal processes that drive these social interactions, has been conceptualized in terms of several interrelated components including social orienting (attending to social stimuli) and social reward seeking (incentive value of social interactions), with each potentially mediated by distinct brain circuits.[1]

Rodent models are widely used to investigate the neural circuitry of social behavior. Specifically, there are well-established assays for quantifying sociability, including the three-chamber social approach test[2] and reciprocal social interaction in an open field.[3] While valuable for the assessment of specific areas of social behavior, neither of these tasks quantify social motivation directly (i.e., the amount of work an animal will exert for access to social interaction). The rewarding properties of social contact can be evaluated via a social conditioned place preference (CPP) assay, in which animals learn to associate social interaction with a particular environmental context and are then given a choice between the socially associated environment or one associated with isolation. Preference for the socially conditioned side is thought to reflect the rewarding value of social contact.[4–6] However, this assay does not measure the social orienting aspect of social motivation, and it suffers from other

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limitations as well: a recent review concluded that social CPP is highly variable, transiently expressed, and occurs only in specific conditions.[7] Thus, while well-established procedures exist to investigate social interaction and limited aspects of social reward, methods for direct quantitative assessment of social motivation and its subcomponents are lacking. Such methods would allow more precise mapping of social motivation circuits and studies of their regulation by genes associated with human conditions.

Better understanding of social motivation circuits is important because while challenges with social functioning are defining characteristics of autism, the underlying circuits remain unclear. Recent human genetic studies have identified hundreds of autism-associated genes,[8] prompting rodent studies to explore how mutations in these genes might affect conserved social circuits in the mammalian brain.[9–11] The social motivation theory suggests that social interaction may be less motivating to autistic individuals[1,12] and that this reduced motivation may lead to poor acquisition of social skills and further social disengagement. In line with this theory, children with autism demonstrated reduced behavioral and neural responses to social rewards[12,13] and deficits in orienting to social stimuli (i.e., eye tracking).[14,15] However, mechanistic studies defining the circuitry underlying each of these functions in humans are challenging, especially considering the heterogeneity of autism: gathering enough participants with the same autism-associated mutations, controlling for environmental confounds and genetic interactions, and interrogating neural mechanisms in a causal manner are all difficult to achieve in human research.

Thus, mouse models of autism-associated mutations provide the opportunity to systematically investigate the role of specific genes in social motivation circuits under controlled conditions and enable application of mechanistic circuit mapping tools not available in humans.[9–11] For example, mice with homozygous deletion of the *Shank3B* gene in the autism-associated Phelan-McDermid locus show reduced social approach behavior, though it remains unclear whether this is due to a lack of social motivation.[16] Interestingly, such sociability reductions have only been reported in homozygous *Shank3B*-null mice, lacking two functional copies of the gene, while mice heterozygous for *Shank3B* mutation failed to show this effect despite more closely modeling the haploinsufficiency that clinically defines Phelan-McDermid syndrome.[17] In the absence of specific behavioral assays to measure both social orienting and reward seeking aspects of social motivation, the true extent of social impairment in these mice remains unknown.

Pharmacological manipulation of neural circuitry provides a complementary approach to genetic studies. Notably, the peptide oxytocin has been shown to mediate social reward in the context of pair bonding and social preference, as well as to enhance the salience of social signals in a variety of other circuits across the brain.[18,19] Blocking oxytocin via administration of a receptor antagonist can reduce social reward learning (related to reward seeking behavior) in rodents.[20] Evidence in humans suggests oxytocin may increase social orienting under specific conditions.[21,22] Thus, oxytocin is thought to play a critical role in the neural mechanisms underlying social motivation.

What behavioral tasks might enable more direct investigation of motivational circuits? Operant conditioning has long been employed to test motivation for many types of non-social stimuli such as food, sucrose, and drugs of abuse. However, only a few previous studies have attempted to use social rewards.[23] Female mice have been shown to lever press for access to their pups[24,25] or for time with a sexual partner.[20] Interestingly, the latter study found that mice were also motivated to access a sex-matched stimulus animal, suggesting that social reward is sufficient to drive operant responding in the absence of parental or sexual drivers. Notably, Martin et al. developed a social operant task using lever pressing and found that BTBR inbred mice exert less effort to obtain social contact compared with C57BL/6J mice, consistent with known differences in overall sociality between these strains.[26,27] A similar approach has recently been used to map social motivation circuits in wild-type mice as well.[10]

Here, we present in depth a method for parallel quantitative assessment of multiple aspects of social motivation—social reward seeking and concurrent social orienting—by building on existing methods to couple a social operant conditioning paradigm with automated video tracking of mice. We designed a simple, inexpensive, add-on device compatible with existing operant conditioning systems and developed a significantly abbreviated conditioning protocol compared with previous studies. The training sessions are fully automated, combining automated quantification of rewarded hole-poking behavior to measure social reward seeking. Simultaneous automatic video tracking of the animal's movement is used to measure social orienting, which is quantified by time spent in proximity to and interacting with the social partner during rewards. We first demonstrate that mice will work harder for access to a conspecific than the non-social condition of simply opening a door, validating the social specificity of this reward. We then assess reproducibility of the assay, noting remarkable intraindividual stability of the day-to-day measurement of social motivation. Finally, we establish construct validity and further demonstrate the sensitivity of the task with the disruption of social motivation via genetic mutation of *Shank3B* or antagonism of oxytocin receptors. We also provide links to detailed protocols for assembly and operation of the equipment, parts lists, and annotated scripts for data processing and analysis. This method represents an important tool for more nuanced phenotyping of rodent social behavior and will further contribute to the mapping of social motivation circuits in the brain. Ultimately, this assay will aid in understanding the consequences of gene mutations on social circuit functions.

RESULTS

Mice will work for access to a social partner

We developed an approach to quantify social reward by adapting a classic operant conditioning paradigm (Figure 1A) to deliver transient visual, olfactory, and limited tactile access to a social partner in response to a conditioned behavior (i.e., nose poking; Figure 1B). We hypothesized that mice could learn to distinguish an active hole, which results in access to a social partner, from an inactive hole. We further hypothesized that the conditioned

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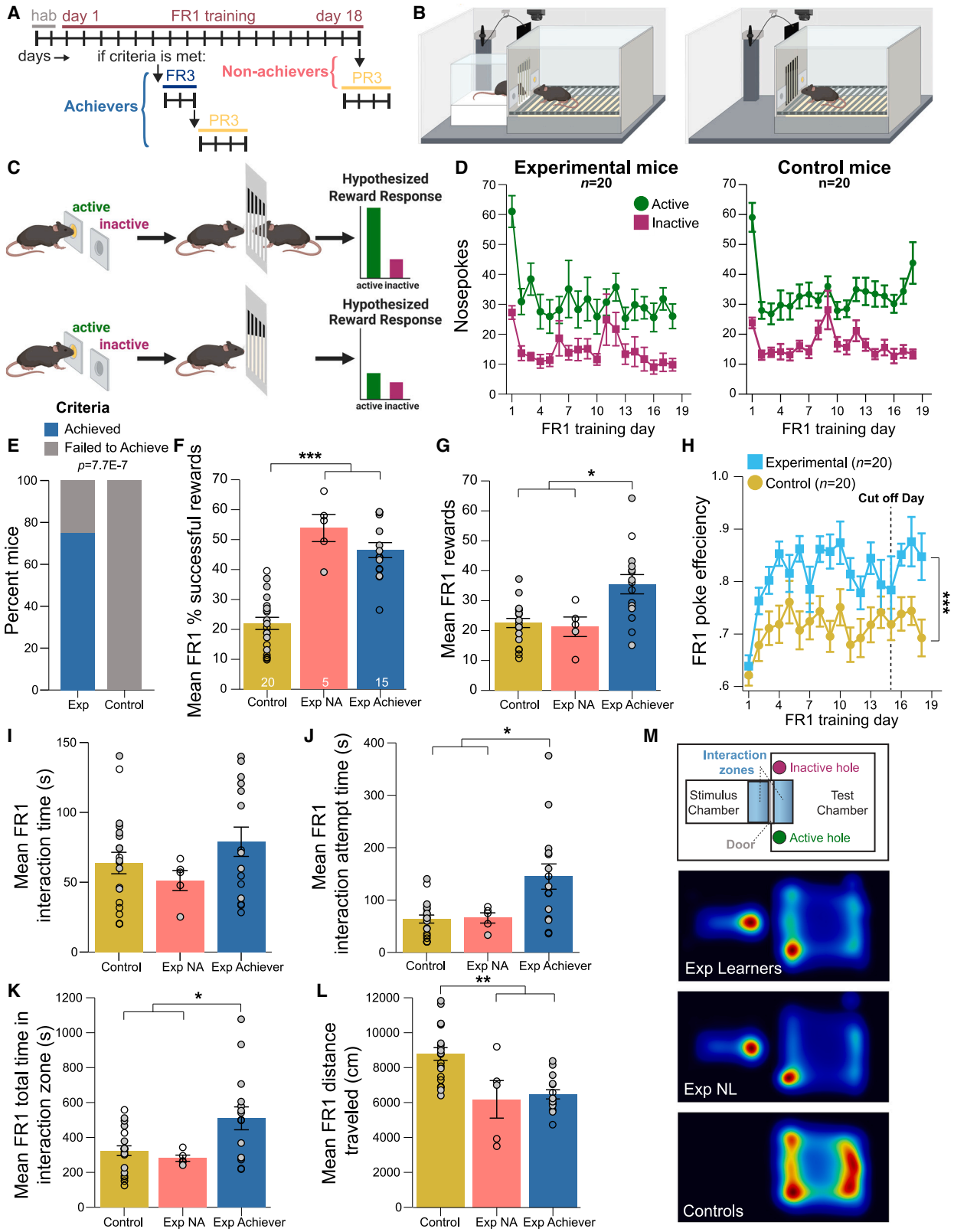

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response would be greater in mice rewarded with a social partner than those that were not (Figure 1C). Thus, in our first experimental cohort (cohort 1), we examined the behavior of two groups of mice. In the experimental group, following a correct nose poke into the active hole, a vertical door opened to reveal a novel age- and sex-matched social partner for a duration of 12 s. In the control group, the door opened to reveal a blank wall for 12 s. This group distinction allowed us to confirm that the performance of the experimental group was indeed due to the social reward.

It is important here to operationally define the components of social motivation assessed in this task. As defined in Chevallier et al., social reward seeking is the behavioral manifestation of the incentive value of a social reward.[1] Thus, we defined outcomes related to reward seeking behavior in our task as conditioning criteria achievement, number of rewards achieved, poke efficiency (rewards/active nose pokes), percentage of successful rewards, poke accuracy (active/total nose pokes), and breakpoint (all defined in Table 1). Social orienting is the prioritization of attention to social signals,[1] operationally defined here as time engaged in a social interaction with the stimulus mouse, social interaction attempts, and total time in the interaction zone regardless of the location of the stimulus mouse and entries into the interaction zone (also defined in Table 1).

To determine the point at which animals were successfully conditioned to nose poke for the reward, we defined conditioning criteria of at least 40 active nose pokes, 75% poke accuracy, and 65% successful rewards during a single session. We included the criterion of the percentage of successful rewards to ensure that the behavior of the mice was driven by engagement with the reward, i.e., interaction with a social partner or the blank wall. All mice in both groups learned to distinguish the active from inactive holes (Figure 1D) within 15 days of training on a fixed ratio schedule of reinforcement in which one active nose poke was required to receive one reward (fixed ratio 1 [FR1]). However, while the majority of experimental mice (6/10 females, 9/10 males) reached conditioning criteria, none of the control mice did (Figure 1E). The control mice had a lower percentage of successful rewards on average per session compared with all experimental mice (Figure 1F). In addition, the control mice received significantly fewer rewards on average per session than the subset of experimental mice who successfully met criteria (achiever), performing comparably to the subset of experimental mice who did not meet criteria (non-achiever; Figure 1G; Table S1). Finally, although comparable numbers of active nose pokes were achieved by experimental and control mice (Figure 1D), the poke efficiency was significantly diminished among the control mice (Figure 1H). This indicates that, in contrast to the experimental mice, the control mice continued to poke in the active hole even once a reward had been achieved instead of interacting with the reward stimulus (i.e., the open door). Thus, we can conclude that the performance of the experimental mice was specifically driven by the social reward.

Mice exhibit social orienting during access to a social partner

We next evaluated social orienting in our operant task. We did not observe differences in interaction time between groups (Figure 1I), which is defined here as the time that both the test and stimulus mice are at the open door during a reward (experimental group) or the time the test mice spend at the open door (control group). This may inflate interaction time for the control mice, however, as they do not depend on the behavior of a second animal as in the experimental group (i.e., the stimulus mouse). Therefore, a more adequate comparison with the control group time at the open door may be made with interaction attempt time for the experimental group, measured as the time at the door during a reward regardless of whether the stimulus mouse is present. Here, we see that experimental achievers exhibited greater time attempting an interaction compared with both experimental non-achievers and controls (Figure 1J). In fact, experimental achievers spent significantly more time in the interaction zone regardless of a concurrent reward across the entire session compared with both control and experimental non-achiever mice (Figure 1K). Conversely, the control mice exhibited a greater level of activity in the test chamber during the task compared with both achiever and non-achiever experimental groups (Figures 1L and 1M). Together, these data indicate that

Figure 1. Mice exhibited both reward seeking and social orienting in the social motivation operant task

(A) Social motivation operant task timeline schematic.

(B) Social motivation operant apparatus provides access to transient access to a social partner for the experimental condition (left panel; n = 20) and access to a non-social door raising for the control condition (right panel; n = 20).

(C) Schematized hypothesis that the response to reward would be greater in mice that received a social reward compared with those that did not.

(D) Nose pokes into active and inactive holes for experimental mice (left panel) and control mice (right panel) across FR1 training.

(E) Ratio of mice in experimental (15/20) and control (0/20) conditions that achieved conditioning criteria during training.

(F) Across training, the average percentage of successful rewards that included an interaction with the reward stimulus for experimental achievers (Exp achievers), experimental non-achievers (Exp NAs), and control non-achievers (controls).

(G) The average number of rewards received per day for all groups.

(H) Poke efficiency across FR1 days. Cutoff day signifies the day after which no additional mice reached conditioning criteria (experimental mice, n = 20; control mice, n = 20).

(I) The duration of time spent interacting with the reward stimulus during the 12 s reward periods averaged across FR1 days.

(J) The average time spent attempting to interact (either successfully or unsuccessfully) during FR1 training.

(K) The total time spent in the interaction zone per day averaged across FR1 training.

(L) Total distance traveled, on average, in the apparatus during FR1 training.

(M) Schematic of apparatus (top panel) and heatmaps of animals' body positions in the apparatus across FR1 training (bottom three panels). Warmer colors represent greater time spent in that position.

(D and F–L) Grouped data are presented as means ± SEM with individual data points as open circles (females) and gray circles (males). Statistical significance, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Sample sizes for all panels are Exp achievers (n = 15), Exp NAs (n = 5), and controls (n = 20) unless otherwise stated.

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Table 1. Glossary of outcomes

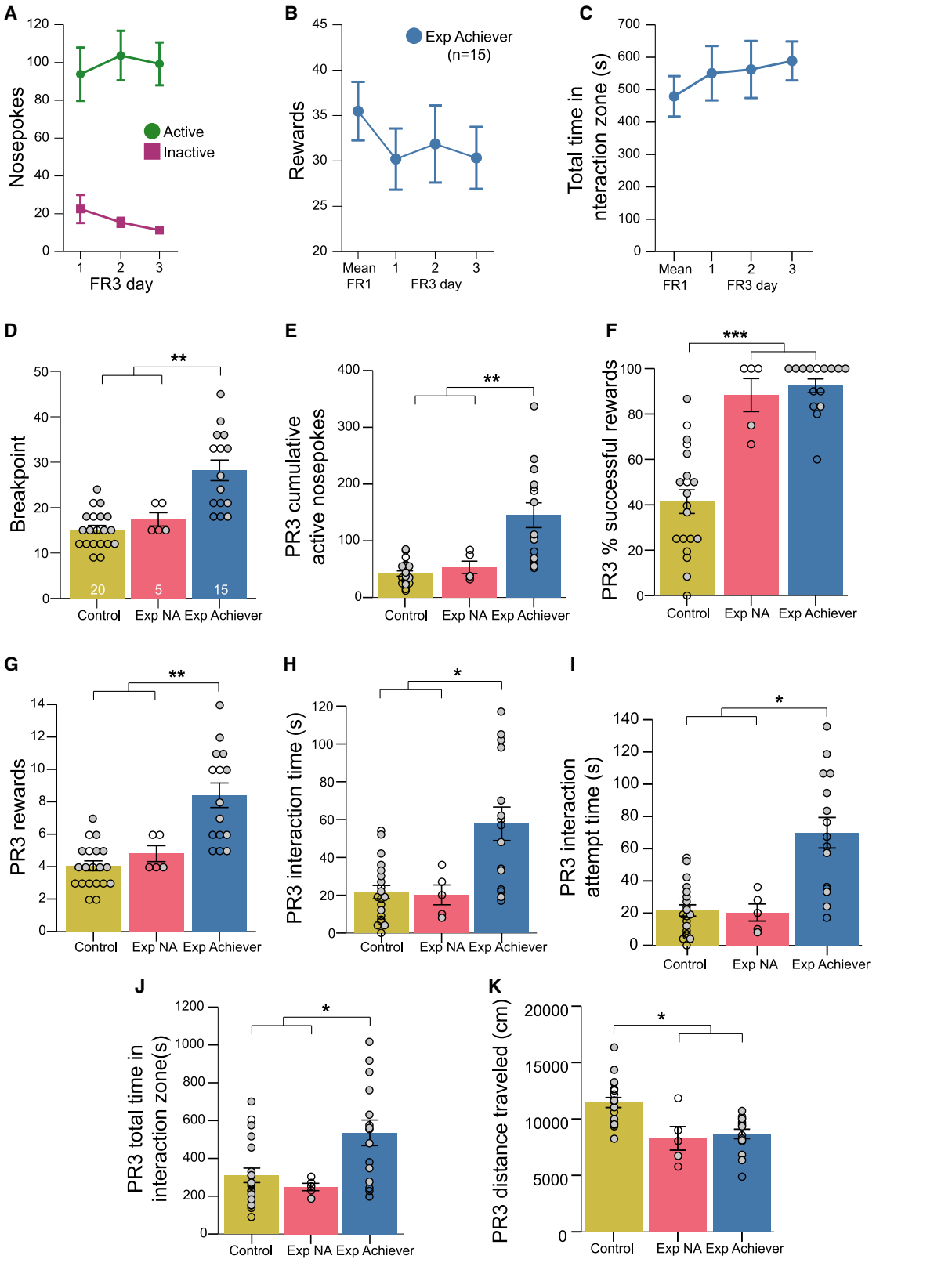
Social motivation component	Outcome	Definition
Seeking/liking	conditioning criteria reached	animal showed conditioning to the reward stimulus (criteria include at least 40 active nose pokes, 75% poke accuracy, and 65% successful rewards during a single session)
	reward	the door opening in response to the required number of active nose pokes, presenting access to a social partner and allowing for a social interaction to occur
	poke efficiency	rewards per active nose pokes, where a 1 indicates one reward was received per one active nose poke (rewards/active nose pokes)
	percentage of successful rewards	percentage of rewards where a social interaction occurred (or an interaction with the door for cohort 1 control mice)
	poke accuracy	percentage of active nose pokes (active nose pokes/total nose pokes)
	breakpoint	maximum nose pokes performed to elicit a reward during progressive ratio 3 testing
Social orienting	interaction	duration of time the test and stimulus mice are simultaneously at the open door (in their respective interaction zones) during a reward
	interaction attempts	duration of time the test mouse is in the interaction zone during a reward when the door is open, which can be both successful (an interaction occurs) or unsuccessful (the stimulus mouse is not at the door and no interaction occurs)
	total time in interaction zone	total duration of time the test mouse is in the interaction zone during a 1 h session, including time during and not during rewards, regardless of the stimulus mouse location
	interaction zone entries	entries into the interaction zone at the door

the experimental achievers exhibited social orienting, spending time in close proximity to the social reward, while the control mice exhibited much more general exploration of the test chamber. Summary statistics can be found in Table S1.

Mice are highly motivated to obtain social rewards under effortful conditions

Since the majority of experimental mice were successfully conditioned to nose poke for a social reward during training under an FR1 reinforcement schedule, we next increased the effort required to obtain this reward. In the session following their achievement of conditioning criteria, achievers were moved up to an FR3 schedule of reinforcement, requiring three active nose pokes to achieve one reward. During FR3 training, the mice continued to show a high poke accuracy (Figure 2A) and achieved comparable numbers of rewards per day as during FR1, despite the required effort increasing 3-fold (Figure 2B). In addition, the mice spent a similar amount of time in the interaction zone on each day of FR3 compared with the average time per day during FR1 (Figure 2C). Thus, when the task became more effortful, the mice continued to display high levels of social reward seeking and social orienting.

Finally, after three sessions at FR3, we further increased the effort required to obtain social rewards by moving to a progressive ratio 3 schedule of reinforcement (PR3), in which the number of nose pokes required increases by 3 pokes after each reward (i.e., 3, 6, 9, 12 …). By quantifying the breakpoint, or the number of nose pokes at which the animal stops pursuing further rewards, we directly measured how hard the animal was willing to work for access to a social partner. Experimental achiever mice reached a significantly greater breakpoint compared with control mice and experimental non-achiever mice (Figure 2D), reaching up to 350 active nose pokes within a 1 h session (Figure 2E), suggesting a strong motivation to seek a social reward. Furthermore, the experimental mice, independent of achiever status, achieved 88% successful rewards (achievers, 92.4%; non-achievers, 88.3%), whereas the control mice only achieved 41.4% successful rewards (Figure 2F). Due to the much higher number of rewards received by the experimental achiever group relative to the other two groups (Figure 2G), the interaction time with the stimulus (social partner or door) and the time attempting an interaction (when a social partner was not at the door during a reward) were significantly greater in the experimental achiever group compared with both experimental non-achievers and controls (Figures 2H and 2I). These data suggest that mice are more highly motivated to interact with a social stimulus in this paradigm compared with a control stimulus, regardless of achiever status, although there appears to be a dichotomy in social motivation between individual mice driven at least in part by social orienting differences. As observed in FR1 training, the experimental non-achievers and the controls spent significantly less total time in the interaction zone during the PR3 testing compared with experimental achievers (Figure 2J), while the



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control mice traveled a further total distance overall (Figure 2K). Summary statistics can be found in Tables S1 and S2.

Social motivation measurements show high reliability across days

We next explored the measurement reliability of the task within individuals across time. To do this, we continued PR3 testing for an additional 3 days in cohort 1. The breakpoint remained consistent across sessions for all groups (Figure S1A) and was highly correlated within an individual (Figure S1B). Further, measures of social orienting remained consistent: each group showed similar percentages of successful rewards across all four PR3 days, with the two experimental groups both significantly higher than controls (Figure S1C). Similar consistency was observed for interaction time across PR3 days (Figure S1D), as well as for total time in the interaction zone (Figure S1E). Summary statistics can be found in Table S1.

Male mice exhibit more robust social reward seeking and social orienting than females

Understanding innate sex differences in social behavior can help us decipher the origins of sex biases observed in conditions like autism.[28] Thus, we examined the influence of sex on social motivation in our data, collapsing across achiever and non-achiever experimental groups to enhance statistical power. Both females and males learned to distinguish the active from inactive holes (Figure S2A). Males achieved significantly more rewards on average during FR1 training and PR3 testing compared with females, though this difference was non-significant for FR3 training (Figures S2B–S2D). Correspondingly, males reached a higher PR3 breakpoint (Figure S2E). Males showed greater interaction time, interaction attempt time, and total time in the interaction zone (Figures S2F–S2N). Males also demonstrated non-significant increases in the percentage of successful interactions during FR1 and PR3 (Figures S2O–S2Q). In contrast, no effect of sex was observed for poke accuracy or distance traveled (Figures S2R–S2X). An increase in total nose pokes was observed in males for PR3 (Figures S2X–S2Z), likely a reflection of the increased nose poking conducted to achieve the higher breakpoint. Achiever status was not associated with sex, nor was the average number of days to reach conditioning criteria different between sexes (females mean [M] = 5 days, SD = 1.9; males M = 6.4 days, SD = 4.5). Together, these data indicate that learning and general activity levels in this task were comparable between the sexes, yet reward seeking and social orienting behaviors were greater in male mice compared with females. Summary statistics can be found in Tables S1.

Shank3B mutation disrupts social motivation

Shank3 haploinsufficiency is a highly penetrant monogenic cause of autism.[29] Mouse models of SHANK3 loss have been well validated, with knockout (KO) mice demonstrating self-injurious repetitive grooming and deficits in social approach, social novelty, and communication.[16,30,31] To provide insights into social motivation in an autism-associated model, we assessed the performance of both male and female *Shank3B* homozygous (KO) and heterozygous (Het) mutants alongside wild-type (WT) littermates in our social operant task (Figure 3A).

First, we sought to replicate previous findings of social approach deficits among *Shank3B* KO mutants.[16,30,31] Our operant conditioning protocol includes a habituation phase prior to FR1 training, during which the test animal is allowed uninterrupted access to a novel social stimulus mouse, as the door remains raised and the nose-poke holes are inaccessible. Thus, the animal's entries into and total time in the interaction zone provide measures of social approach behavior. As expected, KO mice made fewer entries into the interaction zone compared with WT mice (Figure S3A; Table S2). KO mice also traveled a shorter distance exploring the chamber than WT or Het mice, staying mainly in the corners of the test chamber furthest from the door (Figure S3B). A sex effect was observed for time spent in the interaction zone, with male KO mice spending significantly less time inside it compared with WT and Het males (Figure S3B), a pattern not observed in females. Thus, in the context of freely available access to a social partner, we observed reduced social approach among male *Shank3B* KO mice but not Het mice, similar to established phenotypes.[16,30,31] Summary statistics can be found in Tables S2.

Next, we probed social motivation in *Shank3B* mice. Across FR1 training, all mice eventually learned to distinguish active from inactive nose pokes (Figure 3B). However, more WT mice reached conditioning criteria compared with Het and KO mice, an effect driven by male mice (Figures 3C and 3D). Additionally, WT males obtained significantly more rewards on average across FR1 training than KO males, with Het males exhibiting an intermediate phenotype (Figure 3E). WT mice also exhibited greater overall nose poking than KO mice (Figure 3F), with the KO mice demonstrating a propensity to remain inactive in the apparatus

Figure 2. Mice were highly motivated to obtain a social reward

(A) Nose pokes into active and inactive holes made by Exp achievers (n = 15) across FR3 training.
(B) Rewards achieved by Exp achievers averaged across FR1 days and on each FR3 day.
(C) The total time per day in the interaction zone for Exp achievers averaged across FR1 days and on each FR3 day.
(D) Breakpoint achieved during day 1 of PR3 for Exp achievers, Exp NAs, and controls.
(E) Cumulative number of active nose pokes across groups on day 1 of PR3.
(F) The percentage of successful rewards on PR3 day 1 across groups.
(G) The number of rewards achieved on day 1 of PR3 for all groups.
(H) The duration of interaction time on PR3 day 1 for all groups.
(I) Time an interaction was attempted: test mouse at the door regardless of stimulus mouse location.
(J) Total time in the interaction zone across the entire PR3 day 1 session for all groups.
(K) Total distance traveled during day 1 PR3 for all groups.
For all panels, the grouped data are presented as means ± SEM with individual data points as open (females) and closed (males) circles. Statistical significance, ***p < 0.001, **p < 0.01, *p < 0.05. Sample sizes for all panels: Exp achievers (n = 15), Exp NAs (n = 5), and controls (n = 20).

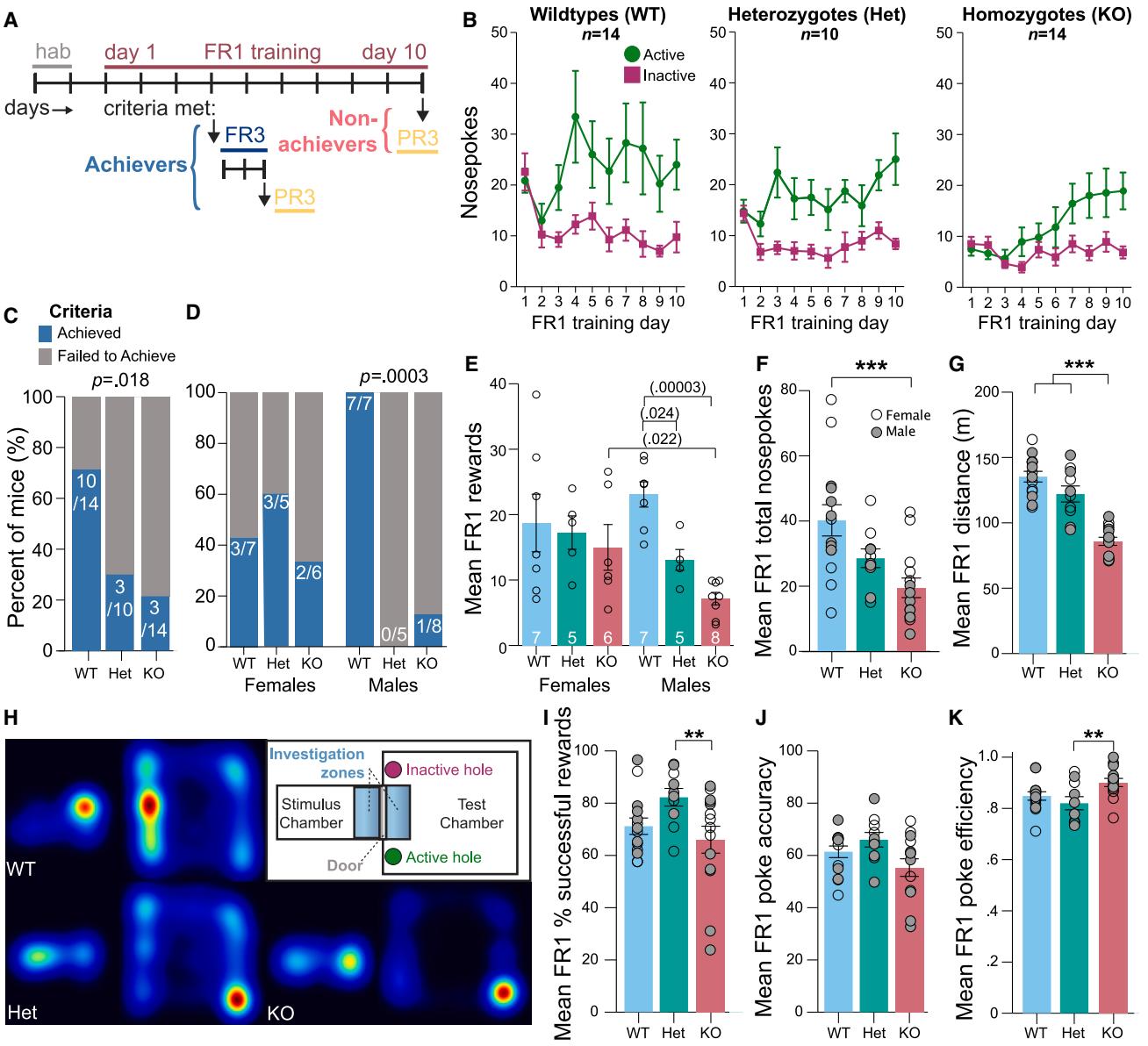

Figure 3. *Shank3B* knockout (KO) and heterozygous (Het) mice exhibited reduced social reward seeking and exploratory behavior relative to wild-type (WT) controls

(A) *Shank3B* social motivation experiment timeline schematic.

(B) Nose pokes into active and inactive holes for WT mice (left panel), *Shank3B* Het mice (middle panel), and KO mice (right panel) across FR1 training.

(C and D) Percentage of mice that achieved conditioning criteria during FR1 training (C) by genotype and (D) by genotype and sex.

(E) The number of rewards, averaged across FR1 days and split by sex, for WT, Het, and KO mice.

(F) Mean number of total nose pokes across FR1 days by WT, Het, and KO mice.

(G) Mean distance traveled by WT, Het, and KO mice across FR1 training.

(H) Apparatus schematic (top right corner) and representative heatmap of the animal's position in the apparatus, with a single animal during a single FR1 session represented in WT (top left), Het (bottom left), and KO (right). Warmer colors represent greater time spent in that position.

(I) Mean percentage of successful rewards attained by WT, Het, and KO mice during FR1 training.

(J) Mean poke accuracy across groups during FR1 training.

(K) Mean poke efficiency across groups in FR1 training.

(D–G and I–K) Grouped data are presented as means ± SEM with individual data points as open (females) and closed (males) circles. Statistical significance, ***p < 0.001, **p < 0.01, *p < 0.05. Sample sizes: WT (n = 14, female = 7), Het (n = 10, female = 5), and KO (n = 14, female = 6).

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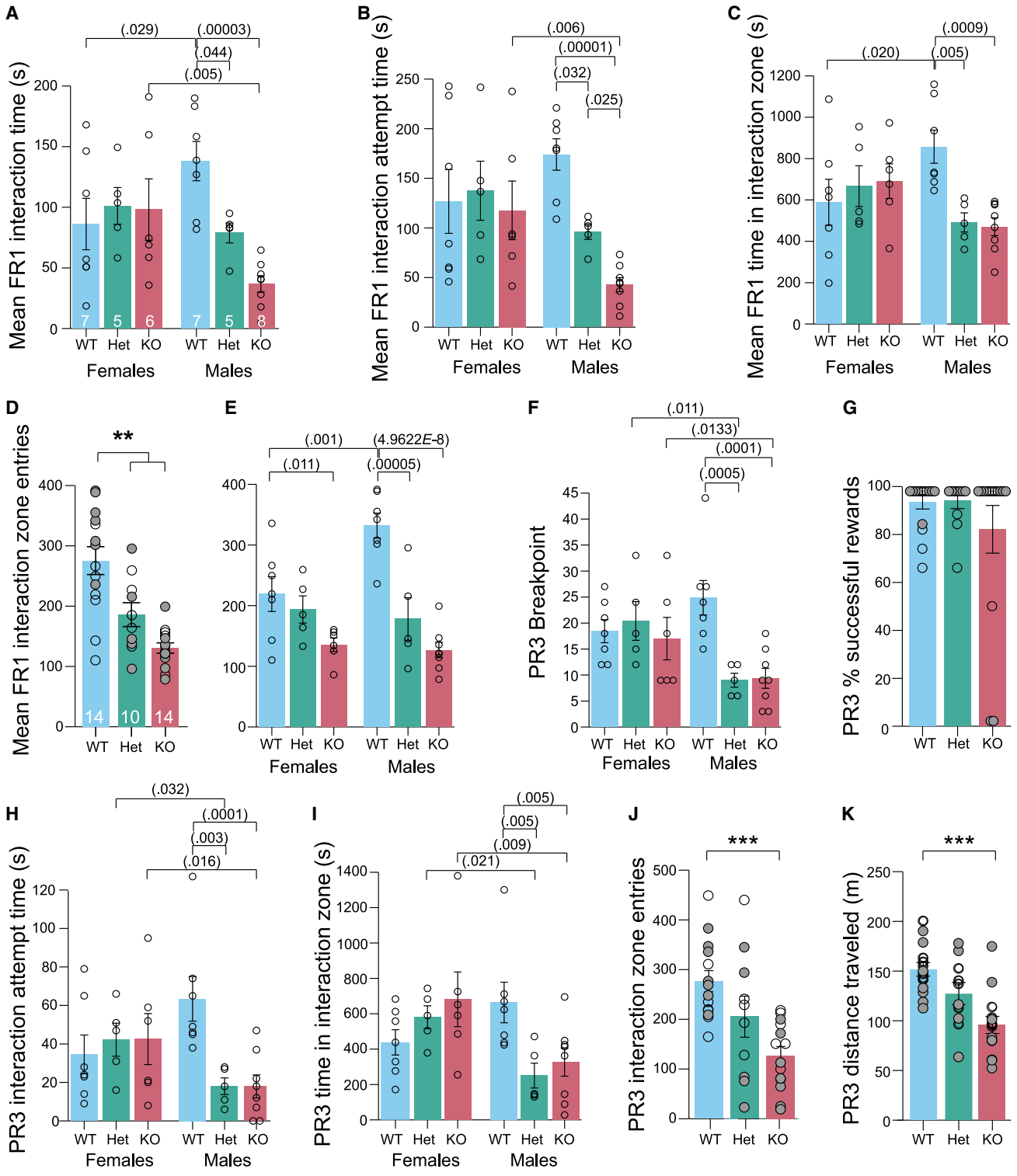

Figure 4. Male *Shank3B* KO and Het mice exhibited reduced social orienting and reduced social motivation relative to WT controls

(A) Mean duration of interaction time across FR1 training, split by sex, for WT, *Shank3B* Het, and KO mice.

(B) Average time attempting interactions in FR1 training.

(C) Total time in the interaction zone in FR1 training.

(D and E) Mean number of interaction zone entries across FR1 days, represented (D) by genotype and (E) by genotype and sex.

(F) Breakpoint achieved during PR3 testing.

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corner, as shown by their shorter distance traveled (Figures 3G and 3H). Still, when accounting for the differences in total distance traveled between the groups, the reduction in rewards attained by Het and KO males (Figure S4A) and trending reduced nose pokes by all KO mice (Figure S4B) remain, indicating that social seeking is diminished in KO mice and Het mice above and beyond hypoactivity. Het mice showed a higher average percentage of successful rewards than KO mice (Figure 3I), an effect that was lost when accounting for distances, indicating that it was largely driven by activity differences between the groups. Poke accuracy was not different among groups (Figure 3J). KO mice did show higher poke efficiency than Het mice (Figure 3K), but this is likely skewed due to their low number of total nose pokes. Together, these data demonstrate reduced social reward seeking among mice with complete loss of *Shank3B*. Summary statistics can be found in Tables S2.

Examination of social orienting behavior revealed that WT mice spent significantly more time interacting during rewards than KO mice, driven by WT males interacting longer than KO males (Figure 4A). Relative to WT males, male Het and KO mice spent less time attempting interactions (Figure 4B) and less total time in the interaction zone (Figure 4C), with no effect observed in females. Furthermore, entries into the interaction zone were significantly greater for WT mice than Het and KO mice (Figure 4D), with WT females entering the interaction zone significantly more than KO females and WT males more than both Het and KO males (Figure 4E). Thus, both male Het and KO mutants exhibited reduced social orienting. All of these effects persisted when controlling for activity levels in the analysis (Figures S4F–S4I).

Finally, when reward value was directly measured in PR3 testing, male WTs achieved higher breakpoints compared with both male Het mice and KO mice (Figure 4F). Interestingly, there was no difference in the percentage of successful rewards, again likely due to the low number of total rewards attained by KO mice; however, the distribution of KO mice was much more variable, while all WT and Het mice exhibited >65% successful rewards (Figure 4G). WT males also spent more time attempting interactions (Figure 4H) and more time in the interaction zone overall (Figure 4I) than Het and KO males in PR3 testing. WT mice entered the interaction zone significantly more throughout PR3 testing, regardless of sex (Figure 4J). Since KO mice did exhibit hypoactivity in PR3 as well (Figure 4K), we reanalyzed the PR3 data controlling for activity levels and found that differences in breakpoint (Figure S4J), interaction attempt time (Figure S4K), and time in interaction zone (Figure S4L) remained. KO mice's reduction in interaction zone entries also remained but was confounded by an interaction with activity levels (Figure S4M). Summary statistics can be found in Table S2.

Overall, these findings suggest that reward seeking and social orienting are both reduced in *Shank3B* Het and KO mice compared with WT mice, particularly among males. The observation of reduced social motivation in *Shank3B* Het mice, which mimic the patient haploinsufficient phenotype, suggests a greater sensitivity of our operant task relative to existing social behavior assays.

Oxytocin receptor blockade reduced social reward seeking but not social orienting behavior

Finally, we tested the sensitivity of this task in the context of pharmacological manipulation of neural circuits involved in social interactions. Specifically, we tested whether the oxytocin system participates in the social reward seeking and/or the social orienting aspects of social motivation by assessing these behaviors in the presence of an oxytocin receptor (OTR) blockade. WT mice were administered either an OTR antagonist (OTRA) or vehicle via intracerebroventricular infusion daily prior to social operant sessions (Figure 5A). During FR1 training, both infusion groups learned to distinguish between the active and inactive holes (Figure 5B), with no difference in the average poke accuracy or average total nose pokes (Figures 5C and 5D; Table S3). However, 61% (6/10 females, 5/8 males) of the vehicle-infused mice reached conditioning criteria while only 7% (0/7 females, 2/8 males) of OTRA mice did so (Figure 5E). Across FR1 training, both male and female OTRA-infused mice achieved significantly fewer average daily rewards compared with vehicle-infused mice (Figure 5F), while only male OTRA mice exhibited a difference in percentage of successful rewards (Figure 5G). Finally, OTRA infusion did not alter poke efficiency (Figure 5H). These data indicate that blocking OTR activity had subtle effects on social reward seeking during training.

We next examined social orienting in the presence of OTR blockade. We found that OTRA infusion did not influence interaction time (Figure 5I), time spent attempting interactions (Figure 5J), or total time in the interaction zone averaged across FR1 training (Figure 5K). However, OTRA-infused mice traveled shorter total distances within the operant chambers on average across days, suggesting less exploration of the apparatus (Figures 5L and 5M). This difference in activity was also reflected in fewer entries into the investigation zone by OTRA-infused mice compared with controls (Figure 5N). This reduction in entries, however, was not substantial enough to alter the average amount of time the OTRA-infused mice spent in the zone per entry (Figure 5O). Thus, OTR antagonism did not influence social orienting in this task but may have reduced overall activity.

To assess the possible impact of altered locomotion on FR1 training performance, we reassessed the data using a model with total distance traveled (Figures 5L and S5A) as a

(G) Percentage of successful rewards in PR3 training by genotype.
(H) Time attempting interactions during PR3 testing.
(I) Total time spent in the interaction zone in PR3.
(J) Number of entries into the investigation zone during PR3 testing.
(K) Distance traveled during PR3 testing.
(A–K) Grouped data are presented as means ± SEM with individual data points as open (females) and closed (males) circles. Statistical significance, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Sample sizes: WT (n = 14, female = 7), Het (n = 10, female = 5), and KO (n = 14, female = 6).

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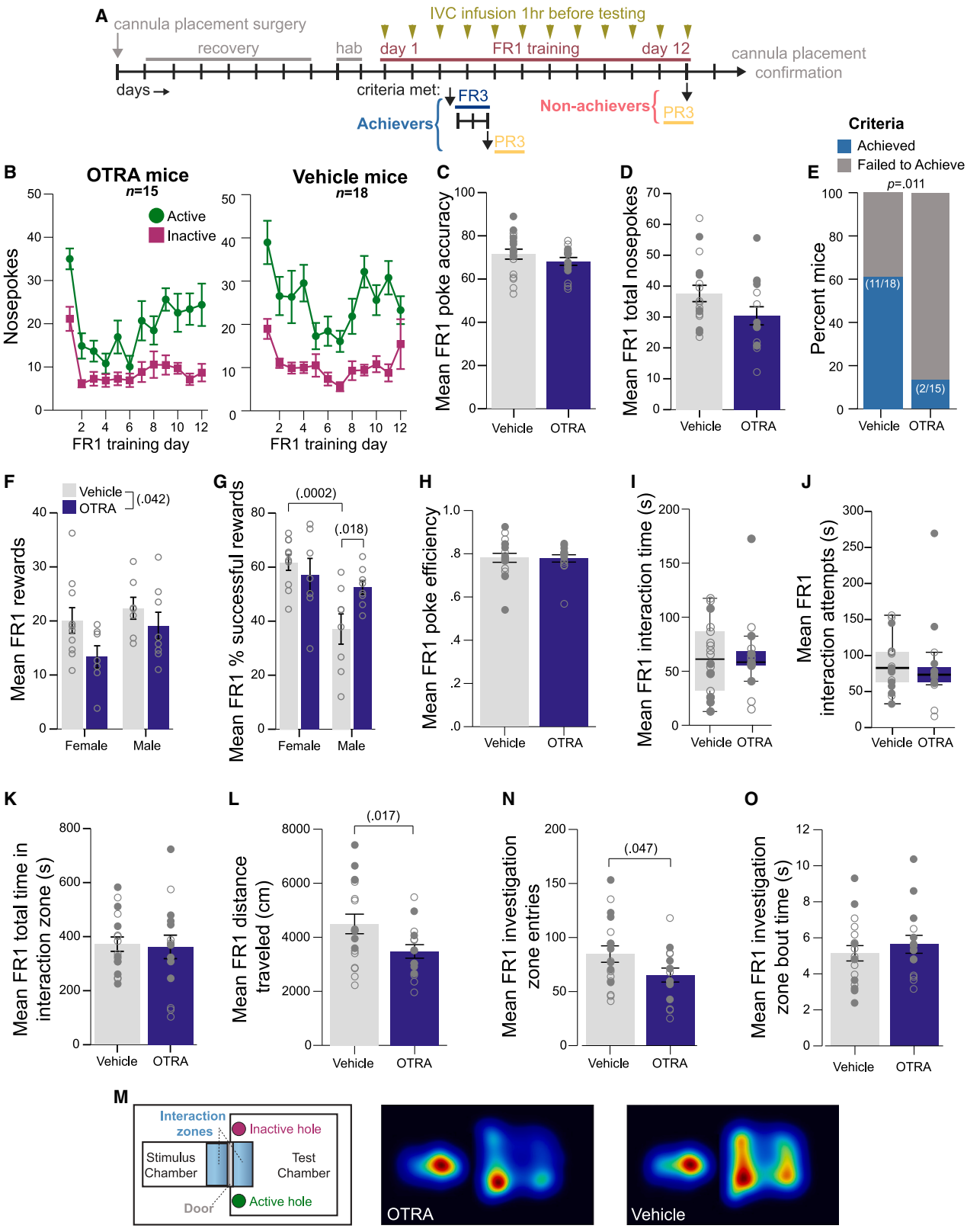

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covariate to regress out the influence of reduced locomotion on reward seeking and social orienting outcomes. In the absence of locomotor differences, we found that the drug effect on the number of rewards no longer reached significance (Figure S5B). However, the change among males for the percentage of successful rewards and interaction time was maintained (Figures S5C and S5D). Accounting for locomotion also removed the significant effect of oxytocin antagonism on entries into the investigation zone (Figure S5E). All other non-significant findings were maintained (Figures S5F–S5J). Thus, the subtle effects on reward seeking during FR1 training are likely not a result of reduced locomotion. Summary statistics can be found in Table S3.

Next, we assessed how blockade of OTR influences an animal's willingness to increase effort for access to a social partner. The PR3 testing revealed a lower breakpoint in the OTRA-infused mice compared with vehicle-infused controls (Figure 6A), which was more pronounced in the females. There was no difference in the percentage of successful rewards between groups (Figure 6B), but OTRA-infused mice did show a reduction in interaction time (Figure 6C) as well as interaction attempt time (Figure 6D). These outcomes were likely influenced by their fewer rewards overall (Figure 6E), which mirrored their lower breakpoint (Figure 6D). Despite this reduction in rewards and breakpoint, blocking OTRs did not influence the total time spent in the interaction zone (Figure 6F), similar to FR1 performance. Unlike in FR1 training, the OTRA-infused mice did not differ in their activity levels as reflected by comparable distances traveled (Figures 6G and S6A) and investigation zone entries (Figure 6H) by the groups during PR3 testing. Regardless, we reanalyzed the data using a covariate approach to quantitatively determine if locomotion was influencing PR3 performance. We found the female-specific effects were maintained (Figure S6B–S6H). Together, these data indicate that blocking OTRs reduces, but does not completely abolish, social motivation by influencing reward seeking and not social orienting, especially in females. Summary statistics can be found in Table S3.

DISCUSSION

Here, we described in depth our social motivation assay that leverages operant conditioning to assess social reward seeking and social orienting, thus establishing a comprehensive assessment of social motivation behavior. We established, in a commonly used WT strain, that mice will work via nose pokes for access to a transient social interaction reward and will work harder for a social interaction than for a non-social control reward. Further, we demonstrated a sex difference in this task, with male mice showing more robust social reward seeking and social orienting behavior compared with females. In addition, we provided two test cases for this assay: the *Shank3B* mutant with established sociability reductions and blockade of the prosocial oxytocin system.

Our task extends previous assays by enabling the dissociation of different aspects of social motivation as well as automating and simplifying the procedure. The addition of simultaneous video tracking allows for the quantification of movement and interactions with the stimulus animal, thus enabling interpretation of nose-poking behavior in light of social engagement—a limitation acknowledged in previous studies.[26] Quantification of social interactions also allows us to disentangle the reward seeking aspects of social motivation from social orienting. This will enable future studies further dissecting the neural circuitry of these different social motivation components and investigating how genetic liabilities may differentially influence them. Extending the video tracking beyond previous work[10] to include that of the stimulus mouse enabled us to quantify additional metrics such as the duration and frequency of interactions between the stimulus and test animals, the percentage of rewards with a successful interaction, and the time spent attempting an interaction independent of the stimulus mouse behavior. This will allow for flexibility in future study designs, permitting differences in stimulus mouse behavioral responses to be explored. In addition, we leveraged the mouse's innate tendency to nose poke in dark holes, a more naturalistic behavior than lever pressing, which allowed us to

Figure 5. Blockade of OTRs had a subtle influence on social reward seeking behavior during training

(A) Experimental timeline for cannula placement surgery, social motivation operant assay with daily OTRA or vehicle infusions, followed by cannula placement confirmation via intracerebroventricular (i.c.v.) dye infusion.

(B) Nose pokes into active and inactive holes for OTRA-infused mice (left panel; n = 15) and vehicle-infused mice (right panel; n = 18) across FR1 training.

(C) Average poke accuracy for FR1 training.

(D) Average total nose pokes for FR1 training.

(E) Ratio of OTRA (2/15) and vehicle (11/18) mice that achieved conditioning criteria.

(F) Number of average rewards received across FR1 days by both infusion groups across males and females.

(G) Across FR1 days, the average percentage of successful rewards across groups for both males and females.

(H) Average poke efficiency across FR1 training.

(I) Average interaction time during FR1 training.

(J) Average interaction attempt time during FR1 training.

(K) Average total time in the interaction zone during FR1 training.

(L) Average daily distance traveled for FR1.

(M) Apparatus schematic (leftmost panel) and heatmaps of animals' body positions in the apparatus across the first three FR1 days. Warmer colors represent greater time spent in that position.

(N) Investigation zone entries averaged across FR1 days.

(O) Average investigation zone bout time across FR1 days.

(C, D, F–H, K, L, N, and O) Grouped data are presented as means ± SEM. (I and J) Data are presented as boxplots with thick horizontal lines as respective group medians, boxes 25th–75th percentiles, and whiskers 1.5 × interquartile range (IQR). Individual data points as open (females) and closed (males) circles. Sample sizes: WT (n = 14, female = 7), Het (n = 10, female = 5), and KO (n = 14, female = 6).

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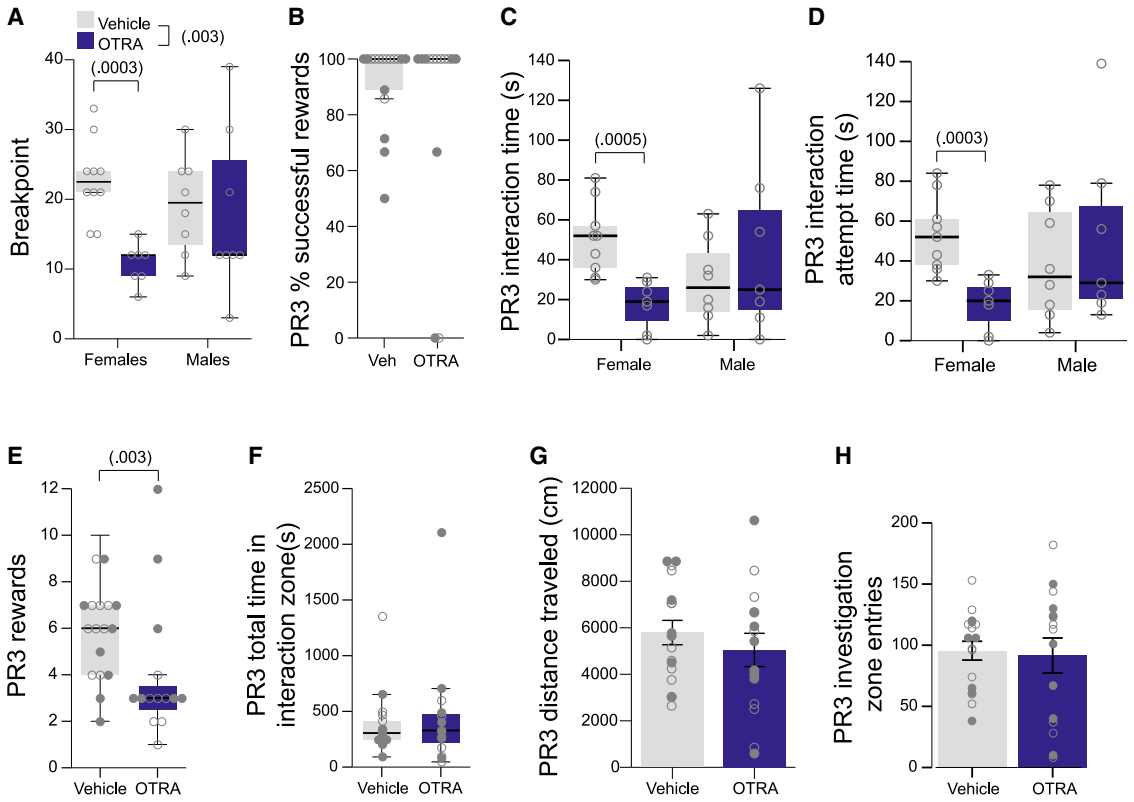

Figure 6. OTR antagonism reduces the motivation to achieve social interaction rewards

(A) The breakpoint achieved during PR3 testing across infusion groups and segregated by sex (vehicle, n = 10 females and n = 8 males; OTRA, n = 7 females and n = 8 males).

(B) Percentage of successful rewards for both drug groups (vehicle, n = 9 females and n = 8 males; OTRA, n = 7 females and n = 6 males).

(C) Interaction time during PR3 testing (vehicle, n = 9 females and n = 8 males; OTRA, n = 7 females and n = 6 males).

(D) Time spent attempting to interact during PR3 (vehicle, n = 9 females and n = 8 males; OTRA, n = 7 females and n = 6 males).

(E) Number of rewards received during PR3 testing (vehicle, n = 10 females and n = 8 males; OTRA, n = 7 females and n = 8 males).

(F) Total time in the interaction zone during PR3 testing (vehicle, n = 10 females and n = 7 males; OTRA, n = 7 females and n = 8 males).

(G) Total distance traveled in the testing chambers by the OTRA and vehicle mice during PR3 testing (vehicle, n = 10 females and n = 7 males; OTRA, n = 7 females and n = 8 males).

(H) Total entries into the investigation zone during PR3 (vehicle, n = 10 females and n = 7 males; OTRA, n = 7 females and n = 8 males).

(G and H) Grouped data are presented as means ± SEM.

(A–F) Data presented as boxplots with thick horizontal lines as respective group medians, boxes 25th–75th percentiles, and whiskers 1.5 × IQR. Individual data points as open (females) and closed (males) circles.

use the shorter 10 day protocol. Thus, our task more easily fits into an extensive autism-model phenotyping pipeline. Further, a shorter protocol allows for social motivation assessment at earlier ages, such as during the social reward critical period,[32] whereas a full month of training would age an animal to early adulthood by the time of PR testing.

While both males and females exhibit clear motivation for social reward in this task, we observed a more robust performance in males. Specifically, males achieved more rewards and higher breakpoints and spent more time interacting with and in the interaction zone compared with females. Sex differences have been reported in other operant-based tasks. For example, female rats exhibited faster punishment-avoidance learning than male rats in a punishment risk task in which a rewarded action is associated with an escalating probability of punishment.[33] Another study reported that female mice were less motivated to engage in an operant-style task on lower-value trials.[34] Specifically, females were slower than males to initiate trials by nose poking to reveal choice levers following unrewarded outcomes. It may be that the social reward was perceived at a lower value in females compared with males in our task, or possibly the females habituated to the social reward more quickly. In a meta-analysis of social approach task performance, males showed a higher approach index than females.[35] Further, another study reported that when males consistently interacted with the social partner, dopamine signal in the nucleus accumbens remained high, while this signal quickly decreased in females, suggesting adaptation.[36] Future studies coupling this social motivation task with calcium imaging (e.g., fiber photometry) and neural activity/inhibition techniques (e.g., DREADDs) are needed to further dissect the sex differences observed here.

Sociability deficits in *Shank3B* mice have been well characterized.[16,30,31] *Shank3B* KO mice display impairments in free socialization, approach of a social partner, and recognition of social novelty, especially in male animals. To assess sociability in *Shank3B* mutants here, we utilized video tracking during the habituation period. We found that, as in the literature, KO mice approached the social stimulus less than WT mice, and KO males spent less time in proximity to the social stimulus door than either WT or Het littermates. Thus, general sociability is indeed reduced in the KO mice.

When tasked with learning to nose poke for social reward, we found that all *Shank3B* genotypes could distinguish between the active and inactive holes, but KO mice nose poke less, attain fewer rewards, and show less exploration of the apparatus. The KO mice fail to show a clear increase in active nose pokes compared with inactive hole pokes until FR1 day 7, whereas both WT mice and Het mice do so by day 3. This suggests a possible slower-paced learning in the KO mice, which would be an interesting phenotype to explore in future work. Further, the experimental timeline of this assay may be altered in future studies to investigate learning speed and efficiency across models. Overall, social reward appears to be less motivating to KO mutants than their Het and WT littermates, even when taking into consideration the KO mutants' overall hypoactivity. Previous work in another SHANK3-deficiency model found that *Shank3* mutants were less incentivized to overcome aversive environments for social reward.[37] While this task was designed to measure social preference rather than motivation, their work aligns with our findings. Unfortunately, their study utilized only male mice, so it is uncertain whether the task would reveal similar sex differences as we observed in the current study.

Interestingly, here we observe a previously unreported phenotype in the Het mutants, which lack only one functional copy of *Shank3B* and thereby better model haploinsufficient patient populations. While we expected that fewer KO mice would reach conditioning criteria, we were surprised that fewer Het mice reached criteria as well. Further, as in the KO males, Het males also exhibited reduced social orienting. Moreover, Het males and KO males both had significantly lower breakpoints than WT males. This suggests that our assay has high sensitivity for detecting social motivation deficits in autism-associated mouse models and represents a valuable addition to existing assays of social behavior. We anticipate that, particularly when measures of social orienting are included, studies of social motivation may broaden the field's understanding of even well-characterized mouse models.

It is an interesting question as to whether overall learning deficits also contribute to the observed phenotypes in the *Shank3B* mutants. For example, a much smaller percentage of mutants achieved learning criteria. Yet, even for those mutants that do, they still show a lower breakpoint, indicating clear social motivation deficits in addition to any challenges with learning in general.

Disruption of oxytocinergic activity blunted WT animals' motivation to work for a social interaction, including reward seeking behavior, yet did not influence social orienting, as the mice spent comparable time near the social stimulus door. This is in line with previous work showing intact social approach behavior in oxytocin-null mice, indicating that this peptide is not essential for general spontaneous social approach in mice.[38] Increasing salience of, and thus attention to, social information via altered inhibitory tone has been suggested as a secondary, neuromodulatory role for oxytocin.[18] Therefore, instead of blunting any social orienting behavior, it is more likely blockade of OTRs during our social motivation operant assay resulted in a reduction in salience of the social reward, thereby decreasing motivation to achieve social interactions, as reflected in fewer rewards. In the mesolimbic reward system, oxytocin has been shown to influence midbrain dopamine activity through modulation of GABAergic neuronal firing. Specifically, oxytocin biases activity toward the ventral tegmental area (VTA) and away from the substantia nigra, thus preferentially activating the reward system.[39] It is possible that preventing this shift in activity results in the reduced reward seeking behavior observed in OTRA mice. Yet, the OTRA mice did still show some social reward-mediated behaviors. This could either be due to additional non-OXT circuits, or the fraction of OXT-responsive VTA dopamine neurons that are insensitive to OTR blockade, likely due to cross-reactivity with the vasopressin receptor.[39] Future work employing circuity mapping tools in OXT neurons will further clarify the role of oxytocin in social motivation.

Another opportunity apparent from our data arises from the fascinating propensity of unmanipulated, WT C57BL/6J littermates to dichotomize into two groups: socially motivated achievers and socially unmotivated non-achievers. Other behavioral paradigms similarly find such apparently inherent dichotomization of littermates into distinct behavioral phenotypes.[40–42] Here, we have further repeated the PR3 test over multiple days, which showed a remarkably stable intraindividual correlation (>0.82), indicating that social motivation, at least under these conditions, exhibits a trait-like stability. This stability highlights an opportunity for investigations comparing highly motivated achievers and their unmotivated non-achiever littermates to further dissect the molecular underpinnings of social motivation.

Finally, although we had selected our manipulations of OTR blockade and *Shank3B* mutation primarily as test cases for this approach, synthesizing across all three experiments included here does lead us to suggest one more conclusion: the circuitry that mediates social motivation may be sexually dimorphic in the mouse brain. Not only do WT males show greater levels of social orienting (e.g., interaction time) and reward seeking (e.g., total nose pokes in PR3), but our two manipulations have disparate consequences on the sexes. Specifically, the female mouse social motivation circuitry appears to be more dependent on OXT signaling in our assay, while only the male circuitry was vulnerable to *Shank3B* mutation. Such a "double dissociation" is a classic demonstration that the circuits in the two sexes are distinct biological systems. Furthermore, of the two autism-associated mouse models we have evaluated so far—*Shank3B* presented here and the previously tested autism/intellectual disability-associated gene *Myt1l*[43]—both showed social motivation deficits only in male mice, while overall sociability measured by social approach was reduced in both males and females. This suggests that this male-specific vulnerability (or interaction between sex and genetic risk[44]) may be present across multiple models of autism-associated gene mutation. This sex difference in circuitry is intriguing given the increased risk of autism in males

and could be further leveraged to understand the neurobiology of sex difference in social motivation, especially if this male bias replicates across many more models of the condition.

Overall, the protocol presented here expands existing assays by coupling automated, quantitative measures of social reward seeking with simultaneous measurement of social orienting. Social operant conditioning protocols have already proven useful in probing inherent social differences between mouse strains and uncovering novel circuits underlying social motivation (Martin et al.,[26] Hu et al.[10]). We anticipate that our assay will provide further avenues for investigations of social motivation circuitry and the impact of autism-associated gene mutations on social motivation. Future studies evaluating the rewarding properties of both social and non-social stimuli across multiple models will enable us to explore whether reduced social motivation is universally present or limited to a subset of autism genetic liabilities and to investigate whether deficits in reward processing are global or specific to the social domain. Finally, using this approach as part of the toolkit to examine the interaction between autism risk factor models and sex might provide insights into the social circuits that are uniquely vulnerable in the male brain.

Limitations of the study

This study presents a method for assessing both social reward seeking and social orienting aspects of social motivation in the mouse model. Limitations of this task should be noted. One is the requirement of intact operant conditioning capabilities. As noted by Martin and Iceberg,[27] this task requires that the mice learn an association between the nose-poking behavior and the social reward. Thus, interpretation of the task will be limited in models with impaired conditioning abilities. However, coupling this task with another non-social operant conditioning paradigm (e.g., for food rewards) would provide a control for general conditioning ability and clarification as to whether any deficits are global or specific to the social domain. Another limitation is the lack of a satiety measurement that is completely independent from motivational valence. Therefore, we cannot separate out reward satiety from lack of motivation in our current data for mice that fail to achieve conditioning criteria or exhibit very low reward numbers. Lastly, an important consideration is the importance of the additional FR3 training on the increased PR3 breakpoints in the achievers compared with non-achievers. To test the influence of the additional FR3 training on PR performance, we conducted an experiment in which FR3 was removed from the paradigm in an independent cohort of C57BL/6J mice at a comparable early adult age to those in cohort 1. Thus all mice, regardless of criteria achievement, received similar types of learning prior to PR3 testing. Once again, we found that the mice segregated into achievers and non-achievers and that the achievers reached a significantly higher breakpoint on average compared with non-achievers (Figure S7). These data suggest that the addition of FR3 training was not the driver of the increased breakpoint among achievers in the cohorts reported above.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Animal models
- METHOD DETAILS
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  - Intracerebroventricular infusion of oxytocin receptor antagonist
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at Zenodo: https://doi.org/10.1016/j.crmeth.2023.100504.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.E.M., J.D.D., and C.W.; methodology, S.E.M., K.B.M, Y.L., and C.W.; software, Y.L.; formal analysis, S.E.M. and S.S.; investigation, S.S., K.B.M., and R.G.S.; resources, S.E.M., J.D.D., C.W., and K.B.M.; writing, S.E.M., C.W., S.S., and J.D.D.; visualization, S.E.M. and S.S.; supervision, S.E.M. and J.D.D.; funding acquisition, J.D.D. and S.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Mouse, Shank3B Heterozygous mutant (B6.129-*Shank3*^{tm2Gfng}/J)	The Jackson Laboratory	#017688; RRID:IMSR_JAX:017688
Mouse, C57BL/6J, Wild-type	The Jackson Laboratory	#000664; RRID:IMSR_JAX:000664
Oligonucleotides		
Shank3B PCR genotyping (WT) primer: F(5′-3′): GAGACTGATCAGCGCAGTTG	The Jackson Laboratory	N/A
Shank3B PCR genotyping (WT) primer: R(5′-3′): TGACATAATCGCTGGCAAAG	The Jackson Laboratory	N/A
Shank3B PCR genotyping (Mut) primer: F(5′-3′): TCTAACTCCCAGAGGCCAGA;	Peça et al.[16]	N/A
Shank3B PCR genotyping (Mut) primer: R(5′-3′): TCAGGGTTATTGTCTCATGAGC	Peça et al.[16]	N/A
Software and algorithms		
IBM SPSS Statistics, v28	IBM, New York, New York USA	https://www.ibm.com/products/spss-statistics?lot=5&mhsrc=ibmsearch_a&mhq=SPSS; RRID:SCR_019096
Excel Office	Microsoft, WA, USA	https://www.microsoft.com/
Python 3.8.3	Anaconda enterprises, TX, USA	https://www.python.org/
Adobe Illustrator 2021	Adobe Systems, CA, USA	https://adobe.com/products/illustrator
BioRender	BioRender, Toronto, ON, Canada	https://www.Biorender.com/
MED PC V	Med Associates, Fairfax, VT USA	https://med-associates.com/; RRID:SCR_012156
Ethovision XT, v15	Noldus, Wageningen, the Netherlands	https://www.noldus.com/; RRID:SCR_000441
Java, v8	Oracle, Redwood Shores, CA USA	http://www.java.com
Annotated custom Java scripts for data processing	This paper.	Zenodo: https://doi.org/10.5281/zenodo.7905220
Annotated Med PC V scripts for data acquisition	This paper.	Zenodo: https://doi.org/10.5281/zenodo.7905220
Annotated SPSS scripts for data processing	This paper.	Zenodo: https://doi.org/10.5281/zenodo.7905220
Other		
Detailed procedural protocol and protocols for assembly and operation of equipment including a parts lists.	This paper.	Zenodo: https://doi.org/10.5281/zenodo.7905220

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Joseph D. Dougherty (jdougherty@wustl.edu).

Materials availability

This study did not generate new unique agents. Detailed protocols for assembly and operation of equipment, parts lists, and annotated scripts for data processing and analysis can be found here: Zenodo: https://doi.org/10.5281/zenodo.7905220.

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Data and code availability

- All behavioral data reported in this paper will be shared by the lead contact upon request.
- All original code created for operant data processing has been deposited at Zenodo: https://doi.org/10.5281/zenodo.7905220 and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animal models

All mice used in this study were maintained and bred in the vivarium at Washington University in St. Louis. For all experiments, adequate measures were taken to minimize any pain or discomfort. The colony room lighting was on a 12:12h light/dark cycle (lights on at 6a.m.); room temperature (~20–22°C) and relative humidity (50%) were controlled automatically. Standard lab diet and water were freely available. Upon weaning at postnatal day (P)21, mice for behavioral testing were group housed according to sex and experimental condition.

Social motivation was assessed in three separate cohorts. The first cohort included unmanipulated wildtype C57BL/6J mice (20 females, 20 males; JAX Stock No. 000664; RRID:IMSR_JAX:000664) that served to validate the task. Twenty mice (10 females, 10 males) served as experimental mice that received transient access to a social partner as a reward during testing. The remaining 20 mice (10 females, 10 males) served as control mice that received as a reward only the opening of the door to expose a metal apparatus wall. The testing procedure for this cohort was as stated above, except that all mice received up to 18 FR1 training sessions and 4 PR3 testing sessions to assess reliability of performance within individuals (Figure 1A). All mice were young adults (*M* = P68, range P56-P82) at the start of testing.

The second cohort consisted of mice modeling genetic liability for Phelan-McDermid Syndrome, a neurodevelopmental disorder characterized by global developmental, speech and motor delay, intellectual disability, and high prevalence of autism.[45] Specifically, these C57BL/6J mice harbor a disruption to the PDZ domain of the *Shank3B* gene.[16] To generate this cohort, we crossed *Shank3B* heterozygous mutants (*Shank3B* Het; JAX Stock No. 017688; RRID:IMSR_JAX:017688) producing 14 wildtype (*Shank3B* WT; 7 females, 7 males), 10 *Shank3B* Het (5 females, 5 males), and 14 *Shank3B* homozygous mutant (*Shank3B* KO; 6 females, 8 males) littermates. The testing procedure for this cohort was as stated above, with 10 days of FR1 testing since more than 85% of experimental mice in the first cohort reached conditioning criteria by then (Figure 3A). All mice were young adults (*M* = P79, range P70-P99) at the start of testing.

The third cohort comprised 33 C57BL/6J wildtype mice administered either an oxytocin receptor antagonist (OTRA; n = 15; 7 females, 8 males) or vehicle-only (n = 18; 10 females, 8 males) on each day of the protocol (Figure 5A). Drug administration details are described below. The testing procedure for this cohort was as stated above, with 12 days of FR1 testing since more than 85% of experimental mice in Cohort 1 reached criteria before then. All mice were young adults (P69-P72) at the start of testing.

METHOD DETAILS

All experimental protocols were approved by and performed in accordance with the relevant guidelines and regulations of the Institutional Animal Care and Use Committee of Washington University in St. Louis and were in compliance with US National Research Council's Guide for the Care and Use of Laboratory Animals, the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.

Social operant conditioning task

Social motivation was evaluated in mice using a social operant conditioning task adapted and extended from previous methods[26,27] by adding continuous video tracking to measure, in parallel, both social reward seeking and social orienting aspects of social motivation.[1] Each standard operant conditioning apparatus was enclosed in a sound-attenuating chamber (Med Associates) and modified to include a 3D printed filament door attached via fishing wire to a motor (Longruner), allowing it to be raised and lowered. This door was controlled by an Arduino (UNO R3 Board ATmega328P; RRID:SCR_017284) programmed with custom code, connected to the Med Associates input panel. A clear acrylic conspecific stimulus chamber (10.2 × 10.2 × 18.4 cm; Amac box, The Container Store) was attached to the side of the operant chamber, centered between the nosepoke holes and separated from the chamber itself by a doorway (10.2 × 6 cm) containing vertical stainless steel bars spaced 6mm apart (Figure 1B). The chamber was illuminated with white light during testing and an additional red chamber light illuminated in the apparatus during testing. The bottom tray of the operant apparatus was filled with one cup of fresh corn cob bedding, which was replaced between mice. Each operant chamber and stimulus chamber were designated for either males or females throughout the experiment. The operant chambers were cleaned with 70% ethanol and the stimulus chambers were cleaned with 0.02% chlorhexidine diacetate solution (Nolvasan, Zoetis) between animals.

Each operant chamber was outfitted with two nosepoke response holes. One was designated the active hole, which triggered illumination of a cue light within that hole and the raising (opening) of the door between the operant and stimulus chambers. The other hole was inactive and did not trigger any events. Designation of active and inactive status to right or left holes was randomized and counterbalanced across groups. When the door opened following a nosepoke in the active hole, the experimental and stimulus animals were able to interact through the bars for a 12-s social interaction reward before the door lowered (shut) and the active hole cue light turned off. The operant apparatus were connected to a PC computer via a PCI interface (Med Associates). MED PC-V software (RRID:SCR_012156) quantified nosepokes as ''active'', ''inactive'', and ''reward'' based on the location of the poke and whether it occurred within an ongoing 12-s reward period (i.e., active nosepokes made while the door was raised were added to the ''active'' count but not the ''reward'' count). CCTV cameras (Vanxse) were mounted above the chambers and connected to a PC computer via BNC cables and quad processors. Ethovision XT v15 software (Noldus; RRID:SCR_000441) was used to track the experimental and stimulus animals' movement to quantify distance traveled, as well as time spent in and entries into the social interaction zones (defined as 6 × 8 cm rectangles in front of the door within both the operant and stimulus chambers). Custom Java and SPSS Statistics (IBM; RRID:SCR_019096) scripts were used to align the Ethovision tracking data with the timing of rewards in the Med Associates data to determine presence or absence of each animal within the interaction zones during each second of every reward period (Zenodo: https://doi.org/10.5281/zenodo.7905220).

The operant conditioning protocol included phases of habituation, fixed-ratio conditioning (''training''), and progressive-ratio conditioning (''testing'') (Figures 1A, 3A, and 4A). For all trials, sex- and age-matched novel C57BL/6J mice served as conspecific stimulus animals. The stimulus mice were loaded into and removed from the stimulus chambers prior to the placement and after removal of the experimental mice into the operant chambers, respectively, to prevent the experimental animals from being in the chambers without a conspecific stimulus animal present. Habituation consisted of 30 min sessions on each of two days, during which the door remained open and the nosepoke holes were inaccessible. This allowed the experimental mice to acclimate to the chamber and to the presence of a stimulus partner in the adjoining chamber, as well as providing an opportunity for a measure of social approach behavior. Fixed- and progressive-ratio conditioning consisted of 1-h daily sessions during which nosepokes in the active hole were rewarded with a 12-s social interaction opportunity. During the 12-s reward period, any additional active nosepokes did not result in another reward.

Several schedules of reinforcement were utilized across the conditioning phases. A fixed-ratio schedule of one reward per one active nosepoke (FR1) was employed for at least the first three days of training, after which mice were individually advanced to the next phase upon achievement of conditioning criteria. Conditioning criteria were defined as, within a single session, achieving at least 40 active nosepokes, a 75% poke accuracy, and 65% successful rewards (defined as both experimental and stimulus mice in their respective social interaction zones simultaneously for at least 1 s of the reward, or time at the door for the control group in cohort 1). Animals who failed to meet these criteria after 10 days of FR1 were designated as non-achievers. For animals that successfully met conditioning criteria, FR1 was followed by three days of a fixed ratio 3 (FR3) training, in which three active nosepokes were required to obtain a single reward. All mice then received 1 to 4 days of a progressive ratio 3 (PR3) testing to determine their breakpoint, or maximum effort the animal will exert for a social interaction. In this phase, rewards became progressively more effortful to obtain, with three additional nosepokes required for each subsequent reward (i.e., 3, 6, 9, 12, etc.). Breakpoint was defined as the number of rewards after which the animal would no longer continue to engage in nosepoking behavior.

Detailed protocols for assembly and operation of equipment, parts lists, and annotated scripts for data processing and analysis can be found here: Zenodo: https://doi.org/10.5281/zenodo.7905220.

Intracerebroventricular infusion of oxytocin receptor antagonist

Surgeries were conducted at P59. Twenty-four hours prior to surgery, each animal was given 0.25 mg of the chewable anti-inflammatory Rimadyl (Bio-Serv, Flemington, NJ) and the surgical area was shaved. Mice were anesthetized with 2.5–5% isoflurane and placed in a stereotaxic apparatus. Mice received a local anesthetic, 1 mg/kg of Buprenorphine SR (ZooPharm, Laramie, WY), an antibiotic, 2.5–5 mg/kg of Baytril (Bayer Healthcare LLC, Shawnee Mission, KS), and 0.5mL of 0.09% sterile saline. Following cleaning of surgical area by alternating 70% Ethanol and Betadine surgical scrub (Purdue Products L.P., Stamford, CT) three times, an incision was made along the skull and skin retracted to visualize bregma to lambda. The periosteum was removed by lightly scratching the surface of the skull and the area was cleaned three times with a Betadine solution, followed by 3% hydrogen peroxide. The guide cannula was cut to a length of 2mm, so that it would enter the lateral ventricle, and placed in a stereotaxic cannula holder (Stoelting, #51636-1). Using a rapid, fluid motion, the 26-gauge unilateral guide cannula (C315GS-5/SPC, Plastics One, Roanoke, VA) was inserted at the following coordinates: M/L = +1, A/p = −0.4, D/V −2.2, based on prior work.[46–48] C&B Metabond dental cement (Parkell, Edgewood, NY) was mixed on a chilled ceramic dish and used to secure the cannula to the skull and seal the surgical area. The dental cement dried completely and a dummy cap was inserted to prevent clogs of the implanted cannula. The dummy cap (C315DCS-5/SPC) and internal cannula (C315IS-5/SPC) were cut to protrude 0.2mm from the end of the guide. The animal was removed from the stereotaxic apparatus and placed in a recovery cage. Animals were housed together after fully awake and provided an additional 0.25mg dose of Rimadyl. During daily monitoring, dummy caps were replaced and tightened as needed. Mice were given seven days to recover prior to testing, and were euthanized at any sign of distress or damage to the surgical area (n = 2 OTRA mice, n = 2 Vehicle mice).

All mice received 4μL infusions at least 1 h before participating in the social operant task each day. Mice were randomly assigned to receive either vehicle (artificial cerebrospinal fluid solution, Tocris Bioscience, Bristol, UK; n = 18) or an oxytocin receptor antagonist (OTRA; desGly-NH_2,d$(CH_2)_5$[Tyr(Me)^{2}Thr^4]OVT, desGly(NH_2),^{9}d$(CH_2)_5$[Tyr(Me)^2 Thr^4]OVT Bachem, Torrance, CA; n = 15). The OTRA, dissolved in vehicle at 0.25 ng/uL, is a peptidergic ornithine vasotocin analog chosen because of its broad applicability and prior use in ICV injections.[49,50] The solutions were delivered into the lateral ventricles through a 33-gage internal cannula (C315IS-5-SPC) via a PlasticsOne Cannula Connector (C313CS) over the course of 120 s using a programmable syringe pump (New Era pump systems #NE-1200). Mice were restrained by neck scruff while the internal cannula was inserted and then were placed in a clean holding cage, allowing free movement during infusion. To ensure complete diffusion, the internal cannula remained inserted for 60–90 s post infusion, after which mice were returned to home cage. Following completion of all behavioral testing, cannula placement was confirmed by infusing 10ul of dye (India Ink, Higgins, Leeds, MA) to flood the ventricles and immediately euthanizing the animal via isoflurane overdose. Brains were extracted and sliced coronally at the injection site with a razor blade. Infusion of the dye into the ventricles was then confirmed by eye. Three OTRA mice had clogged cannula and were excluded from the final analysis.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses and data visualization were conducted using IBM SPSS Statistics (v.28; RRID:SCR_019096)). Prior to analyses, data were screened for missing values and fit of distributions with assumptions underlying univariate analysis. This included the Shapiro-Wilk test on z-score-transformed data and qqplot investigations for normality, Levene's test for homogeneity of variance, and boxplot and *Z* score (±3.29) investigation for identification of influential outliers. One influential outlier was removed from the OTRA Cohort 3 dataset for total nosepokes, rewards, active pokes, interaction attempt time and total time in the interaction zone. Means and standard errors were computed for each measure. Analysis of variance (ANOVA), including repeated measures and covariate analyses, were used to analyze data where appropriate, and simple main effects were used to dissect significant interactions. Sex was included as a biological variable in all analyses across all experiments. Where appropriate, the Greenhouse-Geisser or Huynh-Feldt adjustment was used to protect against violations of sphericity. Multiple pairwise comparisons were subjected to Bonferroni correction, where appropriate. For data that did not fit univariate assumptions, non-parametric tests were used or transformations were applied. Sex by genotype interaction effects are reported where significant, otherwise data are reported and visualized collapsed for sex. The critical alpha value for all analyses was $p < 0.05$ unless otherwise stated. Figure illustrations were generated using BioRender. The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. Details of all statistical tests and results can be found in Tables S1–S3.