

REGULAR RESEARCH ARTICLE

Tandospirone, a Partial 5-HT_{1A} Receptor Agonist, Administered Systemically or Into Anterior Cingulate Attenuates Repetitive Behaviors in Shank3B Mice

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Abstract

Background: Several cases of autism spectrum disorder have been linked to mutations in the SHANK3 gene. Haploinsufficiency of the SHANK3 gene contributes to Phelan-McDermid syndrome, which often presents an autism spectrum disorder phenotype along with moderate to severe intellectual disability. A SHANK3 gene deletion in mice results in elevated excitation of cortical pyramidal neurons that alters signaling to other brain areas. Serotonin 1A receptors are highly expressed on layer 2 cortical neurons and are known to have inhibitory actions. Serotonin 1A receptor agonist treatment in autistic cases with SHANK3 mutations and possibly other cases may restore excitatory and inhibitory balance that attenuates core symptoms.

Methods: A series of experiments investigated the effects of acute tandospirone treatment on spatial learning and self-grooming, subchronic treatment of tandospirone on self-grooming behavior, and the effect of tandospirone infusion into the anterior cingulate on self-grooming behavior.

Results: Only male Shank3B^{+/-} mice exhibited a spatial learning deficit and elevated self-grooming. Acute i.p. injection of tandospirone, 0.01 and 0.06 mg/kg in male Shank3B^{+/-} mice, attenuated a spatial acquisition deficit by improving sensitivity to positive reinforcement and reduced elevated self-grooming behavior. Repeated tandospirone (0.06 mg/kg) treatment attenuated elevated self-grooming behavior in male Shank3B^{+/-} mice. Tandospirone injected into the anterior cingulate/premotor area reduced self-grooming behavior in male Shank3B^{+/-} mice.

Conclusions: These results suggest that stimulation of cortical serotonin 1A receptors may reduce repetitive behaviors and cognitive impairments as observed in autism spectrum disorder, possibly by attenuating an excitation/inhibition imbalance. Further, tandospirone may serve as a treatment in autism spectrum disorder and other disorders associated with SHANK3 mutations.

Key Words: 5-HT_{1A} receptor, tandospirone, autism, repetitive behaviors, learning, Shank3

Introduction

Autism spectrum disorder (ASD) is characterized by restricted interests and repetitive behaviors (RRBs) and social-communicative deficits (Harstad et al., 2015). There is evidence for both environmental (Grabrucker, 2012; Sealey et al., 2016) and genetic factors

conferring risk (Lichtenstein et al., 2010; Robinson et al., 2016) for ASD. Accumulating evidence suggests that the SHANK3 gene is a high-risk gene related to ASD. SHANK3 mutations are associated with ASD (Boccutto et al., 2013; Soorya et al., 2013; Sanders et al.,

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Significance Statement

The SHANK3 gene has been implicated in the pathogenesis of several autism cases as well as an haploinsufficiency syndrome, Phelan-McDermid, which commonly manifests an autism phenotype with intellectual disability. Past studies have predominantly used Shank3 homozygous mice to understand pathophysiology, behavior, and potential drug treatments. The current study characterized learning deficits and repetitive motor behaviors in Shank3 heterozygous mice and determined whether the partial 5-HT_{1A} agonist, tandospirone, could alleviate behavioral impairments. Heterozygous Shank3 mice (Shank3B^{+/-}) exhibited a probabilistic learning deficit and elevated self-grooming behavior. Both acute and chronic treatment with tandospirone alleviated the deficits. Direct infusions of tandospirone into the anterior cingulate region, a brain area found altered in autism, also reduced self-grooming behavior in Shank3B^{+/-} mice. The results suggest that treatment with a partial 5-HT_{1A} agonist may reduce both cognitive and repetitive motor behaviors in autism, at least in part, by acting on cortical circuitry.

2015) that includes Phelan-McDermid syndrome in which a high percentage of individuals meet criteria for an ASD diagnosis and is associated with haploinsufficiency of the SHANK3 gene (Bill and Geschwind, 2009; Bonaglia et al., 2011).

Because SHANK3 mutations have been implicated in contributing to ASD as well as other disorders (Soorya et al., 2013; Guilmatre et al., 2014; Zhang et al., 2017), several Shank3 mutant mouse lines have been developed to understand underlying neuropathophysiology (Bozdagi et al., 2010; Peca et al., 2011; Lee et al., 2015; Jaramillo et al., 2017; Bey et al., 2018; Drapeau et al., 2018). SHANK3 produces Shank3 proteins, which play a large role in anchoring scaffolding protein complexes to the F-actin cytoskeleton of both presynaptic axon terminals and postsynaptic densities (Bozdagi et al., 2010; Verpelli et al., 2011; Halbedl et al., 2016). The autism phenotype has been hypothesized to occur from an excitation/inhibition imbalance in the brain (Rubenstein and Merzenich, 2003). Studies of Shank3 mutations in mice have revealed altered glutamatergic transmission and synaptic plasticity (Bozdagi et al., 2010; Lee et al., 2015; Speed et al., 2015; Jaramillo et al., 2017; Bey et al., 2018) as well as greater activity of cortical neurons in medial prefrontal cortex and elevated self-grooming behavior (Lee et al., 2015; Wang et al., 2016). Therefore, treatments that reduce excitation of cortical neurons may be effective in reducing RRBs in Shank3 mutant mice.

Serotonin 1A receptors (5-HT_{1A}Rs) are expressed in the rodent cortex where activation produces an inhibitory effect on cortical pyramidal neurons (Chalmers and Watson, 1991). Related to RRBs, the partial 5-HT_{1A}R agonist tandospirone reduces marble-burying behavior in Wistar rats (Abe et al., 1998). Other experiments demonstrated that drugs with mixed 5-HT_{1A}/5-HT₂R properties also reduce motor stereotypies in mice (Middlemiss and Fozard, 1983; Wood et al., 2000; Canal et al., 2015). Besides stereotyped motor behaviors, understanding the role of 5-HT_{1A}Rs on learning in Shank3 mutant mice is important as individuals with SHANK3 mutations show learning impairments (LeBlond et al., 2014; Egger et al., 2016). Spatial discrimination tests with probabilistic reinforcement have been developed to understand reward learning in ASD individuals (Solomon et al., 2011; D'Cruz et al., 2013, 2016) and in mouse models of autism (Amodeo et al., 2012, 2018). The same treatments that reduce stereotyped motor behaviors in rodents also enhance learning (Micheau and Van Marrewijk, 1999; Wolff et al., 2004). Taken together, the findings suggest that stimulation of 5-HT_{1A}Rs may improve behaviors in mice comparable with core symptoms in ASD. However, many treatments lack specificity for 5-HT_{1A}Rs and/or lead to significant side effects.

The present set of studies determined whether the partial 5-HT_{1A}R agonist tandospirone attenuates elevated self-grooming and a probabilistic learning deficit in Shank3B^{+/-} mice. Experiments included studying the effects of systemic

treatment, both acute and chronic, as well as infusions into the anterior cingulate, a frontal cortex area identified where altered Shank3 expression may contribute to autism-like behaviors (Guo et al., 2019). Tandospirone possesses a high affinity for 5-HT_{1A} receptors similar to buspirone but a significantly lower affinity for D2 receptors compared with the more commonly studied buspirone (Hamik et al., 1990). Therefore, tandospirone may be a more specific treatment for 5-HT_{1A} receptors.

Methods

Subjects

Male and female Shank3^{+/-} (HET) mice with a B6 background were acquired from the Jackson Laboratory (Bar Harbor, ME) to serve as founders for a Shank3B mouse colony. A trio (1 male, 2 female) breeding system was employed in a temperature-controlled vivarium. After 15 days, female mice were placed in individual housing (28-cm-wide × 17-cm-long × 12-cm-high plastic cage).

Mice genotypes were derived at a typical Mendelian rate. A 3-mm tail tissue sample was obtained from each pup at approximately day 15 for genotyping (Transnetyx, Memphis, TN). Pups were weaned at 21 days of age and group housed with same sex littermates. All mice were placed in individual housing at 5 weeks of age in preparation for behavioral testing procedures, which began when mice reached 7 weeks of age.

Unless noted, mice received food and water ad libitum throughout an experiment. A 12-hour-light/-dark cycle was employed throughout the study. Only Shank3B^{+/-} (wild type [WT]) and HET mice were tested. Shank3B^{-/-} (knockout [KO]) were not tested. Animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Laboratory Animal Care and Use Committee at the University of Illinois at Chicago.

Sex Differences in Learning and Self-Grooming Behavior

Spatial Learning Apparatus

Training and testing were conducted in a black acrylic maze (76 cm long × 50 cm wide × 30 cm high) containing a start area that was distinguished by a center wall separating the start and choice areas. The choice area contained 2 distinct spatial areas separated by an acrylic piece (30 cm long × 16 cm high) extending from the back wall of the maze. Each spatial location contained a food well near the back wall and unique visual cues attached to their respective back and side walls. A small plastic door (10 cm high × 5 cm wide) was inserted into the center of the wall separating the start and choice areas.

Training

WT and HET mice were food restricted until reaching 85% of their ad libitum body weight. Training sessions began by placing a mouse into the start area. The start door was opened, allowing the mouse to enter the choice area. After consuming a one-half piece of Fruity Pebbles (Post Foods, St. Louis, MO) from each food well, the mouse returned to the start area. This sequence was repeated until 15 minutes had elapsed. Mice achieved training criterion after completing 6 or more trials within a 15-minute session for 2 consecutive days. Mice were trained for 2 to 5 days before testing.

Spatial Learning

All mice received an i.p. injection of sterile water 30 minutes prior to testing. Prior to testing, 1 location was designated the “correct” spatial location and contained a one-half piece of cereal on 80% of trials. On the other 20% of trials, the “incorrect” location was baited with a one-half piece of cereal. On each trial, a mouse was allowed to sample only 1 food well per trial. The first 2 trials always contained food reinforcement in the correct reward area. Between trials, the choice area was cleaned with a 2% quatricide solution to minimize the use of odor cues. Learning criterion was achieved when a mouse chose the correct location on 6 consecutive trials (Amodeo et al., 2017, 2018).

Self-Grooming Apparatus

The self-grooming test was conducted using an empty, clear plastic cage (28 cm wide × 17 cm long × 12 cm high) covered with a clear plastic cage filter top.

Procedure

Different WT and HET mice were tested for self-grooming and then used in the spatial learning test. Mice were placed in the apparatus 20 minutes after receiving an i.p. injection of sterile water. Mice were allowed to freely explore for 20 minutes. The first 10 minutes served as a habituation period. The second 10 minutes served as the test. A trained observer recorded the cumulative amount of time spent grooming all body regions. All materials were cleaned or replaced between tests.

Locomotor activity was measured during the self-grooming test. The apparatus was divided into equal 9.33-cm × 17-cm zones. Locomotor activity was measured as the total number of lines crossed, with a line crossing defined as any instance of all 4 paws of a mouse traveling from one zone to a different zone.

Acute Tandospirone Effect on Spatial Learning

Male WT and HET mice receiving vehicle treatment were used as controls in this experiment. All aspects of training and testing were as described above. Mice received an i.p. injection of sterile water or tandospirone at 0.01 or 0.06 mg/kg 30 minutes prior to testing. An error analysis was conducted to assess whether a deficit was driven by an early or late learning impairment (Solomon et al., 2011). Early learning was defined as all trials of the testing period prior to the first instance of 3 consecutive correct choices. All trials following the first instance of a mouse making 3 consecutive correct choices were defined as late learning. All errors made during late learning were scored as late learning errors.

Learning was further analyzed as a function of trial to trial feedback in a manner comparable with that applied to ASD individuals during probabilistic learning (D’Cruz et al., 2013). The number of trials on which a mouse made an error after receiving positive reinforcement for a correct choice on the previous trial

was calculated and termed “win-stay” errors. The number of trials on which a mouse switched its choice to the incorrect spatial location after not receiving positive reinforcement for making a correct choice on the previous trial was calculated and termed “lose-shift” errors.

Acute Tandospirone Treatment on Self-Grooming Behavior

A separate cohort of mice was generated for this experiment as described above. Male WT and HET were tested for self-grooming after receiving treatment of sterile water, 0.01, 0.06, and 0.3 mg/kg tandospirone across 4 sessions following a Latin Square design. Successive tests for a given mouse occurred 1 week following the previous test.

Chronic Tandospirone Treatment on Self-Grooming Behavior

A separate cohort of male WT and HET mice were generated for this experiment as described above. Prior to behavioral testing, mice received either an i.p. injection of sterile water or tandospirone 0.06 mg/kg for 14 consecutive days. This repeated injection procedure was based on a previous study investigating the effects of chronic tandospirone treatment (Uehara et al., 2013). Twenty-four hours after the last injection, each mouse received a self-grooming test. All aspects of testing were as described above.

Tandospirone Injection Into the Anterior Cingulate on Self-Grooming Behavior

A separate cohort of male WT and HET mice were generated for this experiment as described above.

Stereotaxic Surgery

Each mouse (7 weeks of age) received stereotaxic surgery to bilaterally implant cannulae aimed at the anterior cingulate. Before surgery, each mouse received an i.p. injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). A 5-mm stainless-steel guide cannulae (Plastics One, Roanoke, VA) was implanted at a 10-degree angle aimed medially. The stereotaxic coordinates for the anterior cingulate were the following: 1.3 mm anterior to bregma, ±1.1 mm lateral, 1.5 mm below the skull. To minimize pain or discomfort, mice received s.c. administration of the anti-inflammatory meloxicam after surgery and for 2 days subsequently.

Microinfusion Procedure

Mice were first placed in a tapered plastic cone (Braintree Scientific Inc.) with a cut-out that allowed guide cannulae to protrude out. Mice were restrained in the plastic cone throughout the duration of the microinfusion procedure. A 33-gauge injection cannula was inserted into each guide cannula. The injection cannula extended 1 mm beyond the guide cannula tip. The injection cannulae were attached to polyethylene tubes (PE-20) connected to separate 10- μ L syringes. The syringes were driven by a microinfusion pump with solutions infused in a volume of 0.2 μ L per side for 2 minutes. The total volume infused was 0.2 μ L per side. The cannulae were left in place for 30 seconds to allow drug diffusion around the injector tip. After removal of the injection cannulae, mice were removed from the plastic cone and left undisturbed in their home cage for 1 minute. Subsequently, mice were placed in the apparatus for testing.

Self-Grooming Procedure

All aspects of testing were as described above. Male WT and HET mice were tested for self-grooming behavior after receiving a microinfusion of sterile water, 1 $\mu\text{g}/\text{side}$ and 5 $\mu\text{g}/\text{side}$ tandospirone across 3 sessions following a Latin Square design. Successive tests for a given mouse occurred 1 week following the previous test.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism software. In Experiment 1, separate 2-way ANOVAs were conducted to determine if there was a significant difference between genotypes and sex in mice for spatial learning and self-grooming behavior. For all subsequent experiments, a 2-way ANOVA was conducted to determine if there was a significant difference for genotype or treatment in spatial learning or grooming measures. A 3-way ANOVA with repeated measures was conducted for early and late learning errors. Post-hoc Tukey multiple comparisons tests were used to determine significant differences between specific groups.

Results

Sex Differences in Learning and Self-Grooming Behavior

The initial study examined male and female Shank3B HET mice on spatial learning with probabilistic reinforcement. Male HET mice required approximately 100 trials to achieve criterion while other groups required 60 to 65 trials (see Figure 1A). A 2-way ANOVA revealed significant main effects for sex ($F_{1,29}=15.67$, $P<.001$) and genotype ($F_{1,29}=6.76$, $P=.015$) as well as a significant sex \times genotype interaction ($F_{1,29}=4.29$, $P=.047$). Post-hoc comparisons revealed that male HET mice required significantly more trials to criterion compared with that of all other groups ($P<.01$).

The self-grooming results are shown in Figure 1B. There were significant main effects for sex ($F_{1,33}=5.06$, $P=.031$) and genotype ($F_{1,33}=6.18$, $P=.018$) as well as a significant sex \times genotype interaction ($F_{1,33}=5.97$, $P=.020$). Post-hoc comparisons revealed that male HET mice spent significantly more time grooming compared with that of all other groups ($P<.05$).

Tandospirone Effects on Spatial Learning

Because only male HET mice exhibited a spatial learning deficit, tandospirone was examined in only male WT and HET mice (see Figure 2). The analysis revealed significant main effects for genotype ($F_{1,38}=4.61$, $P=.038$) and treatment ($F_{2,38}=13.81$, $P<.001$) as well as a significant genotype \times treatment interaction ($F_{2,38}=3.29$, $P=.048$). A post-hoc test indicated that HET mice were significantly impaired in spatial learning compared with that of WT mice ($P<.05$). Administration of 0.01 and 0.06 mg/kg tandospirone significantly reduced trials to criterion in HET mice compared with that of vehicle-treated HET mice ($P<.001$ and $P<.01$, respectively) and to a level that did not significantly differ from that of vehicle-treated WT mice ($P>.05$).

Analysis of early and late learning errors indicated all groups exhibited a comparable level of errors early in learning. Late in learning, vehicle-treated HET mice exhibited an increase in errors that was decreased by tandospirone treatment (see Figure 2B). This is reflected by a significant genotype \times error interaction ($F_{1,37}=5.62$, $P=.023$). However, tandospirone also decreased late learning errors in WT mice, leading to an overall main effect for treatment ($F_{2,37}=5.33$, $P=.009$). There were no

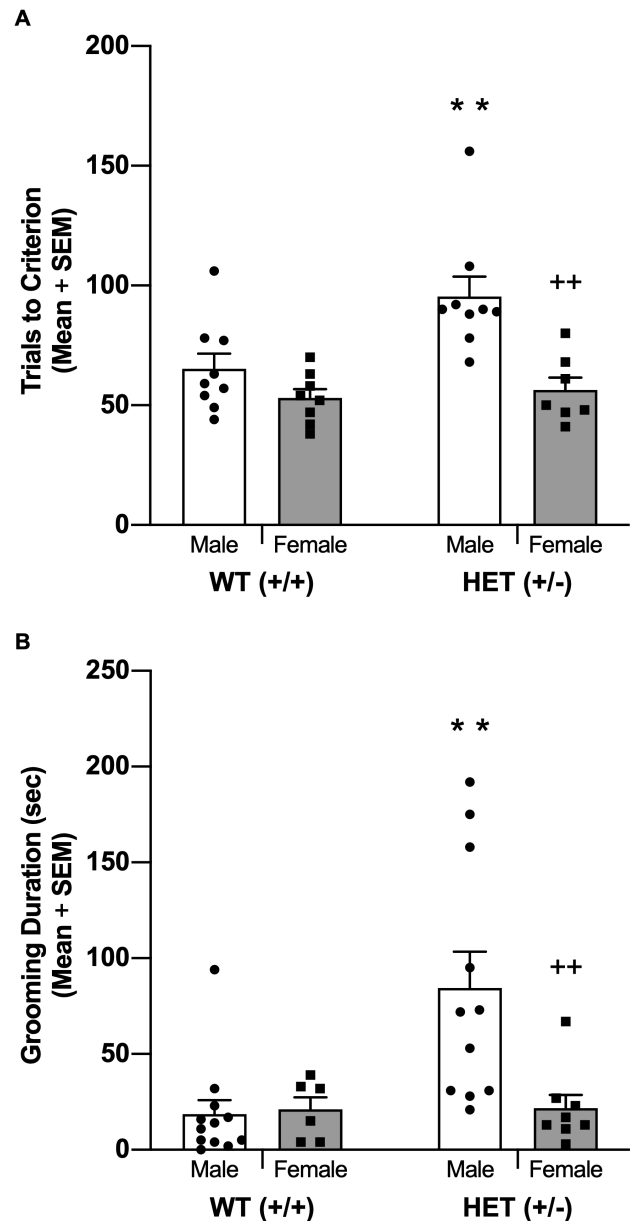


Figure 1. (A) Male^{-/-} (HET) mice required significantly greater number of trials to criterion on a spatial discrimination with probabilistic reinforcement compared with that of wildtype (WT) and female HET mice. (B) Male HET mice exhibited elevated grooming behavior compared with that of WT and female HET mice. ** $P<.01$ vs WT, ++ $P<.01$ vs male HET mice.

other significant main effects or interactions. Thus, male HET mice could initially learn the spatial discrimination at a similar rate as WT mice but were impaired in the later phase of learning that was attenuated by tandospirone.

Analysis of win-stay and lose-shift errors during the late phase of learning revealed that HET mice exhibited an increase in win-stay errors compared with that of WT mice, which was reversed by tandospirone treatment (see Figure 2C). There were significant main effects of genotype, ($F_{1,38}=4.83$, $P=.034$) and treatment ($F_{2,38}=6.29$, $P=.004$). Additionally, there was a significant genotype \times treatment interaction ($F_{2,38}=6.22$, $P=.005$). A post-hoc test indicated that vehicle-treated HET mice committed a significantly greater number of win-stay errors than vehicle-treated WT mice ($P<.01$). In addition, HET mice that received tandospirone treatment at a dose of either 0.01 or

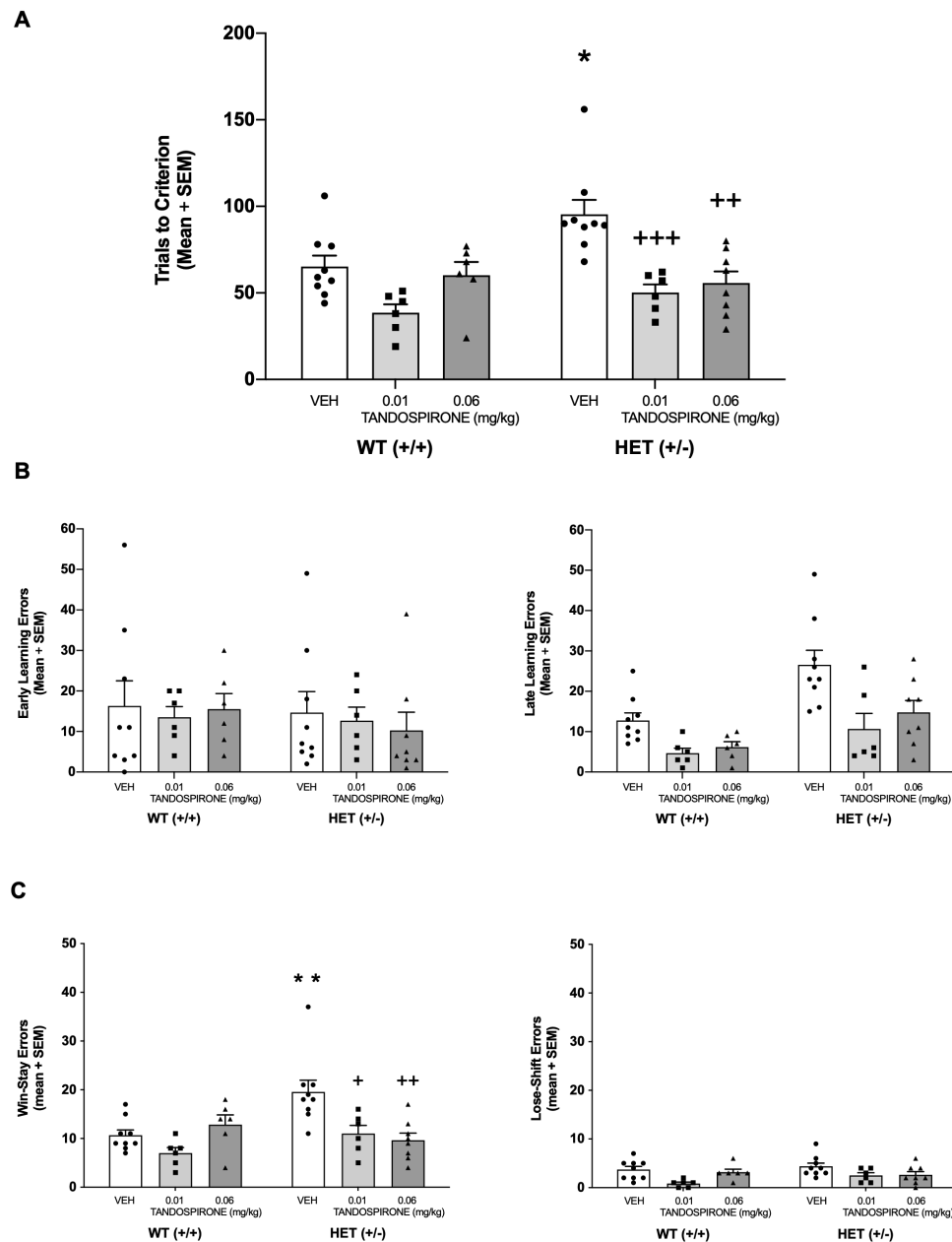


Figure 2. The beneficial effect of tandospirone treatment on spatial acquisition with probabilistic reinforcement in male^{-/-} (HET) mice. (A) Vehicle-treated HET mice required an increased number of trials to criterion compared with that of wildtype (WT) mice. Tandospirone at 0.01 and 0.06 mg/kg significantly reduced the number of trials to criterion in HET mice. (B) HET mice exhibited an increase in late, but not early, learning errors that was attenuated by tandospirone. (C) HET mice had significantly greater number of Win-Stay errors that was attenuated by tandospirone. * $P < .05$ vs WT VEH, ** $P < .01$ vs WT VEH, +++ $P < .001$ vs HET VEH, ++ $P < .01$ vs HET VEH, + $P < .05$ vs HET VEH.

0.06 mg/kg committed significantly fewer win-stay errors than vehicle-treated HET mice ($P < .05$ and $P < .01$, respectively) and to a level that was not significantly different from that of vehicle-treated WT mice ($P > .05$). For lose-shift errors, WT and HET mice made a similar amount of errors. Tandospirone, particularly at the low dose, appeared to reduce lose-shift errors in both WT and HET mice. The analysis revealed there was no significant effect for genotype ($F_{1,38} = 1.21$, $P = .278$), but there was a significant main effect for treatment ($F_{2,38} = 6.30$, $P = .004$). There was no significant genotype \times treatment interaction ($F_{2,38} = 1.26$, $P = .30$). Thus, tandospirone treatment attenuated the learning deficit in male HET mice by improving sensitivity to positive reinforcement late in learning.

Acute Tandospirone Effects on Self-Grooming and Locomotion

Male HET mice groomed for approximately 90 seconds compared with 20 seconds for WT mice. Tandospirone, in a dose-dependent manner, reduced self-grooming behavior in male HET mice (see Figure 3A). The analysis indicated a significant main effect of genotype ($F_{1,84} = 11.53$, $P = .001$) and a significant main effect of treatment ($F_{3,84} = 3.37$, $P = .022$). The analysis also indicated that there was a significant genotype \times treatment interaction ($F_{3,84} = 3.14$, $P = .029$). A post-hoc test revealed that vehicle-treated HET mice had significantly greater grooming behavior than vehicle-treated WT mice ($P < .01$). Tandospirone

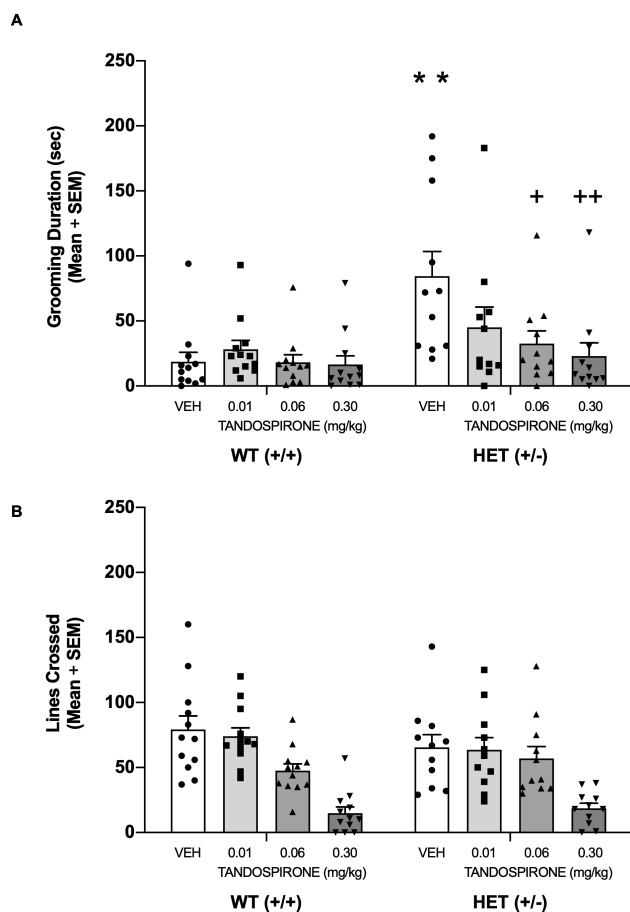


Figure 3. Tandospirone, in a dose-dependent manner, alleviates elevated self-grooming behavior in male^{-/-} (HET) mice. (A) Tandospirone significantly reduced grooming behavior in HET mice at doses of 0.06 and 0.3 mg/kg, but not 0.01 mg/kg. (B) Tandospirone at the 0.30-mg/kg dose reduced the number of line crossings in both wildtype (WT) and HET mice. ** $P < .01$ vs WT VEH, ++ $P < .01$ vs HET VEH, + $P < .05$ vs HET VEH.

treatment at the 0.06- and 0.3-mg/kg doses in HET mice significantly reduced self-grooming compared with that of vehicle-treated HET mice ($P < .05$ and $P < .01$, respectively) and to a level that was not significantly different from that of vehicle-treated WT mice ($P > .05$). In contrast, tandospirone treatment at 0.01 mg/kg in HET mice did not significantly decrease self-grooming behavior compared with that of vehicle-treated HET mice ($P > .05$). Moreover, tandospirone at all doses tested in WT mice did not significantly affect self-grooming behavior compared with that of vehicle-treated WT ($P > .05$).

Acute treatment with tandospirone, particularly at the highest dose, reduced locomotor activity in both genotypes (see [Figure 3B](#)). There was no significant effect for genotype ($F_{1,84} = 0.08$, $P = .78$), but there was a significant effect for treatment ($F_{3,84} = 16.81$, $P < .0001$). The genotype \times treatment interaction was not significant ($F_{3,84} = 1.04$, $P = .38$).

Chronic Tandospirone Treatment on Self-Grooming Behavior

The effects of tandospirone treatment administered for 14 days was examined on self-grooming behavior in male HET and WT mice (see [Figure 4A](#)). The 0.06-mg/kg dose was chosen because acute administration reduced self-grooming without affecting locomotor activity. Prior to chronic treatment, all mice received

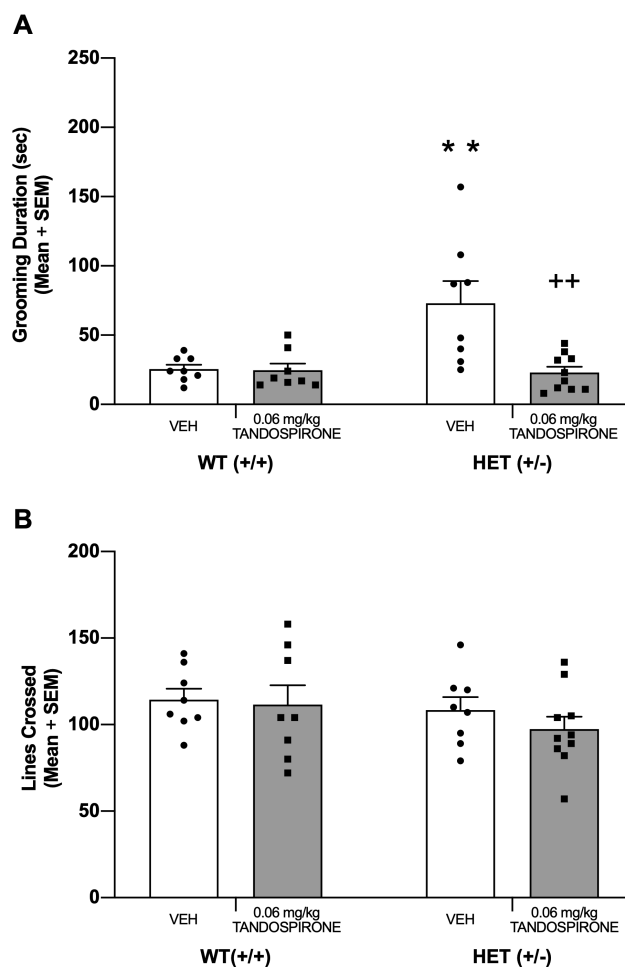


Figure 4. Repeated tandospirone treatment attenuated elevated self-grooming behavior in male^{-/-} (HET) mice. (A) Fourteen days of tandospirone injections significantly reduced grooming behavior in HET mice when tested 24 hours after the last injection. (B) Repeated tandospirone treatment did not affect number of lines crossed during self-grooming test. ** $P < .01$ vs wildtype (WT) VEH, ++ $P < .01$ vs HET VEH.

a self-grooming test because HET mice exhibited a bimodal level of self-grooming in the acute treatment experiment (see [supplementary Figure 1](#)). To ensure that HET mice in the vehicle and tandospirone groups had similar levels of self-grooming behavior prior to treatment, mice were pseudo-randomly assigned to a treatment group. The vehicle-treated group had a mean grooming duration of 65.38 seconds \pm 17.58 SEM, and the tandospirone-treated group had a mean grooming duration of 62.00 seconds \pm 13.15 SEM.

After 2 weeks of treatment, all mice were again tested for self-grooming behavior. There was a significant main effect of genotype ($F_{1,30} = 7.49$, $P = .01$), a significant main effect of treatment ($F_{1,30} = 9.28$, $P = .005$), and a significant genotype \times treatment interaction ($F_{1,30} = 8.48$, $P = .007$). Post-hoc tests revealed that vehicle-treated HET mice had significantly greater grooming behavior than vehicle-treated WT mice ($P < .01$). Chronic administration of tandospirone at 0.06 mg/kg in HET mice significantly reduced self-grooming behavior compared with that of vehicle-treated HET mice ($P < .01$) and to a level not significantly different from that of vehicle-treated WT mice ($P > .05$). Chronic tandospirone treatment in WT mice did not significantly affect self-grooming behavior compared with that of vehicle-treated WT mice ($P > .05$).

Chronic treatment with tandospirone (0.06 mg/kg) did not affect locomotor activity during the self-grooming test (see Figure 4B). There was no significant effect for genotype ($F_{1,30} = 1.48$, $P = .23$), nor was there a significant effect for treatment ($F_{1,30} = 0.70$, $P = .41$). There also was no significant genotype \times treatment interaction ($F_{6,84} = 1.20$, $P = .32$).

Tandospirone Injection Into the Anterior Cingulate on Self-Grooming Behavior

Histological analysis indicated that cannula placements were principally located in the rostral anterior cingulate at the level of forceps minor of the corpus callosum (Figure 5). Some mice had a cannula placement in the premotor area juxtaposed to the anterior cingulate.

Analysis of grooming behavior following injections into the anterior cingulate revealed there was a significant main effect of genotype ($F_{1,36} = 11.07$, $P = .002$), a significant main effect of treatment ($F_{2,36} = 4.92$, $P = .013$), and a significant genotype \times treatment interaction ($F_{2,36} = 3.81$, $P = .032$) (see Figure 6). Post-hoc tests revealed that vehicle-treated HET mice had significantly greater grooming behavior than vehicle-treated WT mice ($P < .01$). Tandospirone 1 μg in HET mice did not affect self-grooming behavior compared with that of vehicle-treated HET mice ($P > .05$). In contrast, tandospirone 5 μg in HET mice significantly reduced grooming behavior compared with that of vehicle-treated HET mice ($P < .01$) and to a level that was not significantly different from that of vehicle-treated WT mice ($P > .05$). Anterior cingulate infusion of tandospirone in WT mice did not significantly affect self-grooming compared with that of vehicle treatment ($P > .05$).

Analysis of locomotor activity during self-grooming revealed a significant effect for genotype ($F_{1,36} = 9.12$, $P = .005$), reflecting that HET mice exhibited fewer line crossings compared with that of WT mice across all treatments. There was no significant effect for treatment ($F_{2,36} = 0.27$, $P = .77$) or significant genotype \times treatment interaction ($F_{2,36} = 0.74$, $P = .48$).

Discussion

The results indicate that male HET mice, but not female HET mice, exhibit a spatial learning deficit when reinforcement contingencies are probabilistic. Male HET mice showed a similar learning rate as WT mice early in acquisition but were impaired later in learning. This acquisition impairment is comparable with that observed in a visual discrimination test using Shank3B HET mice (Copping et al., 2017). The present study extends those past findings by using a probabilistic learning test, as used with ASD individuals (Solomon et al., 2011; D’Cruz et al., 2013), and revealing HET mice display a learning deficit due to reduced positive reinforcement sensitivity. These results are consistent with a study in ASD individuals showing impaired probabilistic learning resulting from a limited ability to use positive feedback for learning (Solomon et al., 2011). Thus, the male Shank3B HET mouse may model some of the learning deficits observed in ASD individuals.

Tandospirone treatment at both doses tested rescued the learning deficit in male HET mice. Further, increased win-stay errors late in learning were alleviated by tandospirone treatment. That tandospirone reduced win-stay errors in male HET mice suggests that activation of 5-HT_{1A}Rs is sufficient to increase reward sensitivity and facilitate learning when outcomes are probabilistic. Future studies that explore whether tandospirone may also alleviate a probabilistic reversal learning deficit in HET mice and whether female HET mice exhibit a

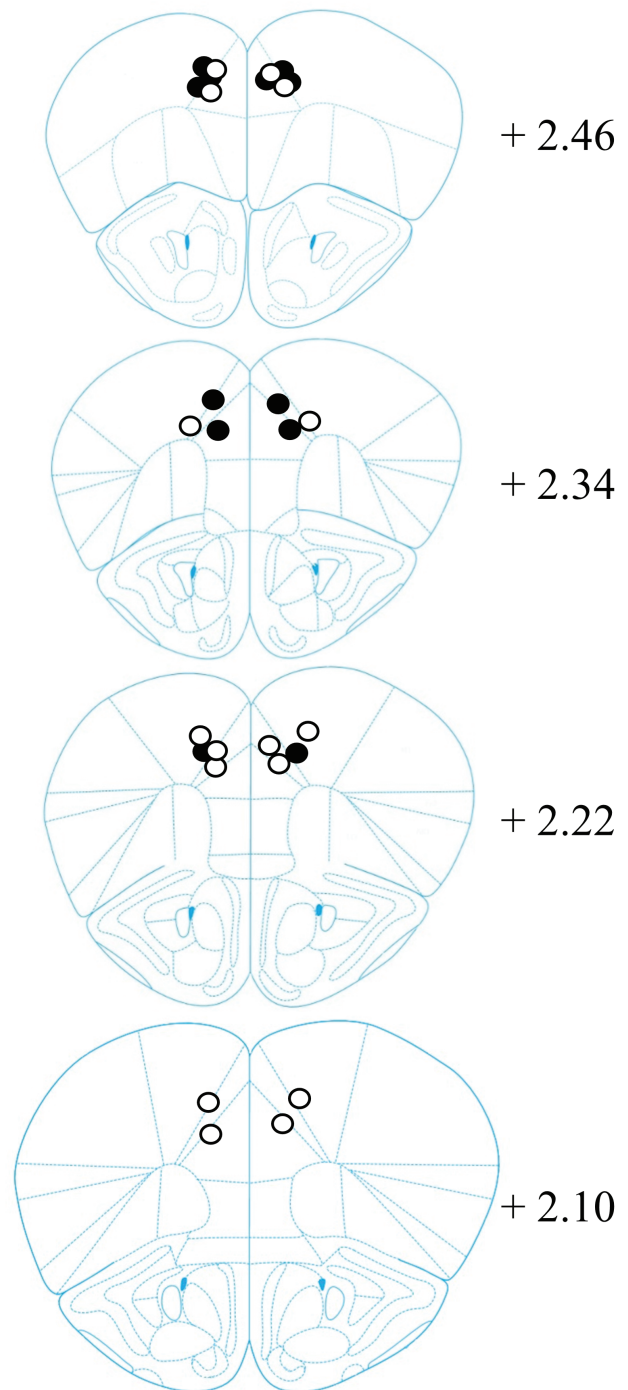


Figure 5. Cannula tip placements in the anterior cingulate and premotor areas of the mouse frontal cortex for mice included in the behavioral analyses. Mouse brain sections adapted from *The Mouse Brain in Stereotaxic Coordinates* (Paxinos and Franklin 2001). Black circle, cannula placements for wildtype (WT) mice; white circle, cannula placements for male^{-/-} (HET) mice.

deficit in probabilistic reversal learning that can be treated with tandospirone will be important to better understand the role of 5-HT_{1A}Rs in cognition related to ASD.

Similar to spatial learning, the self-grooming results indicate that male, but not female, HET mice exhibit elevated self-grooming behavior. Thus, for both learning and grooming, there

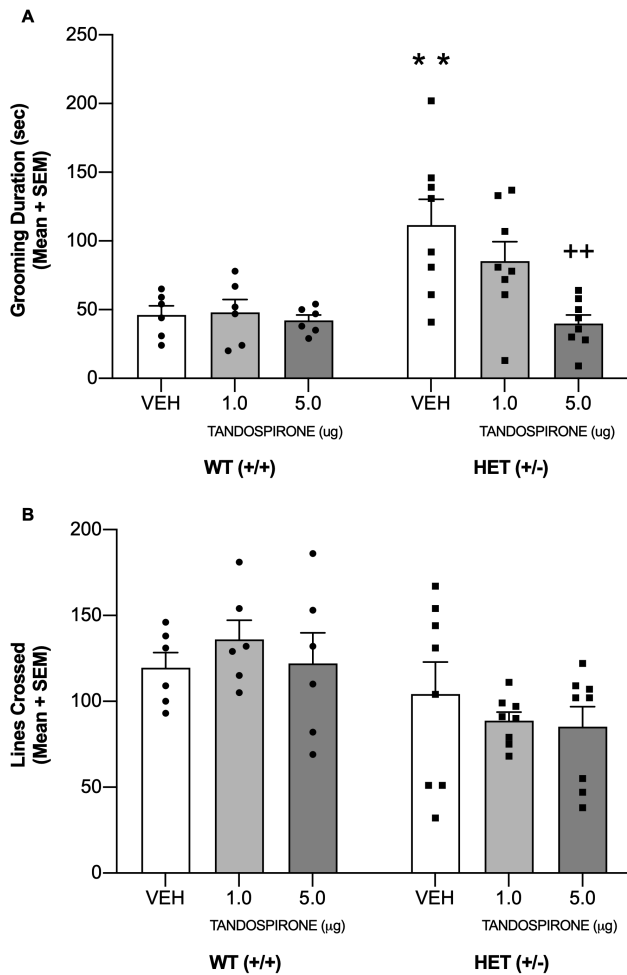


Figure 6. (A) Tandospirone infusion into the anterior cingulate, in a dose-dependent manner, reduced self-grooming behavior in male^{-/-} (HET) mice. (B) HET mice exhibited reduced line crossings compared with that of wildtype (WT) mice. Tandospirone did not affect line crossings in WT or HET mice. ** $P < .01$ vs WT VEH, ++ $P < .01$ vs HET VEH.

was a sex difference. For individuals with haploinsufficiency of the SHANK3 gene, there does not appear to be a sex difference in learning and RRBs (Soorya et al., 2013; Zwanenberg et al., 2016). One possibility is that the sex difference observed in the present study is related to the deletion site in Shank3B mice, and a more extensive deletion or different deletion site may lead to a similar phenotype in female and male HET mice.

Increased self-grooming behavior in male HET mice was bimodal, with approximately one-half of the mice exhibiting grooming around 120 seconds while the other one-half had a grooming duration of 30 seconds (see [supplementary Data](#)). The increased grooming behavior in male HET mice is consistent with a study demonstrating Shank3B^{E13} heterozygous mice also show elevated grooming behavior (Jaramillo et al., 2017). In contrast, another experiment found that HET mice did not display elevated grooming behavior (Drapeau et al., 2014). The difference in behavioral results may be related to the deletion site within the SHANK3 gene. HET mice used by Drapeau et al., (2014) were generated with a deletion of the ankyrin repeat domain (exons 4–9), whereas HET mice in the present experiment possessed a deletion of the PDZ domain (exons 13–16). Six intragenic promoters have been identified in SHANK3, and transcripts for both promoters existing between the ankyrin and PDZ domains continue to be expressed in Shank3^{e4-9} KO mice (Wang et al., 2011).

Thus, deletion of exons 4 to 9 in HET mice could have produced Shank3 proteins with an intact PDZ domain, which is expected to be absent in the HET mice used in the present experiment. By extension, this difference in self-grooming behavior between HET mouse models seems to specifically implicate a relationship between the Shank3 PDZ domain and stereotyped motor behavior. Consistent with this idea, Jaramillo et al., (2017) found disrupting the Shank3 PDZ domain by inserting a transcriptional stop cassette prior to exon 13 led to male Shank3^{E13} heterozygous mice displaying elevated self-grooming behavior similar to the male HET mice in the present study.

The 2 highest doses of tandospirone tested with acute injection reduced grooming behavior in the male HET group. Although tandospirone at 0.3 mg/kg reduced grooming behavior, this dose also significantly decreased locomotor activity, indicating this higher dose had a more general effect on motor activity. In contrast, the 0.06-mg/kg dose decreased grooming without affecting locomotor activity. Moreover, acute treatment also tended to reduce grooming behavior even in male HET mice who were “low groomers.” Although this was not significant, this was likely due to the low grooming values under the vehicle condition. Further, chronic treatment with tandospirone at 0.06 mg/kg was also effective in reducing self-grooming in male HET mice without affecting locomotor activity. Taken together with the findings on probabilistic learning, the results indicate that tandospirone treatment can alleviate both a learning impairment and an elevated stereotyped motor behavior without affecting general activity levels.

Systemic tandospirone administration improving learning and reducing grooming behavior in male HET mice raises the issue of what neural circuitry may be affected by stimulating 5-HT_{1A}Rs to rescue the phenotype. Recent findings indicate that conditional KO of Shank3 in the anterior cingulate region of mice leads to social interaction deficits, while restoration of SHANK3 in the anterior cingulate region rescues the deficit (Guo et al., 2019). Based on these recent findings, we determined whether direct infusions of tandospirone into the anterior cingulate affected self-grooming behavior. The results indicate that tandospirone into the anterior cingulate, in a dose-dependent manner, reduced elevated self-grooming in HET mice without affecting locomotor activity. Thus, the anterior cingulate may be one region in which stimulating 5-HT_{1A}Rs may alter cortical signaling to reduce RRBs.

At a neural systems level, one possibility is that atypical neuronal signaling originating in 1 or more areas of cortex, that is, anterior cingulate, preferentially projects to the basal ganglia direct pathway with greater than typical excitatory drive (Lei et al., 2004). If learning deficits and RRBs in ASD are generated as a product of relatively greater activation of the direct vs indirect pathway of the basal ganglia as previously suggested (Wang et al., 2011; Shepherd, 2013), then tandospirone may rescue the phenotype in Shank3 HET mice by reducing cortical excitatory drive of the striatum (De Almeida et al., 2008). Such a mechanism would be consistent with the findings of Peixoto et al. (2016), who demonstrated that mini excitatory postsynaptic currents of striatal medium spiny neurons in Shank3B KO mice can be reduced by chemogenetic inhibition of layer 5 cortical pyramidal neurons. Because 5-HT_{1A}Rs are primarily expressed on pyramidal neurons in cortical layers 2, 3, and 6, tandospirone might reduce elevated cortico-striatal glutamatergic transmission by reducing excitation of layer 2 pyramidal neurons on target layer 5a neurons (Anderson et al., 2010). Layer 5a pyramidal neurons project exclusively to the striatum (Lei et al., 2004; Reiner et al., 2010). This proposed effect of tandospirone could produce a downstream effect of restoring balance of excitation

and inhibition in the basal ganglia by minimizing glutamatergic input onto direct pathway MSNs.

The ability of tandospirone to alleviate both a stereotyped motor behavior and a learning deficit without affecting motor behavior more broadly suggests the drug may be effective in treating a range of RRBs while having limited side effects. Future studies testing the effects of tandospirone on a wider range of learning and stereotyped motor behaviors in this mouse model can help determine this. If tandospirone is able to improve core symptoms with minimal side effects will be important because the sedative effect of risperidone that accompanies its usefulness in alleviating some symptoms of ASD is problematic to the extent that it often becomes the reason individuals stop treatment (Lemmon et al., 2011). There is no evidence from the present experiments to suggest that a similar undesirable effect is induced by tandospirone even with chronic treatment. On the contrary, the findings from the present experiments demonstrate that treatment with tandospirone effectively and selectively reduces self-grooming behavior and a probabilistic learning deficit in Shank3B HET mice. Thus, this treatment may facilitate daily living in some ASD individuals, such as those that have a SHANK3 mutation. More broadly, the present findings suggest that 5-HT_{1A} agonism could be a viable treatment approach for individuals with ASD or other disorders associated with a SHANK3 mutation that express RRBs or cognitive deficits.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Statement of Interest

None.

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